Microbiological Research 287 (2024) 127833

Contents lists available at ScienceDirect

Microbiological Research

journal homepage: www.elsevier.com/locate/micres

Biocontrol efficacy of *Pseudomonas mediterranea* PVCT 3C against *Plenodomus tracheiphilus*: *In vitro* and *in planta* mechanisms at early disease stages

Giulio Dimaria [a], Angelo Sicilia [a], Francesco Modica [a,1], Marcella Russo [b], Marina Claudia Bazzano [b], Maria Elena Massimino [a], Angela Roberta Lo Piero [a], Patrizia Bella [c], Vittoria Catara [a,*]

[a] Department of Agriculture, Food, and Environment, University of Catania, Via Santa Sofia 100, Catania 95123, Italy
[b] Agrobiotech Soc. Coop, Catania 95121, Italy
[c] Department of Agricultural, Food and Forest Sciences, University of Palermo, Palermo 90128, Italy

ARTICLE INFO

Keywords:
Pseudomonas
Biocontrol
Genomics
Transcriptomics
Citrus
Plenodomus

ABSTRACT

In this study, we investigated the biocontrol activity of the *P. mediterranea* strain PVCT 3C against Mal secco, a severe disease of citrus caused by the vascular fungus *Plenodomus tracheiphilus*. *In vitro*, bacterial diffusible compounds, volatile organic compounds and culture filtrates produced by PVCT 3C reduced the mycelial growth and conidial germination of *P. tracheiphilus*, also affecting the mycelial pigmentation. The application of bacterial suspensions by leaf-spraying before the inoculation with the pathogen on plants of the highly susceptible species sour orange and lemon led to an overall reduction in incidence and disease index, above all during the early disease stage. PVCT 3C genome was subjected to whole-genome shotgun sequencing to study the molecular mechanisms of action of this strain. *In silico* annotation of biosynthetic gene clusters for secondary metabolites revealed the presence of numerous clusters encoding antimicrobial compounds (e.g. cyclic lipopeptides, hydrogen cyanide, siderophores) and candidate novel products. During the asymptomatic disease phase (seven days post-inoculation), bacterial treatments interfered with the expression of different fungal genes, as assessed with an NGS and *de novo* assembly RNA-seq approach. These results suggest that *P. mediterranea* PVCT 3C or its secondary metabolites may offer a potential effective and sustainable alternative to contain *P. tracheiphilus* infections via integrated management.

1. Introduction

Lemon (*Citrus limon*) is the third most important citrus species worldwide, with a global production, together with limes, amounting to 21.5 million tonnes (FAOSTAT, 2022). In the Mediterranean and Black Sea basins, which account for about half of the global lemon production (FAOSTAT, 2022), Turkey is the major producing country (1323,000 t per year), followed by Spain (863,240 t) and Italy (476,310 t). In these areas, lemon cultivation is threatened and severely limited by a devastating vascular disease known in Italian as Mal secco - literally 'dry disease' (Catara and Catara, 2019).

Mal secco disease (MSD), caused by the mitosporic fungus of the

Abbreviations: AHL-QS, acyl-homoserine-lactone-Quorum Sensing; ANIb, average nucleotide identity based on BLAST; AUDPC, Area Under the Disease Progress Curve; BGCs, biosynthetic gene clusters; CDSs, coding sequences; CF, culture filtrate; CLPs, cyclic lipopeptides; DEGs, differentially expressed genes; DI, disease index; GBDP, Genome Blast Distance Phylogeny; HR, hypersensitive response; IMM, Improved Minimal Medium; LB, Luria-Bertani; MSD, Mal secco disease; NDA, Nutrient Agar supplemented with 1 % Dextrose; NRPS, non-ribosomal peptide synthetase; PDA, Potato Dextrose Agar; PGAP, Prokaryotic Genome Annotation Pipeline; PGI, percentage of growth inhibition; PGPR, plant growth-promoting rhizobacteria; *Pme*, *Pseudomonas mediterranea*; *Pt*, *Plenodomus tracheiphilus*; PTI, Pattern-Triggered Immunity; RAST, Rapid Annotations using Subsystems Technology; SDW, sterile distilled water; TYGS, Type Strain Genome Server; T3SS, type-III secretion system; VOCs, volatile organic compounds.

* Corresponding author.
E-mail address: vcatara@unict.it (V. Catara).
[1] present address: Department of Life Sciences, University of Modena and Reggio Emilia, 42122 Reggio Emilia, Italy.

https://doi.org/10.1016/j.micres.2024.127833
Received 9 April 2024; Received in revised form 8 June 2024; Accepted 4 July 2024
Available online 16 July 2024

Leptospheriaceae family, *Plenodomus tracheiphilus* (formerly *Phoma tracheiphila*), is a vascular disease of utmost importance for the citrus industry, both for its significant economic impact and its potential dissemination to disease-free countries (EFSA PLH Panel, 2014; Migheli et al., 2009; Nigro et al., 2011). The main host is lemon, for which the disease is a critical production bottleneck, but other species in the *Citrus* genus and several genera in the Rutaceae, such as *Poncirus*, *Severinia* and their hybrids, can also serve as hosts (Catara and Catara, 2019; Nigro et al., 2011).

The pathogen is considered of quarantine concern by plant protection organizations worldwide, but it is currently present in the majority of countries in the Mediterranean basin and in some of the Black Sea (EFSA PLH Panel, 2014; EPPO; 2024; Picard et al., 2018; Zhao et al., 2021). Infections are initiated by conidia produced in pycnidia on withered twigs or by phialoconidia produced by "free" hyphae grown on exposed wood surfaces and within xylem elements (Migheli et al., 2009). The pathogen enters host tissues through wounds and leaf scars. It then spreads systemically, mostly upward, until it reaches the lumen of the xylem by the conidia transported into the xylem sap (Catara and Catara, 2019; Migheli et al., 2009; Nigro et al., 2011).

Symptoms start as discoloration of the primary and secondary veins that further extends to the whole leaf, leaf drop, and twig and branch dieback, with the name 'mal secco' (dry disease) originating from the characteristic desiccation of part or the whole tree. Typically the wood shows a pink-salmon discoloration (Migheli et al., 2009). Plant debris in orchards can be a source of inoculum for several weeks and cause the infection of wounded roots. In this case two other syndromes have been described: *mal fulminante* (sudden death) and *mal nero* (black disease) due to root infections that differ according to the speed of symptom progression (Perrotta and Graniti, 1988). The tree eventually dies from one to several years after infection (Nigro et al., 2011). Disease development has been mainly attributed to the activity of malseccin, a non-selective complex of toxic glycoproteins, whose major fraction, Pt60, was found to induce typical symptoms of MSD when applied to citrus leaves (Fogliano et al., 1998; Nachmias et al., 1979). Transcriptional analyses have confirmed a multifaceted mechanism of pathogenicity involving the destruction of plant defence metabolites and the optimization of fungus development and pathogenesis (Russo et al., 2021). Additionally, *P. tracheiphilus* infections rewire rhizosphere and endorhizosphere microbial community networks, leading to a depletion of potentially beneficial genera such as *Pseudomonas* and *Burkholderia* (Dimaria et al., 2023).

Management of the disease is challenging and, being a vascular disease, the plant cannot be freed of the pathogen once it has been infected (EFSA PLH Panel, 2014; Nigro et al., 2011). Currently, there are no lemon cultivars or clones resistant to *P. tracheiphilus* with competitive yields and satisfactory bio-agronomic features that can be adopted where the pathogen is present (Abbate et al., 2019; Catalano et al., 2021a). In the Mediterranean region, pruning of withered shoots and suckers, followed by burning, is key to reducing inoculum, along with protective treatments based on the application of copper compounds or other authorized fungicides during the rainy season, after hailstorms, frost damage and pruning (EFSA PLH Panel, 2014; Nigro et al., 2011). However, these methods are currently inefficient and cannot moderate the extremely large economic and social losses (such as loss of jobs, labour income or industry output) caused by the disease. The high susceptibility of the main commercial cultivars of lemon, the absence of authorized systemic agrochemicals and progressive restrictions to the use of copper-based products thus limit effective means to control MSD (EFSA PLH Panel, 2014). Studies on the biocontrol efficacy of different organisms and/or biological commercial products against *P. tracheiphilus* have been conducted on lemon and/or sour orange in a growth chamber (Lima et al., 1994; Grasso et al., 2008) and a glasshouse (Coco et al., 2004; Kalai-Grami et al., 2014) and on Volkamer lemon in a growth chamber (Aiello et al., 2022) and greenhouse (Leonardi et al., 2023).

Most of the biological products tested in these studies are registered for use against bacterial and fungal pathogens (e.g. *Xanthomonas* sp. and *Armillaria* sp.) not including *P. tracheiphilus* and in crops other than citrus, and their mechanism of action was not clarified or addressed only for a specific phytopathogen.

The genus *Pseudomonas* is the largest pool of potential biocontrol agents (BCAs), and diverse strains are effective in controlling vascular diseases both in herbaceous and woody crops (Bubici et al., 2019; Deketelaere et al., 2017; Gomez-Lama Cabanas et al., 2018).

Pseudomonas is a highly diverse and metabolically versatile Gammaproteobacteria genus ubiquitously found in the environment. Multiple *Pseudomonas* species show plant growth-promoting activities and biological control traits, as they are able to efficiently colonise plants, produce antimicrobial compounds and elicit systemic resistance in plants (Bakker et al., 2007; Höfte, 2021; Raaijmakers et al., 2010). Most of these species are included in the *P. fluorescens* complex, which is a taxonomically unresolved group that has been divided into a growing number of different groups and species. In particular, the different species comprising the *P. corrugata* subgroup, including *P. mediterranea*, have been reported to encode a plethora of secondary metabolites with wide-spectrum antimicrobial activities, such as hydrogen cyanide, the antifungal polyketide 2,4-diacetylphloroglucinol, or different cyclic lipopeptides (CLPs) (Höfte, 2021).

Although described as a weak and opportunistic pathogen (Catara, 2007), which causes tomato pith necrosis, *P. mediterranea* strains have shown a strong *in vitro* antimicrobial activity against bacterial, fungal and oomycete phytopathogens (Albert et al., 2024; Gu et al., 2020; Sallam et al., 2023; Strano et al., 2017; Ullah et al., 2020). In particular, preliminary results suggest that *P. mediterranea* PVCT 3C is a promising biocontrol agent for MSD, because it was shown to reduce disease symptoms when applied to the plant before *P. tracheiphilus* inoculations (Coco et al., 2004; Grasso et al., 2008). Recent transcriptomic analyses have shown that strain PVCT 3C also improves the defence response of lemon plants, by preventing the pathogen-induced downregulation of genes involved in effector-triggered immunity and in the biosynthesis and perception of phytohormones (Sicilia et al., 2024, 2023).

The three main aims of this study were to i) evaluate the biocontrol activity of *P. mediterranea* strain PVCT 3C against *P. tracheiphilus*, both *in vitro* and *in planta*; ii) assess its effect on the transcriptome of *P. tracheiphilus* during the early disease stage; and iii) identify potential genetic determinants for the biocontrol activity of this strain.

2. Materials and methods

2.1. Microbial strains, media and culture conditions

The *Pseudomonas mediterranea* strain PVCT 3C (= IPVCT 3C = CFBP 5458), which was isolated from tomato and characterized in different studies (Catara et al., 2002, 1997), was used in all the assays *in vitro* and *in planta* and for genome sequencing. Unless otherwise stated, PVCT 3C was routinely cultured at 25 ± 2 °C on nutrient agar (NA, Oxoid, Hampshire, UK) supplemented with 1 % dextrose (NDA) and stored in Luria-Bertani (LB, Laboratorios Conda S.A., Madrid, Spain) broth supplemented with 20 % (vol/vol) glycerol at −80 °C. Bacterial cells for plant treatments and DNA extraction were grown in LB broth inoculated with a single bacterial colony from a 24-h-old culture on NDA, incubated overnight at 28 °C under continuous shaking (180 rpm) and centrifuged (9000 *x g*, 20 min). The pellets were washed three times and resuspended in sterile distilled water (SDW) up to 10^8 cfu mL^{-1}. Total genomic DNA was purified using a Gentra Puregene bacterial DNA extraction kit (Qiagen, Hilden, Germany), following the manufacturer's protocol.

The *Plenodomus tracheiphilus* isolate PVCT Pt57 was selected for virulence according to a previous study (Oliveri et al., 2022). *Fusarium solani* isolate ABT Fs and *Phytophthora citrophthora* isolate ABT Pc, used in the antagonistic assays *in vitro*, were provided by the diagnostic

laboratory of Agrobiotech Soc. Coop. All isolates were routinely maintained on potato dextrose agar (PDA, Oxoid, Hampshire, UK) at 23 ± 2 °C and stored on PDA slants at 4 °C.

For plant inoculation, phialoconidia of *P. tracheiphilus* were obtained from cultures in carrot broth (Salerno and Catara, 1967). Two four-millimeter plugs of PDA along the actively growing area of seven-day-old fungal colonies were transferred to 250 mL-fluted flasks containing 150 mL of carrot broth (300 g of carrots L^{-1}) and incubated under continuous shaking (220 rpm), at 22 °C and diffused light for up to 4 days. The culture broth was filtered through four layers of sterile gauze in order to eliminate mycelia, and centrifuged at 6000 *x g* for 15 minutes. The pellets were washed three times and resuspended in SDW. Phialoconidia were counted using a hematocytometer and their concentration was adjusted to 10^6 mL^{-1} with SDW.

2.2. Antimicrobial activity in vitro

The antimicrobial activity of the *P. mediterranea* strain PVCT 3C was tested *in vitro* by dual-culture assays (to test diffusible compounds) and by double-plate assays (to test volatile organic compounds, VOCs), as described by Strano et al. (2017). The antimicrobial activity was expressed as percentage growth inhibition (PGI) (Vincent, 1947): PGI (%) = 100 · (GC − GT)/GC, where GC represents the mean value of the pathogen radius in the absence of PVCT 3C (control), and GT represents the mean value of the pathogen radius in the presence of PVCT 3C (treatment). The effect of VOCs on the germination of *P. tracheiphilus* phialoconidia was evaluated according to Strano et al. (2017) and the activity was expressed as percentage germination reduction.

The antimicrobial activity of cell-free culture filtrates (CF) of *P. mediterranea* strain PVCT 3C was tested *in vitro* by the poisoned food technique (Grove and Moore, 1962) against the *P. tracheiphilus* isolate PVCT Pt57. CF concentrations were chosen based on preliminary screenings (data not shown). The CF was obtained by culturing strain PVCT 3C in improved minimal medium (IMM) (Surico et al., 1988) in still culture for four days at 28 °C. After sterilization by filtration using 0.22-μm filters (Millipore, Billerica, MA, U.S.A.), the CF or sterile IMM (control) were added to the PDA medium at final concentrations of 20 % and 50 % (v/v). Bacteriological agar (0.7 % w/v) was added to PDA medium supplemented with 50 % CF or IMM. *P. tracheiphilus* mycelium plugs were placed in the centre of the plate and incubated at 23 ± 2 °C. PGI was calculated as above. The assay in liquid medium was conducted in 100 mL-Erlenmeyer flasks containing 20 mL of potato dextrose broth (PDB, Difco, Le Pont-de-Claix, France) inoculated with *P. tracheiphilus* phialoconidia (final concentration of 10^6 mL^{-1}). Each flask was supplemented aseptically with CF or IMM (control) to a final concentration of 20 % (v/v), and incubated under continuous shaking (150 rpm) at 25 ± 2 °C with diffused light for five days. The weight of the mycelium was determined. All the trials were conducted using three independent replicates and were repeated twice.

2.3. Biocontrol activity of P. mediterranea PVCT 3C against Plenodomus tracheiphilus in planta

The biocontrol activity of *P. mediterranea* PVCT 3C against *P. tracheiphilus* was evaluated in four independent trials on seedlings of *Citrus aurantium* (sour orange) and on *Citrus limon* (lemon) 'Femminello Siracusano 2Kr' plants grafted on sour orange, produced by a local nursery. Plants were grown in pots containing a commercial substrate (60 % volcanic soil + 40 % acid peat) fertilized with Osmocote® 16–09–10 (+2 MgO) (12 g pot^{-1} for sour orange plants and 30 g pot^{-1} for lemon plants), according to their age (Table 1).

Sour orange plants were maintained in an unheated greenhouse (14–25 °C, 60–90 % relative humidity), whereas trials on lemon were conducted both in an unheated greenhouse and in an outdoor shaded area. Plants were treated by spraying the leaves with aqueous bacterial cell suspensions (10^8 cfu mL^{-1}) of *P. mediterranea* PVCT 3C three days and one day before the inoculation with *P. tracheiphilus* PVCT Pt57. Briefly, 10 μL of phialoconidial suspension was placed on the secondary veins of young and fully expanded leaves after they had been wounded by gently pressing with three entomological needles mounted on a rubber handpiece (Dimaria et al., 2023). For each experiment, plants were also treated with either the bacterial suspensions or SDW and mock-infected (negative controls). Plants treated with SDW and inoculated with *P. tracheiphilus* (positive control) were included.

The number of replicates (plants, leaves, inoculation sites) is reported in Table 1. Up to 35 days post-inoculation (dpi), symptoms were monitored every week and evaluated using an arbitrary 5-point scale (Luisi et al., 1979). Weekly incidence was calculated as percentage of positive infections out of total infections. The results were evaluated after processing the disease index (DI) and the area under the disease progress curve (AUDPC) (Oliveri et al., 2022).

After evaluating symptoms with the above 5-rating scale, the disease index was calculated using the following formula: DI = (no. of point 0×0) + (no. of point 1×1) + (no. of point 3×3) + (no. of point 4×4)/ total inoculation points. Using the weekly values of the disease index of the inoculated leaves, the AUDPC was determined for each experiment according to Campbell and Madden (1990): AUDPC= $\Sigma_i^{n-1}(y+y_{i+1})/2$ $(t_{i+1}-t)$,where "n" is the number of measures, "y" is the DI, and "t" is the number of days after *P. tracheiphilus* inoculation.

2.4. Assays on leaf discs and detached leaves of lemon

2.4.1. Isolation of P. mediterranea PVCT 3C from lemon leaves

The presence of PVCT 3C on lemon leaves after treatment was assessed. Leaves from lemon plants treated with *P. mediterranea* PVCT 3C and inoculated with SDW or treated and inoculated with SDW of the trial performed in the greenhouse were sampled. Weekly, leaf discs (8-mm diameter) were cut from both the wounded and non-wounded portions of lemon leaves with a cork-borer and placed on PDA plates. Three replicates of 10 discs were assayed. The plates were scored for the presence or absence of *P. mediterranea* PVCT 3C after three days of incubation at 25 ± 2 °C. At least five colonies randomly selected per plate

Table 1
Citrus plants used in biocontrol assays *in planta* and number of replicates (plants, leaves and inoculation sites) per treatment.

Species and age[a]	Pots, growth condition[b]	Plants	Leaves $plant^{-1}$	Inoculation sites $leaf^{-1}$	Total inoculation sites
Citrus aurantium					
10-month-old	7x7x8 cm, 0.27 L, G	5	6	4	120
18-month-old	10x10x22 cm, 2 L, G	4	8	4	128
C. limon					
Two-year-old[c]	⌀ 28 cm, 15 L, G	8	18	6	864
Two-year-old	⌀ 28 cm, 15 L, O	7	18	6	756

[a] *C. limon* plants (variety 'Femminello Siracusano 2Kr') were grafted onto sour orange.
[b] Measures and volume of the pots are reported. Plants were maintained in a greenhouse (G) or outdoors (O). [c] Leaves from the plants of this trial were sampled for RNA-seq analysis.

were tested by PCR with species-specific primers (Catara et al., 2000). In addition, the PDA plates containing the leaf discs were sprayed with a suspension of *P. tracheiphilus* phialoconidia (10^6 mL^{-1} in SDW). The suspension was sprayed 48 h after the application of the leaf discs on the plates or at the same time. The plates were incubated at 25 ± 2 °C. Three replicates of 4–5 discs were assayed. All the experiments were performed at least twice.

2.4.2. Antimicrobial activity assay on detached leaves

The ability of *P. mediterranea* PVCT 3C to combat *P. tracheiphilus* infection locally was also investigated through a detached leaf assay, using a protocol based on the detached leaf assay for *Alternaria alternata* (Reis et al., 2007), with some modifications. Young but fully expanded leaves with petioles were excised from two-year-old greenhouse-grown 'Femminello Siracusano 2Kr' lemon plants. Leaves from three different trees were washed under running tap water, surface-sterilized by immersion for 30 s in 70 % ethanol followed by 30 s in 0.5 % (vol/vol) sodium hypochlorite, rinsed three times in SDW, and then dried under sterile conditions. Leaves were wounded at the midvein with a sterile scalpel and 20 μl of PVCT 3C cell suspensions (10^8 cfu mL^{-1}) were pipetted onto the wound. When the drop dried, a PDA plug from a fresh culture of *P. tracheiphilus* isolate PVCT Pt57 was placed inverted on the wound, allowing the mycelium to make contact with the leaf tissues. Controls consisted of leaves wounded and treated with SDW or wounded and inoculated only with the pathogen. Leaves were acclimated in humid chambers in plastic boxes with three layers of wet filter paper, avoiding the direct contact of the leaves with paper using plastic grids, and incubated at 25 ± 2 °C. The assay was repeated at least twice.

2.5. Whole-genome shotgun sequencing of the P. mediterranea strain PVCT 3C

2.5.1. P. mediterranea PVCT 3C genome sequencing, assembly and annotation

Sequencing libraries were constructed at the Beijing Novogene Bioinformatics Technology Co., Ltd using a NEBNext® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA), with a 350-bp insert size, following the manufacturer's recommendations. After purification and quantification, the libraries were sequenced to an average depth of 222× using an Illumina NovaSeq 6000 platform and PE150 sequencing strategy, generating a total of 12,706,667 bp of raw reads. The raw reads were demultiplexed, recorded in FASTQ file using Illumina bcl2fastq v2.19 and checked for quality using the tool Fastp v0.23.1 (Chen et al., 2018). The resulting clean reads (10,033,333 bp, Q_{30} = 94.69 %) were assembled with the software SOAPdenovo v2.04 (Luo et al., 2012), SPAdeS v3.11.1 (Bankevich et al., 2012), Abyss v1.5.2 (Simpson et al., 2009) and CISA v1.3 (Lin and Liao, 2013) to integrate the assembly results of the four software programs. The completeness of the assembled draft genome was estimated at 98.5 % using BUSCO v5.6.1 (Manni et al., 2021) and the pseudomonadales_odb10 (2024–01–08) lineage dataset.

The genome assembly annotation was performed remotely on the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v6.6 with GeneMarkS-2 v.1.14_1.25 (Li et al., 2021) and on the Rapid Annotations using Subsystems Technology (RAST) server, including the construction of a metabolic model (Aziz et al., 2008; Overbeek et al., 2014). For each software tool, default parameters were used except where otherwise noted.

The draft genome assembly of the *P. mediterranea* strain PVCT 3C was deposited at the NCBI under the accession number GCF_032208645.1 and SRA accession number SRR27587393. The 16S rRNA gene region of *P. mediterranea* PVCT 3C was amplified with the universal primer set 27F/ 1492R and 530F/ 1492R (Lane, 1991) and 1 × GoTaq® G2 Hot Start Colorless Master Mix (Promega). The PCR program consisted of an initial denaturation step at 95 °C for 5 min, followed by 35 cycles of 1 min at 94 °C, 1 min at 50 °C, 2 min (1 min for primer set 27F/ 1492R) at 72 °C, and a 10-min extension at 72 °C. All reactions were performed in a GeneAmp® PCR System 9700 thermal cycler. The DNA amplicons were sequenced by BMR Genomics (Padua, Italy). The partial 16S rRNA gene sequence was deposited in GenBank (accession no. PP066855.2). A circular map of the *P. mediterranea* PVCT 3C draft genome assembly was constructed and visualized using the Proksee Server (https://proksee.ca) (Grant et al., 2023).

2.5.2. Phylogenetic and taxonomic analyses

A whole genome-based phylogenomic tree was constructed on the Type (Strain) Genome Server (TYGS; https://tygs.dsmz.de) (Meier-Kolthoff et al., 2022). The tree was inferred with FastME 2.1.6.1 (Lefort et al., 2015) from Genome Blast Distance Phylogeny (GBDP) distances calculated from publicly available genomes of type strains of species belonging to the *P. corrugata* phylogenomic subgroup and of *P. mediterranea* strains retrieved from the NCBI RefSeq genome database. The *P. aeruginosa* type strain DSM 50071^T was used as an outgroup. The average nucleotide identity based on BLAST (ANIb) values between the genome sequences of the analysed strains was estimated by using PYANI software v0.2.12 (Pritchard et al., 2016).

2.5.3. Comparative genomic analysis

Intraspecific comparisons of the deduced proteomes and metabolic reconstructions were performed with RAST (Aziz et al., 2008; Overbeek et al., 2014), and the results were visualized on SEED Viewer v2.0. Candidate prophage sequences were annotated within the genomes of *P. mediterranea* strains using PHASTER (Arndt et al., 2016; Zhou et al., 2011).

2.5.4. Analysis of genes of interest in biocontrol

Biosynthetic gene clusters (BGCs) for secondary metabolites were predicted using antiSMASH bacterial version v7.1.0 (Blin et al., 2023), the "relaxed" detection strictness setting, and the "extra features" setting. BGCs showing no sequence match with known BGCs for secondary metabolites were designated as orphans, according to a previous study (Balthazar et al., 2022).

P. mediterranea PVCT 3C genes involved in plant growth promotion and in bacteria-plant interactions were predicted using respectively the PGPT-Pred and PIFAR-Pred tools in the PLaBAse v.1.02 platform (Ashrafi et al., 2022; Martínez-García et al., 2016; Patz et al., 2024, 2021). For the PGPT-Pred tool, BLASTp v2.10.1+ and HMMER v3.3 software package (Potter et al., 2018) were used.

2.6. Analysis of P. tracheiphilus gene expression in inoculated lemon leaves as influenced by P. mediterranea strain PVCT 3C treatment

To evaluate the effect of the *P. mediterranea* treatment on the *P. tracheiphilus* transcriptome, leaf samples from all treatments in the trial on lemon 'Femminello Siracusano 2Kr' plants grafted onto sour orange and maintained in a greenhouse were used for RNA extraction using the RNeasy Plant MiniKit (Qiagen, Venlo, The Netherlands), as detailed in Sicilia et al. (2023).

Library preparation and RNA sequencing were performed as reported in Sicilia et al. (2023). *De novo* assembly of clean reads yielded 156,377 transcripts and 38,831 unigenes. The unigenes whose expression level changed upon pathogen infection were identified as differentially expressed genes (DEGs) (Sicilia et al., 2023). To retrieve transcripts belonging to *P. tracheiphilus*, DEGs of the leaves inoculated with *P. tracheiphilus* and those treated with PVCT 3C were scanned. All the sequences were validated by mapping the reads back to the fungal genome (https://mycocosm.jgi.doe.gov/Photr1/Photr1.info.html accessed 7 September 2023).

RNA sequencing data are available at NCBI (https://www.ncbi.nlm.nih.gov/geo/) under the accession number GSE227934.

2.7. Statistical analysis

The data were subjected to ANOVA using Statgraphics PLUS v5.1 software (StatPoint Technologies, Inc., Warrenton, VA, USA) after the normality of the distribution had been checked by the Shapiro-Wilk normality test. Mean values were compared according to the Student-Newman-Keuls test and Tukey's HSD test (analysis of *P. tracheiphilus* gene expression *in planta*).

3. Results

3.1. In vitro antimicrobial activity of P. mediterranea PVCT 3C

As a first step, we tested the ability of the *P. mediterranea* strain PVCT 3C to inhibit the growth *in vitro* of *P. tracheiphilus* PVCT Pt57 (Oliveri et al., 2022) and two other citrus pathogens, *Fusarium solani* ABT Fs and the oomycete *Phytophthora citrophthora* ABT Pc. To explore different modes of action, we evaluated the production of diffusible compounds by strain PVCT 3C. Using a standard dual-culture technique, we analysed the effect of culture filtrates (CFs) on liquid cultures, and the effect of volatile organic compounds (VOCs) by growing the pathogens or germinating phialoconidia on plates above a culture of the bacterium.

Diffusible compounds inhibited the growth on plates of the three tested plant pathogens, although to different extents (26–47 % PGI) (Fig. 1A, Fig. 2, Figure S1). Similarly, cell-free CF of the PVCT 3C strain grown in a minimal medium also led to a reduction in *P. tracheiphilus* radial growth when added to a solid medium (Fig. 1B). This inhibition was more evident when the CF was added to PDB, leading to an 85 % reduction in the dry weight of the fungal mycelium (Fig. 1B).

VOCs inhibited the mycelial growth of *P. tracheiphilus* (Fig. 1A, Fig. 2) and also led to a reduction (25 ± 6 %) in the germination of *P. tracheiphilus* phialoconidia (Fig. 2). Notably, the pigmentation of *P. tracheiphilus* mycelium was reduced or completely inhibited when exposed to bacterial diffusible compounds or VOCs (Fig. 2).

3.2. Antimicrobial activity on detached lemon leaves

The application of *P. mediterranea* PVCT 3C suspensions on wounds of detached lemon leaves, immediately before the inoculation of *P. tracheiphilus*, delayed the onset of leaf alterations caused by the fungus. At seven dpi, most of the leaves (77 %) inoculated only with the pathogen showed either wilting or curling and sometimes midvein discoloration. At this time point, no alterations were observed in leaves treated with PVCT 3C and inoculated with the pathogen (Fig. 3). At 14 dpi, the percentage of leaves inoculated only with the pathogen showing alterations increased to 89 % (Fig. 3). At the same time point, only 5 % of the leaves pre-treated with PVCT 3C showed symptoms that were limited to midvein chlorosis (Fig. 3). At the end of the trial (21 dpi), leaves inoculated only with the pathogen showed extended leaf chlorosis, necrosis or desiccation, while 16 % of leaves pre-treated with PVCT 3C showed chlorosis of the lamina (Fig. 3).

3.3. Biocontrol activity of P. mediterranea PVCT 3C in planta

To assess the efficacy of the *P. mediterranea* strain PVCT 3C to control *P. tracheiphilus* infections in whole plants, sour orange seedlings and lemon plants grafted onto sour orange were sprayed with bacterial suspensions of PVCT 3C, and subsequently wound-inoculated with phialoconidia of the pathogen.

Foliar treatments with *P. mediterranea* PVCT 3C before the inoculation with *P. tracheiphilus* reduced the disease significantly in most of the trials and with a similar trend. Ten-month-old sour orange seedlings showed the highest susceptibility (Fig. 4). Compared with those of the control plants, the seedlings treated with the bacterium showed a significantly reduced incidence at seven ($p = 0.0207$) and 14 dpi ($p = 0.0124$) and a lower DI throughout the experiment (35 dpi) compared to the control (Fig. 4) ($p = 0.0175$ at 35 dpi). AUDPC values were significantly ($p = 0.0175$) reduced following bacterial treatments (21.1) compared to the control treatment (37.7) (Fig. 4). Inoculations on 18-month-old sour orange seedlings at the end of the trial resulted in a low percentage of positive infections regardless of the treatment. The overall DI for the treated and control plants at the end of the trial was 1.0

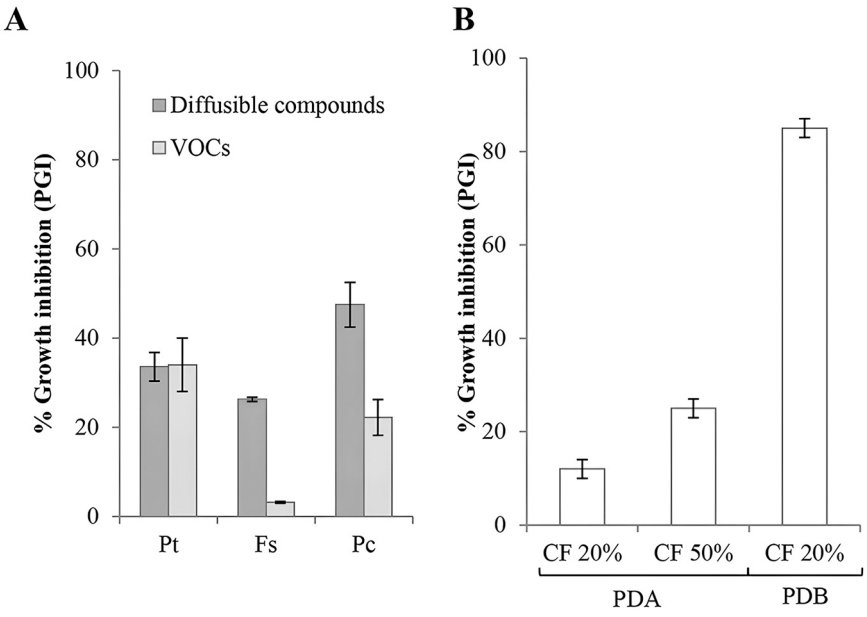

Fig. 1. Antagonistic assays *in vitro*. **A**: Activity of diffusible and volatile organic compounds (VOCs) produced by *P. mediterranea* PVCT 3C against *Plenodomus tracheiphilus* PVCT Pt57 (Pt), *Fusarium solani* ABT Fs (Fs) and *Phytophthora citrophthora* ABT Pc (Pc). **B**: Activity of cell-free culture filtrate (CF) of *P. mediterranea* PVCT 3C tested at 20 % and 50 % final concentrations (v/v) on potato dextrose agar (PDA) medium plates and at 20 % (v/v) on potato dextrose broth (PDB) against Pt. The antimicrobial activity is expressed as the percentage growth inhibition (PGI). The trials were conducted using three independent replicates and repeated twice. Each bar represents the mean, and the error bars indicate the standard errors of the mean.

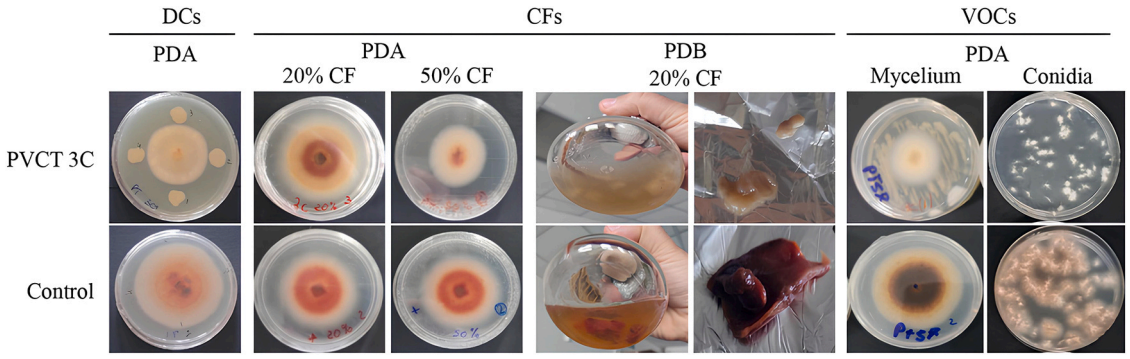

Fig. 2. *In vitro* antimicrobial activity of diffusible compounds (DCs), culture filtrates (CFs) and volatile organic compounds (VOCs) produced by *P. mediterranea* PVCT 3C. The activity was tested against *P. tracheiphilus* isolate PVCT Pt57. The potato dextrose agar (PDA) medium was supplemented with CF at final concentrations of 20 % and 50 % (v/v) and the potato dextrose broth (PDB) with CF at final concentration of 20 %. Control, only fungus (mycelium or conidia suspension). Inhibition of mycelial growth and conidia germination by VOCs is shown.

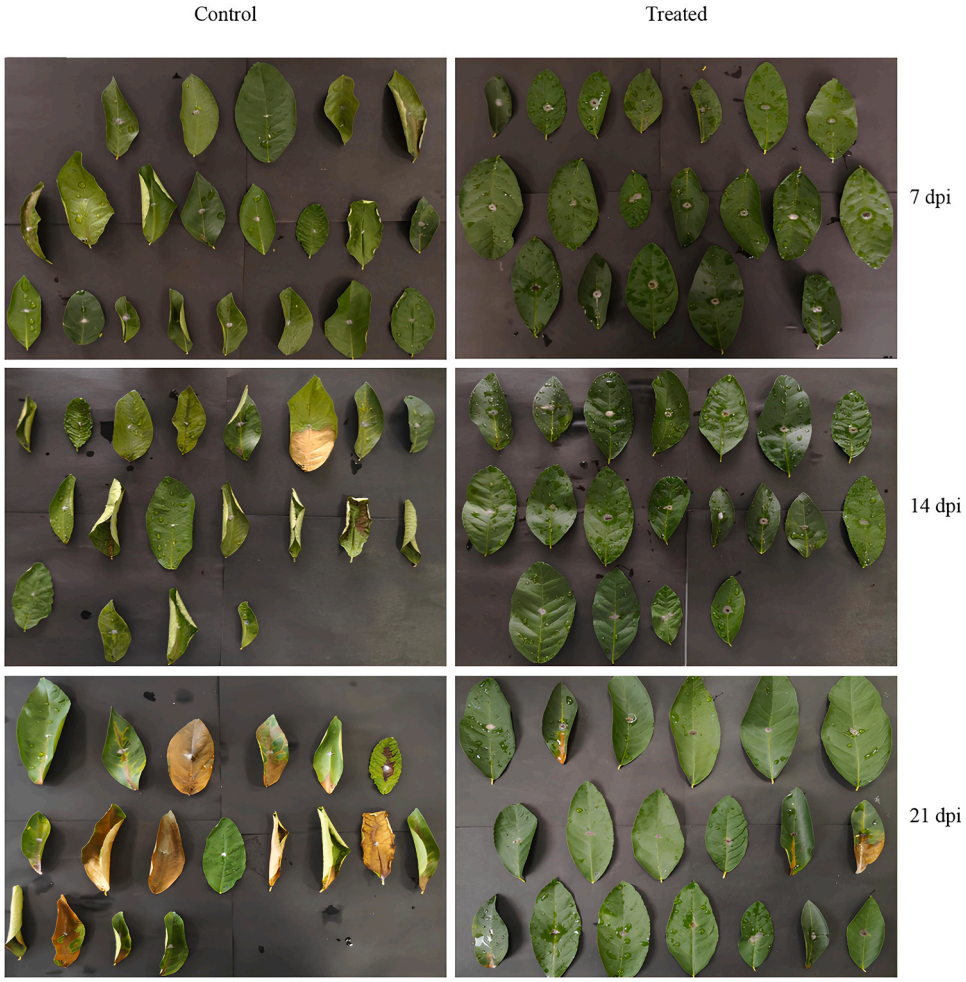

Fig. 3. Antimicrobial activity assay in detached 'Femminello Siracusano 2Kr' lemon leaves. Leaf alterations at 7, 14, 21 days post-*P. tracheiphilus* inoculation (dpi) are shown. Control: leaves were wounded and inoculated with an agarose plug of *P. trachephilus*. Treated: leaves were wounded, treated with a drop of *P. mediterranea* PVCT 3C suspension on the wound and inoculated with *P. tracheiphilus*.

and 1.1 respectively although no significant differences were recorded at this time point (Fig. 4).

On lemon plants, symptoms first appeared 14 dpi regardless of the growth condition (greenhouse or outdoors) and treatment. In the plants maintained in the greenhouse, treatments with PVCT 3C significantly reduced incidence at 14 dpi ($p = 0.0158$) compared to the control plants (3.3 vs 18.3, respectively). In addition, the DI values were always significantly lower than those of the untreated control up to 28 dpi ($p = 0.0301$ at 28 dpi). The mean AUDPC was lower in treated plants than in the control plants (13.4 vs. 23.7, respectively, $p = 0.0218$) (Fig. 4).

On the plants maintained outdoors, the incidence was significantly lower in treated plants compared to the control plants inoculated only with *P. tracheiphilus* up to the end of the trial (35 dpi), with percentages of 5.5 and 21.6 for treated and control plants respectively at 14 dpi ($p =$

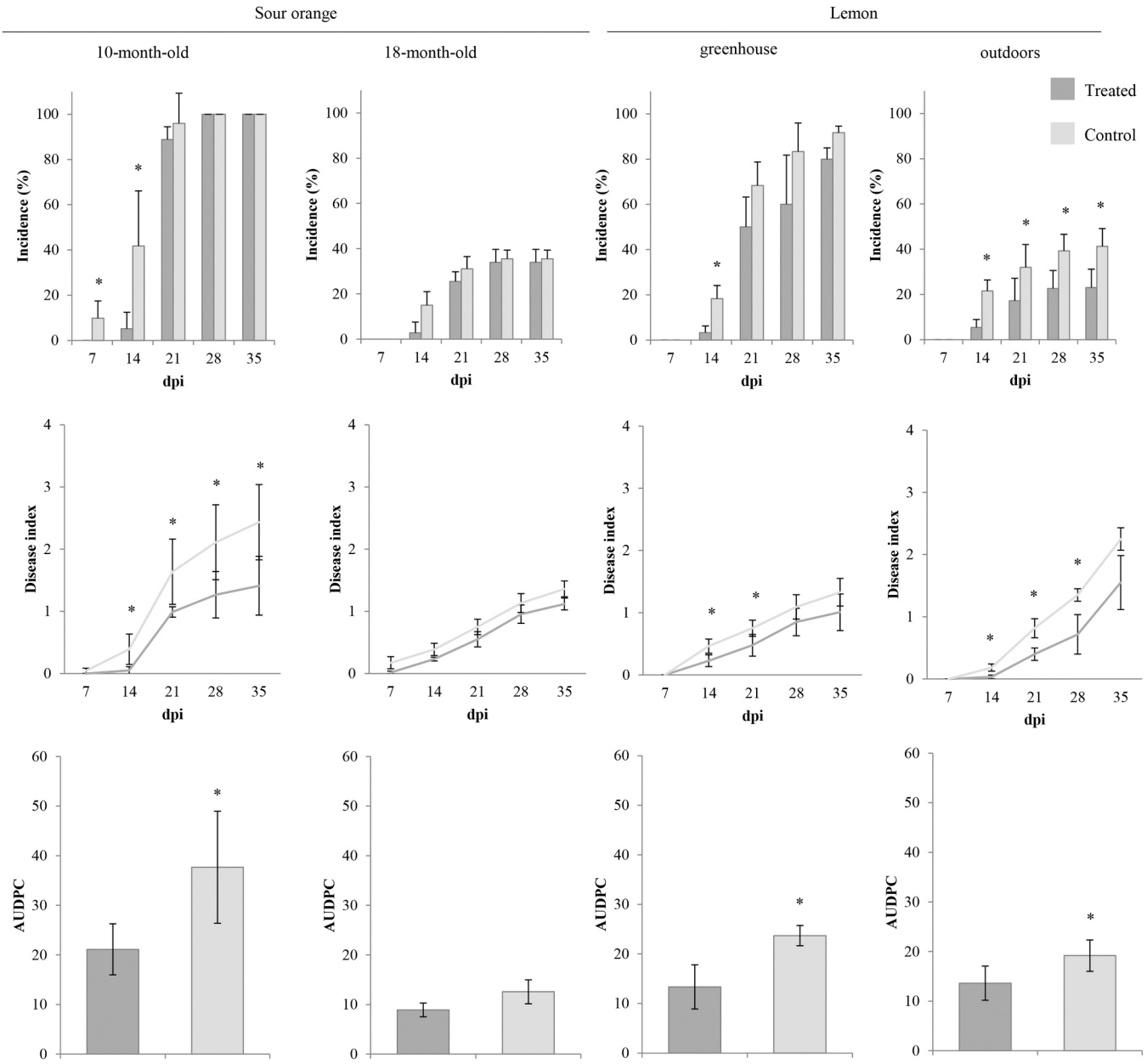

Fig. 4. Effect of treatments with *Pseudomonas mediterranea* strain PVCT 3C on citrus plants inoculated with *Plenodomus tracheiphilus* isolate PVCT Pt57. The weekly disease index and incidence (%) are reported. The area under the disease progress curve (AUDPC) was measured as disease index at 7, 14, 21, 28 and 35 days post-inoculation (dpi). Treated: plants leaf-sprayed with *P. mediterranea* strain PVCT 3C and leaf-inoculated with *P. tracheiphilus* isolate PVCT Pt57. Control: plants only leaf-inoculated with *P. tracheiphilus* isolate PVCT Pt57. Mean values are statistically different at *$p < 0.05$ according to the Student-Newman-Keuls test. Each bar represents the mean, and the error bars indicate the standard errors of the mean.

0.0000). Along with incidence, DI was significantly reduced upon treatment with PVCT 3C at 14 ($p = 0.0031$) and 21 dpi ($p = 0.0074$) (Fig. 4). The AUDPC values were lower (13.6) in plants treated with PVCT 3C suspensions compared to the control plants (19.2) ($p= 0.0085$).

For all the experiments, citrus plants treated only with the *P. mediterranea* PVCT 3C suspension or with SDW and mock-infected plants, used as controls, showed no symptoms (data not shown).

For the latter group of samples, *P. mediterranea* PVCT 3C was re-isolated from 100 % of the discs obtained from the wounded leaf portions at seven dpi and from approximately 70 % of them up to 21 dpi, while at the same time points. The bacterium was isolated from 40 % and 23 % of the discs obtained from the non-wounded leaf portions at seven and 21 dpi, respectively (Table S1). No growth of *P. mediterranea* PVCT 3C was observed from leaves treated and inoculated with SDW. The identity of the colonies was confirmed by PCR (data not shown).

When the leaf discs were placed on PDA plates and the plates were sprayed with a phialoconidia suspension of *P. tracheiphilus*, conidia germination was not observed in the presence of bacterial colonies (Figure S2 A), whereas the mycelium colonised the whole plate with the discs from leaves treated and inoculated with SDW (Figure S2 B). If the discs were placed on the plates 48 h before spraying the conidia suspension, larger inhibition zones were observed compared to plates where the leaf discs and *P. tracheiphilus* conidia suspension were applied at the same time (data not shown).

3.4. Draft genome sequence of P. mediterranea PVCT 3C

3.4.1. General genomic features and functional annotation

To further investigate the mechanism of action of the *P. mediterranea* strain PVCT 3C, a draft genome was obtained, consisting of 31 scaffolds (>500 bp; genome coverage, 100×; N_{50} length, 249,124 bp), with genome properties similar to those of other *P. mediterranea* strains (Fig. 5A; Table S2). The protein-coding genes of strain PVCT 3C were assigned to 24 COG categories, with the majority related to amino acid transport and metabolism (10.38 %), transcription (9.25 %), and signal transduction mechanisms (7.84 %) (Fig. 5B).

3.4.2. Phylogeny and taxonomy of P. mediterranea PVCT 3C

A phylogenomic analysis using the TYGS server showed that strain PVCT 3C was included in a monophyletic clade with all the other sequenced genomes of *P. mediterranea* (Fig. 6). As part of its routine comparison with type strains (Meier-Kolthoff et al., 2022), the TYGS software automatically identified *P. corrugata* as the valid species closest to *P. mediterranea* (Fig. 6), confirming previous phylogenetic analyses (Garrido-Sanz et al., 2016; Mulet et al., 2010; Trantas et al., 2015). However, a genome-wide analysis revealed that some strains clustered between the two species and could represent new species (Garrido-Sanz et al., 2021). ANIb values of valid species were consistent with the phylogenomic analysis (Figure S3).

3.4.3. Comparative genomic analysis

P. mediterranea strains DSM 16733^T and S58 were chosen as reference strains as they represent the type strain of the species and a well-studied biocontrol agent (Gu et al., 2023, 2020), respectively.

According to the RAST annotation based on subsystems, defined as a set of abstract functional roles (Aziz et al., 2008), strain PVCT 3C shared 1920 and 1910 subsystem-associated genes with strains DSM 16733^T and S58, respectively (data not shown). Fifty-nine and 56 genes were unique to strains DSM 16733^T and S58, respectively, compared to strain PVCT 3C (Tables S3-S4). Conversely, 10 and 17 genes were found to be unique to strain PVCT 3C in comparison with strains DSM 16733^T and S58 respectively (Tables S5-S6). These unique genes were mainly related to metabolism, DNA repair and phage DNA, and are likely not involved in the production of antagonistic secondary metabolites or in interactions with plants.

Comparison of the deduced proteomes of *P. mediterranea* strains against that of strain PVCT 3C showed that strains CFBP 5444 and TEIC 1105 had the highest similarity with PVCT 3C in terms of the entire proteome (Fig. 7A), consistently with the phylogenomic analysis.

On the other hand, the proteome comparison of strain PVCT 3C with strains DSM 16733^T (Fig. 7B) and S58 (Fig. 7C) highlighted gaps, which also correspond to deletions in phage regions, according to previous observations (Trantas et al., 2015). Our annotation of candidate prophage regions in the *P. mediterranea* genomes revealed both intact and incomplete regions accounting for a total of 116.4 kb, 60.0 kb and 150.2 kb in *P. mediterranea* strains PVCT 3C, S58 and DSM 16733^T, respectively (Table S7). The plasmid-like prophage VP882 (accession no. NC_009016.1) from *Vibrio parahaemolyticus* strain O3:K6 was the only intact prophage in common between the three genomes (Table S7), and was detected in all the *P. mediterranea* genomes available (data not shown).

3.4.4. Analysis of genes of interest in biocontrol

The analysis of the *P. mediterranea* PVCT 3C genome with the antiSMASH pipeline predicted a total of 17 candidate biosynthetic gene clusters (BGCs) for secondary metabolites, distributed across 11 types, mainly consisting of non-ribosomal peptide synthetases (NRPSs), hydrogen cyanide or hybrid clusters (Table S8). Among these BGCs, six showed no similarity to clusters encoding known bioproducts and were designated as orphans (Table S8). A repertoire of compounds potentially involved in the antagonistic activity of strain PVCT 3C was predicted, including CLPs from the mycin and peptin families, siderophores, hydrogen cyanide and putative novel bioproducts. A hybrid cluster (Hserlactone + NRPS) was predicted to encode an AHL-QS (acyl-homoserine-lactone-Quorum Sensing) system and the NRPS for the biosynthesis of CLPs similar to corpeptin A-B.

Analysis with the PIFAR-Pred tool predicted a total of 567 genetic traits involved in bacteria-plant interactions in the PVCT 3C genome (Table S10). These involved genes related to adhesion (5.3 %), detoxification (10.2 %), production of exopolysaccharides (EPS, 13.9 %), phytohormones (7.6 %) and pigments (2.8 %) (Table S10).

A total of 6174 genetic traits related to direct and indirect plant growth-promoting activities were predicted with the PGPT-Pred tool (Table S11). The others included genes involved in plant colonisation (25 %), biocontrol (21 %), competitive exclusion (21 %) and

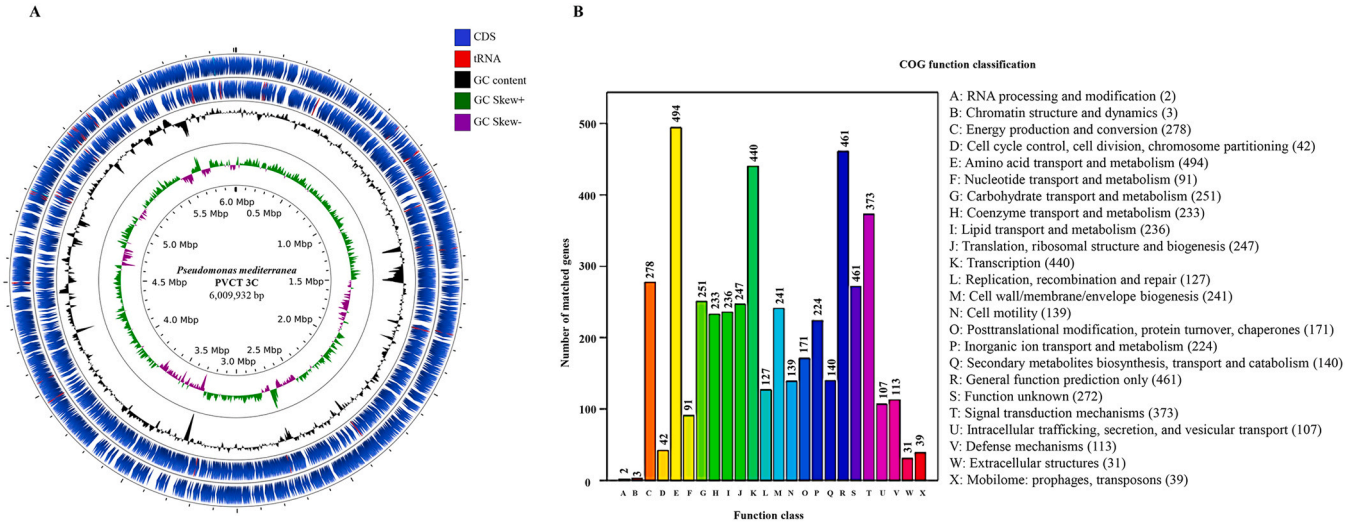

Fig. 5. Genome features and annotation of *Pseudomonas mediterranea* strain PVCT 3C. **A**: Circular map of the draft genome sequence of *P. mediterranea* PVCT 3C created with Proksee (https://proksee.ca). Starting from the innermost ring, the first represents the scale in Mbp, the second the GC skew in the forward (green) and reverse (purple) strand, the third one GC content (black), and the fourth and fifth the location of coding sequences (CDSs, blue) and transfer RNA (tRNA, red) on the reverse and forward DNA strands respectively. **B**: Annotation of *P. mediterranea* strain PVCT 3C genes against the clusters of orthologous groups (COGs) database. X-axis, COG function type; Y-axis, number of annotated genes.

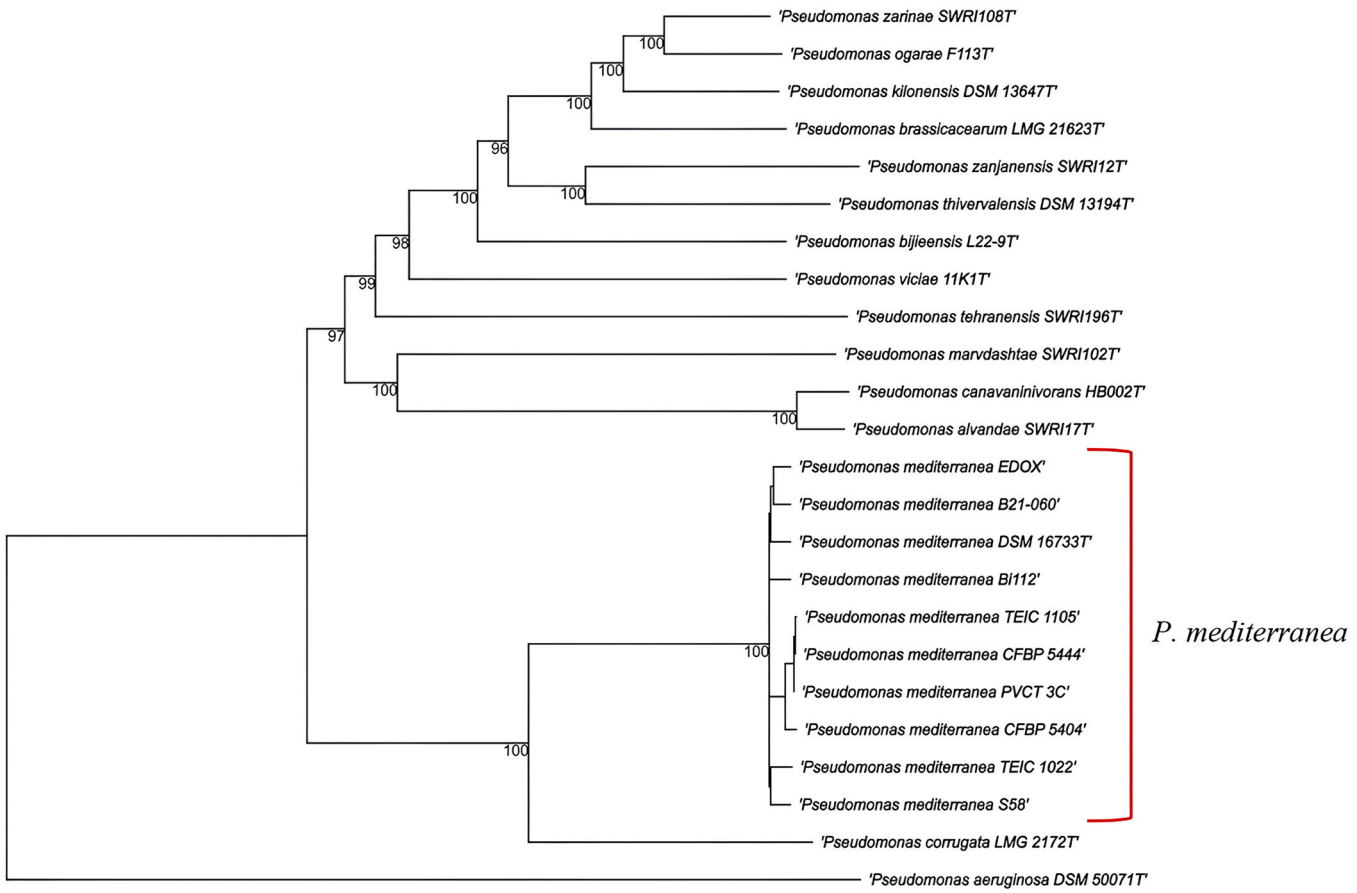

Fig. 6. Phylogenomic tree of *P. corrugata* subgroup strains. The tree was constructed on the TYGS (https://tygs.dsmz.de/) and inferred with FastME 2.1.6.1 from the genome BLAST distance phylogeny (GBDP) distances calculated from genome sequences of *P. corrugata* subgroup type strains (T) and of *P. mediterranea* strains available in NCBI. The branch lengths are scaled in terms of the GBDP distance formula d5. The numbers above branches are GBDP pseudo-bootstrap support values > 60 % from 100 replications. The *P. aeruginosa* type strain DSM 50071 ^{T} was used as an outgroup.

phytohormone production (11 %). Interestingly, among biocontrol genes, 36 genes were identified as encoding antifungal compounds (e.g. thanamycin and brabantamide A) and chitinolytic enzymes. Among genes for phytohormones, several volatile plant signals (e.g. 2,3-butanediol) were identified.

Among motility genes, many genes for chemotaxis and components of the bacterial flagellum and fimbriae were predicted (Table S11).

3.5. *P. tracheiphilus gene expression in inoculated lemon leaves as influenced by P. mediterranea strain PVCT 3C treatment*

As reported in the Materials and Methods, a global transcriptome analysis had previously been performed on lemon leaves challenged by fungal inoculation during the disease onset. At the same time, the effect of *P. mediterranea* pre-treatment on fungus-induced modifications of lemon leaf transcriptome was also evaluated (Sicilia et al., 2024, 2023).

Table S9 reports the number of DEGs retrieved in the following three comparisons: *P. tracheiphilus* inoculated vs. control plants (*Pt* vs. CK), *P. mediterranea* treated and *P. tracheiphilus* inoculated vs. control plants (3C*Pt* vs. CK) and *P. mediterranea* treated vs. control plants (3C vs. CK). As regards the *Pt* vs. CK data set, a total of 2760 differentially expressed genes (DEGs) were identified. Of these, only 182 clusters belonged to the fungal transcriptome, the majority being lemon leaf transcripts (2578 DEGs) (Table S9). Similarly, a total of 373 DEGs were recruited in the 3C*Pt* vs. CK dataset, most of which were lemon leaf transcripts (357) and only 16 clusters belonged to the *P. tracheiphilus* transcriptome (Table S9). All these 16 clusters are also included in the list of the *Pt* vs. CK samples, representing the fungal core gene group still detected despite the *P. mediterranea* treatment. The majority of these clusters encode uncharacterized or unknown proteins (data not shown). Finally, the number of lemon leaf DEGs in the 3C vs. CK comparison was very low (13 DEGs) and, notably, no clusters belonging to *P. mediterranea* were identified in the 3C vs. CK data set (Table S9) (Sicilia et al., 2023). Considering the *Pt* vs. CK comparison, 182 fungal transcripts exhibited $\log_2$fold change values ranging from +1.40 to +9.34 indicating that they were highly expressed inside the plant tissues during the asymptomatic phase of the infection (data not shown).

As expected, most of these genes encode ribosome proteins and enzymes involved in the Krebs cycle and mitochondrial respiration, suggesting that the fungus has a high metabolic activity in terms of protein biosynthesis and energy supply (data not shown). Fig. 8 reports the $\log_2$fold change of some fungal clusters that are considered crucial during the onset of host-pathogen interactions.

Interestingly, two clusters encoding pectin lyase were highly expressed in the inoculated leaves during the asymptomatic phase of the disease ($\log_2$fold change = +7.268 and +6.638, respectively) (Fig. 8). Cluster −1255.0 encodes norsolorinic acid reductase A-like (log2fold change = +7.816), as it is part of the gene cluster that mediates the biosynthesis of aflatoxins, a group of polyketide-derived furanocoumarins and some of the most toxic and carcinogenic compounds among known mycotoxins (Yu et al., 2004) (Fig. 8). Transcripts encoding the ECM33 protein ($\log_2$fold change = +6.509), a glycosylphosphatidylinositol (GPI)-anchored protein, and an ergothioneine biosynthesis protein 1-like ($\log_2$fold change = +5.467) were also highly expressed in lemon plants infected with *P. tracheiphilus* (Fig. 8). In contrast, none of the aforementioned transcripts were detected in the

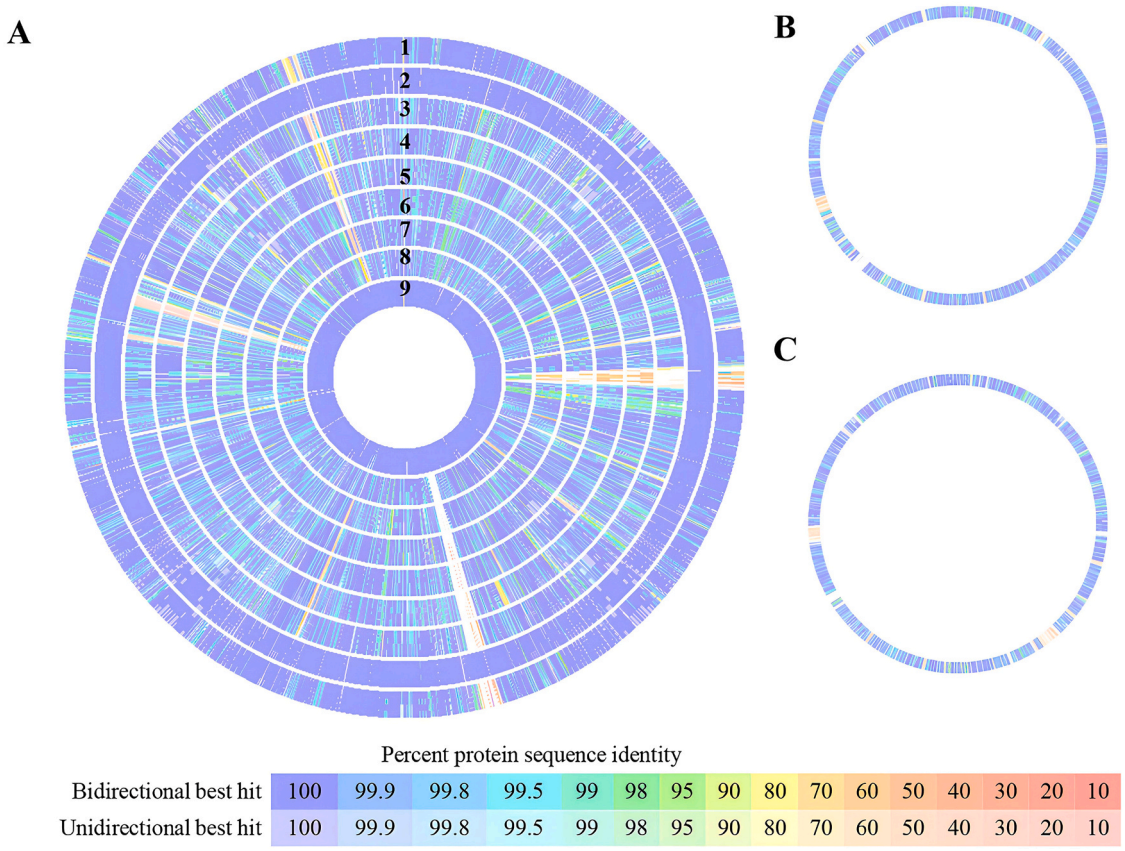

Fig. 7. Circle plot showing the proteome comparison of *Pseudomonas mediterranea* (*Pme*) strains based on RAST genome annotation. **A**: Comparison of the proteome of *Pme* strains against the reference proteome of the following strains: PVCT 3C; 1, *Pme* CFBP 5404; 2, *Pme* CFBP 5444; 3, *Pme* B21–060; 4, *Pme* Bi112; 5, *Pme* DSM 16733^T; 6, *Pme* EDOX; 7, *Pme* S58; 8, *Pme* TEIC 1022; 9, *Pme* TEIC 1105. B-C: Comparison of PVCT 3C proteome against the proteomes of strain DSM 16733^T (**B**) and strain S58 (**C**), used as references. The areas of white space indicate deletions.

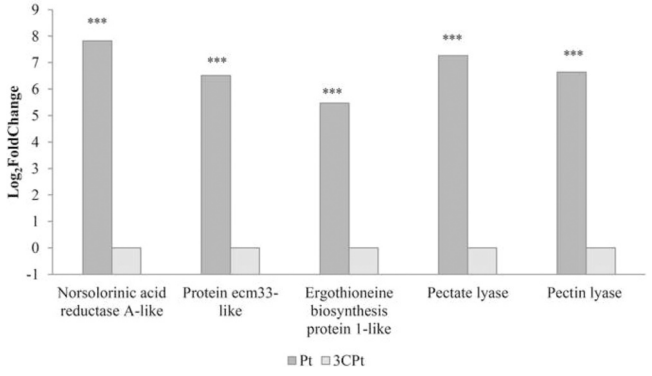

Fig. 8. *P. tracheiphilus* differential expressed genes (DEGs) in plants inoculated with the fungus (*Pt*) and in plants pre-treated with *P. mediterranea* PVCT 3C and inoculated with the pathogen (3C*Pt*). Tukey's HSD test was performed at significance levels (p values) of 0.05 (*), 0.01 (**) and 0.001 (***).

3C*Pt* vs. CK dataset suggesting that they were not expressed in response to *P. mediterranea* pre-treatment (Fig. 8).

4. Discussion

In this study the biocontrol activity of the *Pseudomonas mediterranea* PVCT 3C strain was evaluated to mitigate infection by *P. tracheiphilus,* the causal agent of citrus Mal secco disease which has a considerable negative impact on lemon production.

Multiple *Pseudomonas* species are recognized as plant growth-promoting rhizobacteria (PGPR) and have been included mainly within the *P. fluorescens* complex which is currently divided into nine subgroups (David et al., 2018; Garrido-Sanz et al., 2016; Mulet et al., 2010). Species from this complex, often isolated from the plant microbial 'sphere', have shown plant growth-promoting activities and biological control traits related to the ability to efficiently colonise plants by producing antimicrobial compounds and eliciting systemic resistance (Bakker et al., 2007; Höfte, 2021; Raaijmakers et al., 2010).

Among *Pseudomonas*, there has been increasing interest in the biocontrol activity of *P. mediterranea* strains (*P. corrugata* subgroup) (Albert et al., 2024; Gu et al., 2020; Sallam et al., 2023; Ullah et al., 2020; Zhou et al., 2021). The limited availability of efficient control methods for MSD, the continuous decrease in active ingredients for chemical control, and the interest in biological control agents that are respectful to the environment, motivated us to exploit preliminary results obtained with *Pseudomonas* spp. which belong to this taxonomic group (Coco et al., 2004; Grasso et al., 2008; Strano et al., 2017).

In vitro, both volatiles and diffusible compounds showed that indirectly PVCT 3C had strong antifungal activity, limiting *P. tracheiphilus* mycelial growth and conidia germination. Clear activity was evident not only in dual-culture tests but also using bacterial culture filtrates or challenging *P. tracheiphilus* with *P. mediterranea* volatiles. In liquid medium supplemented with the 20 % bacterial culture filtrate, and inoculated with the phialoconidia of the fungus, a reduction of up to 85 % of the biomass was obtained.

In line with our results, the culture filtrate at different concentrations (20, 40 and 60 %) of the *P. mediterranea* strain HS-4 significantly reduced the dry weight of the fungal pathogens *Alternaria solani*, *A. alternata* and *Curvularia lunata*, which are causal agents of early blight

disease of tomato, with a 60 % concentration determining the lowest fungal dry weight (Sallam et al., 2023).

The exposure of *P. tracheiphilus* to VOCs and culture filtrates produced by PVCT 3C affected conidia germination and fungal pigmentation on PDA and PDB media, suggesting an interference with secondary metabolites involved in the pigmentation. Chromogenic and less frequently nonchromogenic isolates of the fungus were reported, the latter showing reduced virulence (Graniti, 1969; Magnano di San Lio and Perrotta, 1986). Although several anthrachinone derivative pigments, namely helmintosporin, cinodontin, chrysophanol, emodin and islandicin have been isolated from chromogenic isolates, they appeared to play no role in the virulence of *P. tracheiphilus* (Migheli et al., 2009; Nigro et al., 2011).

Biocontrol bacteria affecting fungal pigmentation have already been reported. VOCs of *P. mediterranea* CFBP 5447^T and *P. corrugata* CFBP 5454 inhibited the growth and pigmentation of the fungal pathogen *Botrytis cinerea* (Strano et al., 2017). The VOCs produced by *P. aurantiaca* ST-TJ4 inhibited the mycelial pigmentation of *Verticillium dahliae*, reducing the expression of genes related to melanin biosynthesis (Ni et al., 2022). The same effect on mycelial pigmentation was observed for the culture filtrate of *Bacillus subtilis* isolate W3.15 on *F. oxysporum* (Putri et al., 2021) and the antifungal metabolite salvianolic acid B from *Streptomyces* sp. M4 on *Alternaria brassicicola* (Sharma and Manhas, 2022).

Conidia germination inhibition was most probably also exploited in the wounds of detached leaves where *P. mediterranea* PVCT 3C suspensions were applied before inoculation with *P. tracheiphilus,* thus suggesting that infection did not take place. Plant-associated *P. mediterranea* strains isolated from different matrices have demonstrated the great potential of this species as a biocontrol agent against phytopathogenic fungi and oomycetes (Höfte, 2021). The type strain CFBP 5447^T, isolated from tomato pith, inhibited the growth of *F. oxysporum* and *B. cinerea* (Strano et al., 2017). *P. mediterranea* S58, isolated from tobacco rhizosphere, showed antimicrobial activity *in vitro* against the fungal plant pathogens *Magnaporthe oryzae*, *F. graminearum*, *Rhizoctonia solani* and the oomycete *P. nicotianae* (Gu et al., 2020). *P. mediterranea* HU-9 reduced the growth *in vitro* of *F. oxysporum* and *F. moniliforme*, the causal agents of crown and root rot of wheat (Ullah et al., 2020).

More recently, the use of new strains HS-4 and B21–060 isolated from the tomato endosphere and from the lettuce rhizosphere, respectively, has widened the range of fungi to which *P. mediterranea* is active. These strains demonstrated a strong inhibitory activity, namely against *Alternaria solani*, *A. alternata* and *Curvularia lunata*, which are causal agents of early blight disease in tomato (strain HS-4, Sallam et al., 2023) and *Sclerotinia sclerotiorum* in lettuce (strain B21–060, Albert et al., 2024).

Foliar treatments with *P. mediterranea* PVCT 3C before inoculation with *P. tracheiphilus* induced a delay in symptom appearance both in young sour orange seedlings and in lemon plants grafted on sour orange treated with bacterial suspensions. In general, the response of the two species to artificial inoculation with *P. tracheiphilus* in control plants was in agreement with previous studies in a growth chamber, in which symptoms appeared 7–14 days after the inoculation (Oliveri et al., 2022). The reduction in positive infections in plants treated with *P. mediterranea* PVCT 3C was significant in the majority of trials up to 14 days post-inoculation. It should be noted that bacterial treatments were not repeated and that the experiment was carried out favouring the fungus *P. tracheiphilus,* which penetrates the plant surface through wounds. Pin-point small leaf wounds remain receptive from 4 to 5 days, while larger ones, such as those performed to increase the chance of infection, remain receptive for up to 10 days (Catara and Catara, 2019).

The results suggest that the conidia inoculation drops penetrated directly into the wounds, and found the ideal conditions for germination. Those that remained outside were challenged by *P. mediterranea*, resulting in reduced conidia germination or mycelial growth, which is in line with *in vitro* results. However, *P. tracheiphilus* hyphae that successfully penetrated after reaching the xylem, spread infection rapidly and could not be reached by the biocontrol agent. Therefore, any positive infection at the wound site, although delayed, would lead to xylematic infection. Some differences were observed between experiments which could be attributed to the citrus species, plant age and growth conditions (i.e. greenhouse or outdoors). In line with our results, the susceptibility of sour orange was found to be greater in young plants than in older ones (Demontis et al., 2008).

At the end of the trials, the incidence and DI values were higher in lemon plants grown in the greenhouse than in lemon plants maintained outdoors, regardless of whether they were treated or not. Studies on fungal penetration have demonstrated that conidia that remain outside the plant only germinate with 100 % relative humidity, producing abundant hyphae that penetrate inside the host through wounds and initiate the infection process. However, without humid conditions the conidia degenerate (D'Anna et al., 1986).

To further address how *P. mediterranea* PVCT 3C behaves, the genome was sequenced. Genome sequencing is crucial for the development of *Pseudomonas*-based biocontrol inoculants (Zboralski et al., 2023). This approach enables the phylogenetic relationships between strains to be identified, to understand the biological control mechanisms, to contribute to the discovery of antimicrobial compounds, and to identify and map the repertoire of biocontrol-related traits across species and strains (Zboralski et al., 2023).

The biocontrol capacity of *Pseudomonas* strains of different species was analysed to investigate the potential related to the genetic determinants involved in biocontrol (Albert et al., 2024; Balthazar et al., 2022; Berendsen et al., 2015; De Vrieze et al., 2020; Ghadamgahi et al., 2022; Jing et al., 2024; Meng et al., 2024; Nelkner et al., 2019; Takeuchi et al., 2023; Zhang et al., 2021). Integrating genomic exploration with studies on the ecological competence of *Pseudomonas* strains could help accelerate the development and commercialization of successful field-efficient and safe bacterial inoculants (Zboralski et al., 2023). Taking advantage of the availability of genome sequences, the construction of mutant strains for individual antibiotics has provided new insights into the contribution of secondary metabolites produced by bacterial biocontrol agents to the induction of host plant defences (Dimkić et al., 2022).

To confirm their non-toxicity to humans and animals, and to evaluate their efficacy against *P. tracheiphilus* and other pathosystems, future research should focus on isolating and characterizing the bioproducts mined through genome analysis, and on determining their biochemical structures.

Genome analysis confirmed that *P. mediterranea* PVCT 3C was correctly identified although it showed the smallest genome reported to date for the ten strains already sequenced (NCBI). The variability of *P. mediterranea* genomes seems largely dependent on numerous prophage sequences as already observed by Trantas et al. (2015).

The analysis of secondary metabolite BGCs highlighted the similarity with other *P. mediterranea* strains and with *P. corrugata* due to the presence of an AHL-QS genomic island encoding a LuxI/R QS system and the LuxR regulator Rfia and the biosynthetic genes for the CLPs of the mycin and peptin family (Girard et al., 2020; Licciardello et al., 2018, 2012; Oni et al., 2022).

CLPs of the mycin and peptin families are produced from both plant pathogens and biocontrol *Pseudomonas* spp. strains, and have a strong biological activity (Girard et al., 2020; Oni et al., 2022; Raaijmakers et al., 2010). Their antifungal activity has been linked to their ability to form pores in the cell membranes, which affects their integrity and results in compromised growth and spore germination (Girard et al., 2020; Oni et al., 2022).

Cormycin and corpeptins have been purified from *P. corrugata* (Emanuele et al., 1998; Scaloni et al., 2004), whereas to date only peptins (medipeptins and medpeptins) have been characterised in *P. mediterranea* strains (Gu et al., 2023; Zhou et al., 2021). However, the production of both families of CLPs has been observed in *P. mediterranea*

by MALDI-TOF mass spectrometry analysis (Licciardello et al., 2012). The antimicrobial activity of these compounds was confirmed by the analysis of supernatant cell filtrates and purified CLPs, sometimes combined with loss-of-function mutants (Emanuele et al., 1998; Gu et al., 2023; Licciardello et al., 2012; Scaloni et al., 2004; Zhou et al., 2021).

A BGC putatively encoding thanafactin A, which is a structurally novel linear octalipopeptide identified in *Pseudomonas* sp. SH-C52 and is widespread among *P. corrugata* subgroup strains, has also been identified in the PVCT 3C genome (Cesa-Luna et al., 2023).

In addition, the *P. mediterranea* PVCT 3C genome harbors the BGC for the poisonous volatile compound hydrogen cyanide, which could account for the inhibition of *P. tracheiphilus* cultures growth when challenged by avoiding direct contact with the bacterium. Similar results were obtained by challenging the fungus *B. cinerea* with *P. mediterranea* strain CFBP 5447 ^{T} (Strano et al., 2017). In addition, cyanide production was responsible for *B. cinerea* mycelial growth and conidia germination reduction in *P. corrugata* CFBP 5454 (Strano et al., 2017).

P. mediterranea does not produce fluorescent pigments (Catara et al., 2002). The PVCT 3C genome was predicted to encode a BGC for the siderophore histicorrugatin described in *P. thivervalensis* LMG 21626 ^{T} and similar to *P. corrugata* corrugatin (Matthijs et al., 2016; Risse et al., 1998), which is likely involved in competition for iron.

Overall, the results obtained with the PLaBAse platform suggest that a complex interaction of PVCT 3C with the plant and the pathogen *P. tracheiphilus* can occur. The predicted volatile compound 2,3-butanediol has been reported to induce resistance in creeping bentgrass to turfgrass brown patch caused by *R. solani* by regulating the content of phytohormones and antioxidant compounds (Shi et al., 2018).

It has been shown that *P. mediterranea* pre-treatment reduces plant transcriptome changes in thesamples inoculated with the fungus, and prevents the de-regulation of important plant biological processes and metabolic pathways otherwise affected by fungal infection (e.g. the photosynthetic process and phytohormone biosynthesis and signalling) (Sicilia et al., 2023).

Exploiting the RNAseq experiment detailed in Sicilia et al. (2023), transcripts belonging to *P. tracheiphilus* were retrieved from *Pt* vs. CK (*P. tracheiphilus* inoculated vs. control samples) comparison. The number of clusters attributable to fungal transcripts was lower (182 fungal transcripts) than that observed in a previous experiment performed on rough lemon (*C. jambhiri*) (2438 fungal transcripts) in the symptomatic phase of the disease (15 days after inoculation) (Sicilia et al., 2022). However, in both experiments, most of the fungal transcripts encoded ribosome proteins and enzymes involved in the Krebs cycle and mitochondrial respiration (Sicilia et al., 2022).

Among the 182 clusters belonging to the fungal transcriptome, two clusters encoding pectin and pectate lyase were identified. *P. tracheiphilus* produces pectinolytic enzymes (e.g. pectinmethylesterase) in artificial media and *in planta* and their inoculation leads to the production of gums in sour orange shoot wood and electrolyte leakage in leaves (Migheli et al., 2009; Nigro et al., 2011). Down-regulation of the expression of genes encoding pectinesterases and pectin lyase in the fungal pathogen *R. solani* after challenge *in vitro* with the bacterial biocontrol agents *Serratia proteamaculans* S4 or *S. plymuthica* AS13 has been described (Gkarmiri et al., 2015). A similar phenomenon was described in *Crocus sativus* for pectin lyase genes of *Fusarium oxysporum* R1 when plants were treated with the biocontrol agent *Bacillus* sp. strain D5 (Bhagat and Vakhlu, 2024).

A norsolorinic acid reductase A-like gene was identified among the fungal transcripts. BLASTx analysis revealed that it encoded a product that is 100 % identical to an aryl-alcohol dehydrogenase-like protein from *P. tracheiphilus* IPT5 (GenBank accession no. KAF2845479.1). This gene is putatively involved in the biosynthesis of sterigmatocystin, a dibenzo-γ-pyrone mycotoxin produced by *Aspergillus* spp. and considered a precursor in the biosynthesis of aflatoxins (Groopman et al., 2012; Xu et al., 2021). Although a role in the disease development has been demonstrated for malseccin, which is a non-selective complex of toxic glycoproteins, several secondary metabolites have been described from *P. tracheiphilus* culture filtrates (Nigro et al., 2011; Xu et al., 2021).

Of these, two pyrone compounds, the polypropionate α-pyrone phomenins A and B (Tringali et al., 1993) and the benzo-α-pyrone mellein (Parisi et al., 1993) induced phytotoxicity in tomato cuttings. Pyrone compounds act as phytotoxins in several plant pathogenic fungi, including *Neofusicoccum* sp., *Fusarium proliferatum*, *Alternaria solani*, *Colletotrichum nicotianae* (Xu et al., 2021).

Both pectin and pectate lyase as well as norsolorinic acid reductase A-like genes were found to be highly expressed 15 days after inoculation in rough lemon (Sicilia et al., 2022). This suggests that they start to be early expressed *in planta* during fungal infection and that their expression might be maintained later during disease progression, likely playing key roles in pathogenesis.

The ECM33 glycosylphosphatidylinositol (GPI)-anchored protein, which is important for fungal vegetative growth and virulence by regulating cell wall integrity (Huang et al., 2022) was also among the fungal transcripts, along with a transcript encoding ergothioneine biosynthesis protein 1-like. This latter protein enables pathogens to withstand hostile environments within the host by rendering cellular antioxidant recycling more rapid, removing metabolic waste and ROS, and repairing oxidative damage (Cumming et al., 2018). None of these genes, which probably facilitate the onset of the disease, are included in the 3C*Pt* vs. CK samples, suggesting that *P. mediterranea* biocontrol activity is exerted through direct action on the pathogen gene expression.

The limited number of lemon leaf DEGs observed in the 3C vs. CK comparison (13 DEGs), demonstrated that *P. mediterranea* treatment did not lead to transcriptomic reprogramming in plant tissues (Sicilia et al., 2024, 2023). These results, along with *in vitro* and *in planta* PVCT 3C activities, suggest that the biocontrol activity was due to the impairment of fungal development at the early disease phase. The decrease in amount of fungal DNA detected in the *P. mediterranea* pre-treated lemon leaves (Sicilia et al., 2024, 2023) strongly supports this inference.

P. mediterranea strains can elicit a hypersensitive response (HR) in *Nicotiana* spp. despite not harbouring a type-III secretion system (T3SS) (Catara et al., 2002; Trantas et al., 2015). In *N. benthamiana* treated with *P. mediterranea* S58, 12 days post-inoculation, the expression of two pattern-triggered immunity (PTI) marker genes (NbPti5 and NbWRK7) was upregulated (Gu et al., 2020). Although flagellin has been demonstrated to be the major PTI-ROS inducer in *P. mediterranea* S58, the CLP medpeptin seems to be key in modulating tobacco plant cell death immunity through a non-canonical mechanism of CLP sensing based on cell-wall perception and cytoplasmic signalling (Gu et al., 2023). In our study, at the time of sampling, genes involved in SAR or ISR were not induced in lemon plants treated with *P. mediterranea* PVCT 3C or inoculated with *P. tracheiphilus*. This suggests that the bacterium slowed the onset of the disease probably by competing with the pathogen for space and nutrients and not by inducing plant resistance, as also observed in Sicilia et al. (2023). However, it cannot be ruled out that at different time points post-treatment, the *P. mediterranea* strain PVCT 3C may induce the basal defences of citrus leaves.

In conclusion, *P. mediterranea* PVCT 3C delays the initial phase of leaf infection caused by the fungal pathogen *P. tracheiphilus* and, overall, mitigates citrus Mal secco symptom severity on sour orange and lemon plants. These results, along with the antimicrobial activity observed *in vitro* against the pathogen due to the production of diffusible and extracellular metabolites and volatile organic compounds, suggest that *P. mediterranea* PVCT 3C may restrict *P. tracheiphilus* germination on the leaf before it enters the wounds. In addition to the inhibition of the growth of the hyphae produced by conidia germination, the tests carried out *in planta* highlight the existence of molecular interactions between the pathogen, the host and the biocontrol agent that slow down the process of infection at least in the initial phase of the disease.

The limited understanding of the genetic factors underlying lemon resistance to Mal secco has hindered progress in developing genetically

improved varieties. As a result, no commercially available solutions currently exist for this issue. However, new biotechnological breeding techniques and marker-trait association analyses hold promise for marker-assisted breeding programs. The ongoing programs focus on selecting lemon varieties that combine disease resistance with high fruit quality (Abbate et al., 2019; Catalano et al., 2021a; Catalano et al., 2021b).

Our results suggest that the development of a formulation with the bacterium or its secondary metabolites may contain the disease in a sustainable manner in a short time, eliminating the impact of agro-chemical formulations. This solution could also facilitate the spread of varieties with a partial tolerance that may require containment.

To develop bioproducts, further validation is necessary to ensure large-scale usage and consistent efficacy under field conditions (Bonaterra et al., 2022). Therefore, *P. mediterranea* PVCT 3C also needs to be evaluated considering different environmental factors, disease pressures, lemon cultivars. In addition, the number of applications needs to be finetuned for a real assessment of its applicability.

The assessment of the ecological behaviour of biocontrol agents and their interaction with other organisms is pivotal for marketing microbial inoculants (Berg, 2009). However, the ecological interactions of *P. mediterranea* PVCT 3C in agroecosystems need to be investigated to ensure its environmental sustainability and minimize unintended consequences. Biosafety studies should be carried out to assess the adverse effects of the formulated bioproduct in plants and non-target organisms, including humans (Bonaterra et al., 2022).

A metagenomic approach has been proven to be useful for investigating the ecological impact and measuring the colonisation of bacterial biocontrol agents in different plant compartments (Dimkić et al., 2022; Romano et al., 2020).

P. mediterranea PVCT 3C putatively acts on *P. tracheiphilus* through different modes of action, including competition for nutrients and space and production of a wide range of secondary metabolites with different chemical structures. Further investigations of the mode of action responsible for the protective effect will facilitate the optimization of biocontrol efficacy and help establish appropriate formulations and application methods (Bonaterra et al., 2022).

The different mechanisms underlying biocontrol activities potentially mitigate the risk of resistance development (Bardin et al., 2015). Variations in the sensitivity to different secondary metabolites produced by *Pseudomonas* biocontrol agents have in fact been reported for both bacterial and fungal phytopathogens (Ajouz et al., 2011, 2010; Clough et al., 2024; Mazzola et al., 1995; Schouten et al., 2004). Long-term efficacy studies will therefore be essential for assessing the durability of the biocontrol efficacy of *P. mediterranea* PVCT 3C.

Author agreement

All authors have read and approved the manuscript.

Funding

This work was supported by the project S.I.R.P.A. funded by PO FESR 2014–2020 Sicilia action 1.1.5 [grant number 08CT7211000254].

CRediT authorship contribution statement

Vittoria Catara: Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Patrizia Bella:** Writing – review & editing, Validation, Supervision, Software, Methodology. **Francesco Modica:** Methodology, Investigation, Formal analysis. **Angelo Sicilia:** Writing – review & editing, Validation, Software, Resources, Methodology, Investigation, Formal analysis. **Giulio Dimaria:** Writing – review & editing, Writing – original draft, Software, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Angela Roberta Lo Piero:** Writing – review & editing, Supervision, Software, Methodology. **Maria Elena Massimino:** Methodology, Investigation. **Marina Claudia Bazzano:** Methodology, Investigation. **Marcella Russo:** Methodology, Investigation.

Declaration of Competing Interest

Vittoria Catara reports financial support was provided by Sicilian Region General Assembly. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Each accession number reported in the manuscript has been hyperlinked to the publicly available data record.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.micres.2024.127833.

References

Abbate, L., Mercati, F., Del Bosco, S.F., 2019. An overview on citrus mal secco disease: approaches and strategies to select tolerant genotypes in *C. limon*. Crop Breed. Genet Genom. 1, e190018.

Aiello, D., Leonardi, G.R., Di Pietro, C., Vitale, A., Polizzi, G., 2022. A new strategy to improve management of citrus mal secco disease using bioformulates based on *Bacillus amyloliquefaciens* strains. Plants 11, 446.

Ajouz, S., Nicot, P.C., Bardin, M., 2010. Adaptation to pyrrolnitrin in *Botrytis cinerea* and cost of resistance. Plant Pathol. 59, 556–566.

Ajouz, S., Walker, A.S., Fabre, F., Leroux, P., Nicot, P.C., Bardin, M., 2011. Variability of *Botrytis cinerea* sensitivity to pyrrolnitrin, an antibiotic produced by biological control agents. BioControl 56, 353–363.

Albert, D., Zboralski, A., Biessy, A., Blom, J., Beaulieu, C., Filion, M., 2024. Identification and genomic characterization of *Pseudomonas* spp. displaying biocontrol activity against Sclerotinia sclerotiorum in lettuce. Front. Microbiol. 15, 1304682.

Arndt, D., Grant, J.R., Marcu, A., Sajed, T., Pon, A., Liang, Y., Wishart, D.S., 2016. PHASTER: a better, faster version of the PHAST phage search tool. Nucleic Acids Res 44, W16–W21.

Ashrafi, S., Kuzmanović, N., Patz, S., Lohwasser, U., Bunk, B., Spröer, C., Lorenz, M., Elhady, A., Frühling, A., Neumann-Schaal, M., 2022. Two new Rhizobiales species isolated from root nodules of common sainfoin (*Onobrychis viciifolia*) show different plant colonization strategies. Microbiol. Spectr. 10, e01099-22.

Aziz, R.K., Bartels, D., Best, A.A., DeJongh, M., Disz, T., Edwards, R.A., Formsma, K., Gerdes, S., Glass, E.M., Kubal, M., 2008. The RAST Server: rapid annotations using subsystems technology. BMC Genom. 9, 1–15.

Bakker, P.A.H.M., Pieterse, C.M.J., Van Loon, L.C., 2007. Induced systemic resistance by fluorescent *Pseudomonas* spp. Phytopathology 97, 239–243.

Balthazar, C., Novinscak, A., Cantin, G., Joly, D.L., Filion, M., 2022. Biocontrol activity of *Bacillus* spp. and *Pseudomonas* spp. against *Botrytis cinerea* and other cannabis fungal pathogens. Phytopathology® 112, 549–560.

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V. M., Nikolenko, S.I., Pham, S., Prjibelski, A.D., Pyshkin, A.V., Sirotkin, A.V., Vyahhi, N., Tesler, G., Alekseyev, M.A., Pevzner, P.A., 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J. Comput. Biol. 19, 455–477.

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.

Berendsen, R.L., van Verk, M.C., Stringlis, I.A., Zamioudis, C., Tommassen, J., Pieterse, C. M.J., Bakker, P.A.H.M., 2015. Unearthing the genomes of plant-beneficial *Pseudomonas* model strains WCS358, WCS374 and WCS417. BMC Genom. 16, 1–23.

Berg, G., 2009. Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl. Microbiol. Biotechnol. 84, 11–18.

Bhagat, N., Vakhlu, J., 2024. Effects of biocontrol *Bacillus* sp. strain D5 on the pathogenic *Fusarium oxysporum* R1 at the microscopic and molecular level in *Crocus sativus* L. (saffron) corm. FEMS Microbes 5, xtad025.

Blin, K., Shaw, S., Augustijn, H.E., Reitz, Z.L., Biermann, F., Alanjary, M., Fetter, A., Terlouw, B.R., Metcalf, W.W., Helfrich, E.J.N., 2023. antiSMASH 7.0: new and improved predictions for detection, regulation, chemical structures and visualisation. Nucleic Acids Res. 51, W46–W50.

Bonaterra, A., Badosa, E., Daranas, N., Francés, J., Roselló, G., Montesinos, E., 2022. Bacteria as biological control agents of plant diseases. Microorganisms 10, 1759.

Bubici, G., Kaushal, M., Prigigallo, M.I., Gómez-Lama Cabanás, C., Mercado-Blanco, J., 2019. Biological control agents against Fusarium wilt of banana. Front. Microbiol. 10, 445720.
Campbell, C.L., Madden, L.V., 1990. Introduction to plant disease epidemiology, first ed. John Wiley & Sons, New York.
Catalano, Chiara, Di Guardo, M., Distefano, G., Caruso, M., Nicolosi, E., Deng, Z., Gentile, A., La Malfa, S.G., 2021a. Biotechnological approaches for genetic improvement of lemon (*Citrus limon* (l.) burm. f.) against mal secco disease. Plants 10, 1002.
Catalano, C., Seminara, S., Licciardello, G., Modica, F., Musumeci, S., Lo Cicero, L., Di Guardo, M., Tropea Garzia, G., Biondi, A., Catara, V., 2021b. Phenotyping and genotyping of a lemon segregating population to mining genetic resistance to biotic stresses. : III Int. Org. Fruit. Symp. . I Int. Org. Veg. Symp. 1354, 87–94.
Catara, V., 2007. *Pseudomonas corrugata*: plant pathogen and/or biological resource? Mol. Plant Pathol. 8, 233–244.
Catara, V., Arnold, D., Cirvilleri, G., Vivian, A., 2000. Specific oligonucleotide primers for the rapid identification and detection of the agent of tomato pith necrosis, *Pseudomonas corrugata*, by PCR amplification: evidence for two distinct genomic groups. Eur. J. Plant Pathol. 106, 753–762.
Catara, A., Catara, V., 2019. Catara, A., Catara, V., 2019. Il "mal secco" degli agrumi, da un secolo in Sicilia. Mem. e Rend. USPI Assoc. all'Unione Stamp. Period. Ital. Giovanni Battista RM, Italy 3, 33–58.
Catara, V., Gardan, L., Lopez, M.M., 1997. Phenotypic heterogeneity of *Pseudomonas corrugata* strains from southern Italy. J. Appl. Microbiol. 83, 576–586.
Catara, V., Sutra, L., Morineau, A., Achouak, W., Christen, R., Gardan, L., 2002. Phenotypic and genomic evidence for the revision of *Pseudomonas corrugata* and proposal of *Pseudomonas mediterranea* sp. nov. Int. J. Syst. Evol. Microbiol. 52, 1749–1758.
Cesa-Luna, C., Geudens, N., Girard, L., De Roo, V., Maklad, H.R., Martins, J.C., Höfte, M., De Mot, R., 2023. Charting the lipopeptidome of nonpathogenic *Pseudomonas*. mSystems 8, e0098822.
Chen, S., Zhou, Y., Chen, Y., Gu, J., 2018. Fastp: An ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34, i884–i890.
Clough, S.E., Elphinstone, J.G., Friman, V.-P., 2024. Plant pathogenic bacterium *Ralstonia solanacearum* can rapidly evolve tolerance to antimicrobials produced by *Pseudomonas* biocontrol bacteria. J. Evol. Biol. 37, 225–237.
Coco, V., Grimaldi, V., Catara, V., Licciardello, G., Cirvilleri, G., Grasso, S., Catara, A., 2004. Inhibition of *Phoma Tracheiphila* by Pseudomonads in Citrus Seedlings. : Proc. Int. Soc. Citric. 2, 729–732.
Cumming, B.M., Chinta, K.C., Reddy, V.P., Steyn, A.J.C., 2018. Role of ergothioneine in microbial physiology and pathogenesis. Antioxid. Redox Signal. 28, 431–444.
D'Anna, R., Trigila, P.L., Catara, A., 1986. Aspetti della morfologia e della biologia di *Phoma tracheiphila* esaminati al microscopio elettronico a scansione. Atti Giornate Fitopatol. 1986, 105–114 (Riva del Garda, Italy).
David, B.V., Chandrasehar, G., Selvam, P.N., 2018. *Pseudomonas fluorescens*: a plant-growth-promoting rhizobacterium (PGPR) with potential role in biocontrol of pests of crops. In: Prasad, R., Gill, S.S., Tuteja, N. (Eds.), Crop Improvement through Microbial Biotechnology. Elsevier, pp. 221–243.
De Vrieze, M., Varadarajan, A.R., Schneeberger, K., Bailly, A., Rohr, R.P., Ahrens, C.H., Weisskopf, L., 2020. Linking comparative genomics of nine potato-associated *Pseudomonas* isolates with their differing biocontrol potential against late blight. Front. Microbiol. 11, 857.
Deketelaere, S., França, S.C., Höfte, M., 2017. Desirable traits of a good biocontrol agent against Verticillium wilt. Front. Microbiol. 8, 276684.
Demontis, M.A., Cacciola, S.O., Orrù, M., Balmas, V., Chessa, V., Maserti, B.E., Mascia, L., Raudino, F., Magnano di San Lio, G., Migheli, Q., 2008. Development of real-time PCR systems based on SYBR® Green I and TaqMan® technologies for specific quantitative detection of *Phoma tracheiphila* in infected *Citrus*. Eur. J. Plant Pathol. 120, 339–351.
Dimaria, G., Mosca, A., Anzalone, A., Paradiso, G., Nicotra, D., Privitera, G.F., Pulvirenti, A., Catara, V., 2023. Sour orange microbiome is affected by infections of *Plenodomus tracheiphilus* causal agent of Citrus mal secco disease. Agronomy 13, 654.
Dimkić, I., Janakiev, T., Petrović, M., Degrassi, G., Fira, D., 2022. Plant-associated *Bacillus* and *Pseudomonas* antimicrobial activities in plant disease suppression via biological control mechanisms-A review. Physiol. Mol. Plant Pathol. 117, 101754.
EFSA PLH Panel, 2014. Scientific Opinion on the pest categorisation of *Plenodomus tracheiphilus* (Petri) Gruyter, Aveskamp & Verkley [syn. *Phoma tracheiphila* (Petri) LA Kantschaveli & Gikashvili]. EFSA J. 12, 3775–3808.
Emanuele, M.C., Scaloni, A., Lavermicocca, P., Jacobellis, N.S., Camoni, L., Di Giorgio, D., Pucci, P., Paci, M., Segre, A., Ballio, A., 1998. Corpeptins, new bioactive lipodepsipeptides from cultures of *Pseudomonas corrugata*. FEBS Lett. 433, 317–320.
EPPO. European and Mediterranean Plant Protection Organization. 2024. ⟨https://gd.eppo.int/⟩ (accessed 13 March 2024).
FAOSTAT. Food and Agriculture Organization of the United Nations. 2022. ⟨http://www.fao.org/faostat/en/#home⟩ (accessed 14 March 2024).
Fogliano, V., Marchese, A., Scaloni, A., Ritieni, A., Visconti, A., Randazzo, G., Graniti, A., 1998. Characterization of a 60 kDa phytotoxic glycoprotein produced by *Phoma tracheiphila* and its relation to malseccin. Physiol. Mol. Plant Pathol. 53, 149–161.
Garrido-Sanz, D., Meier-Kolthoff, J.P., Göker, M., Martín, M., Rivilla, R., Redondo-Nieto, M., 2016. Genomic and genetic diversity within the *Pseudomonas fluorescens* complex. PLoS One 11, e0150183.
Garrido-Sanz, D., Redondo-Nieto, M., Martin, M., Rivilla, R., 2021. Comparative genomics of the *Pseudomonas corrugata* subgroup reveals high species diversity and allows the description of *Pseudomonas ogarae* sp. nov. Microb. Genom. 7, 593.
Ghadamgahi, F., Tarighi, S., Taheri, P., Saripella, G.V., Anzalone, A., Kalyandurg, P.B., Catara, V., Ortiz, R., Vetukuri, R.R., 2022. Plant growth-promoting activity of *Pseudomonas aeruginosa* FG106 and its ability to act as a biocontrol agent against potato, tomato and taro pathogens. Biology 11, 140.
Girard, L., Höfte, M., Mot, R.De, 2020. Lipopeptide families at the interface between pathogenic and beneficial *Pseudomonas*-plant interactions. Crit. Rev. Microbiol. 46, 397–419.
Gkarmiri, K., Finlay, R.D., Alström, S., Thomas, E., Cubeta, M.A., Högberg, N., 2015. Transcriptomic changes in the plant pathogenic fungus *Rhizoctonia solani* AG-3 in response to the antagonistic bacteria *Serratia proteamaculans* and *Serratia plymuthica*. BMC Genom. 16, 1–17.
Gomez-Lama Cabanas, C., Legarda, G., Ruano-Rosa, D., Pizarro-Tobias, P., Valverde-Corredor, A., Triviño, J.C., Roca, A., Mercado-Blanco, J., 2018. Indigenous *Pseudomonas* spp. strains from the olive (*Olea europaea* L.) rhizosphere as effective biocontrol agents against *Verticillium dahliae*: From the host roots to the bacterial genomes. Front. Microbiol. 9, 297478.
Graniti, A., 1969. Host-parasite relations in *Citrus* diseases as exemplified by *Phytophthora* gummosis and *Deuterophoma* "mal secco". : Proc. 1st Int. Citrus Symp. ., Univ. Calif. 1187–1200 (Riverside, USA).
Grant, J.R., Enns, E., Marinier, E., Mandal, A., Herman, E.K., Chen, C., Graham, M., Van Domselaar, G., Stothard, P., 2023. Proksee: in-depth characterization and visualization of bacterial genomes. Nucleic Acids Res 51, W484–W492.
Grasso, F.M., Coco, V., Grimaldi, V., Catara, V., 2008. Contenimento delle infezioni *Phoma tracheiphila* in piante di limone mediante trattamenti con *Pseudomonas* spp. e Acibenzolar-S-methyl. Giornate Fitopatol. 2, 191–195.
Groopman, J.D., Kensler, T.W., Wu, F., 2012. Food safety: mycotoxins-occurrence and toxic effects. In: Encyclopedia of Human Nutrition. Elsevier Inc, pp. 337–341.
Grove, R.K., Moore, J.D., 1962. Toximetric studies of fungicides against brown rot organism *Sclerotina fruticola*. Phytopathology 52, 876–880.
Gu, Y.-L., Li, J.-Z., Li, Y., Cong, S., Wang, J., Ma, Y.-N., Wei, H.-L., 2023. *Pseudomonas* cyclic lipopeptide medpeptin: biosynthesis and modulation of plant immunity. Engineering 28, 153–165.
Gu, Y., Wang, J., Xia, Z., Wei, H.-L., 2020. Characterization of a versatile plant growth-promoting rhizobacterium *Pseudomonas mediterranea* strain S58. Microorganisms 8, 334.
Höfte, M., 2021. The use of *Pseudomonas* spp. as bacterial biocontrol agents to control plant disease. In: Köhl, J., Ravensberg, W.J. (Eds.), Microbial bioprotectants for plant disease management. Burleigh Dodds Science Publishing, Cambridge, UK, pp. 1–74.
Huang, H., Zhang, X., Zhang, Y., Yi, G., Xie, J., Viljoen, A., Wang, W., Mostert, D., Fu, G., Peng, C., 2022. FocECM33, a GPI-anchored protein, regulates vegetative growth and virulence in *Fusarium oxysporum* f. sp. *cubense* tropical race 4. Fungal Biol. 126, 213–223.
Jing, X., Su, L., Yin, X., Chen, Y., Guan, X., Yang, D., Sun, Y., 2024. Genome analysis of *Pseudomonas viciae* G166 conferring antifungal activity in grapevine. J. Fungi 10, 398.
Kalai-Grami, L., Ben Slimane, I., Mnari-Hattab, M., Rezgui, S., Aouani, M.A., Hajlaoui, M. R., Limam, F., 2014. Protective effect of *Bacillus amyloliquefaciens* against infections of *Citrus aurantium* seedlings by *Phoma tracheiphila*. World J. Microbiol. Biotechnol. 30, 529–538.
Lane, D.J., 1991. 16S/23S rRNA sequencing. In: Stackebrandt, E., Goodfellow, M. (Eds.), Nucleic Acid Techniques in Bacterial Systematics. Wiley, New York, pp. 115–175.
Lefort, V., Desper, R., Gascuel, O., 2015. FastME 2.0: a comprehensive, accurate, and fast distance-based phylogeny inference program. Mol. Biol. Evol. 32, 2798–2800.
Leonardi, G.R., Polizzi, G., Vitale, A., Aiello, D., 2023. Efficacy of biological control agents and resistance inducer for control of mal secco disease. Plants 12, 1735.
Li, W., O'Neill, K.R., Haft, D.H., DiCuccio, M., Chetvernin, V., Badretdin, A., Coulouris, G., Chitsaz, F., Derbyshire, M.K., Durkin, A.S., 2021. RefSeq: expanding the Prokaryotic Genome Annotation Pipeline reach with protein family model curation. Nucleic Acids Res 49, D1020–D1028.
Licciardello, G., Caruso, A., Trantas, E.A., Sarris, P.F., Almeida, N.F., Catara, V., 2018. The LuxR regulators PcoR and RfiA co-regulate antimicrobial peptide and alginate production in *Pseudomonas corrugata*. Front. Microbiol. 9, 319727.
Licciardello, G., Strano, C.P., Bertani, I., Bella, P., Fiore, A., Fogliano, V., Venturi, V., Catara, V., 2012. N-acyl-homoserine-lactone quorum sensing in tomato phytopathogenic *Pseudomonas* spp. is involved in the regulation of lipodepsipeptide production. J. Biotechnol. 159, 274–282.
Lima, G., Ippolito, A., Nigro, F., Salerno, M., 1994. Tentativi di lotta biologica contro il mal secco degli agrumi (*Phoma tracheiphila*) a mezzo di batteri endofiti. La Difesa delle Piante 17, 43–49.
Lin, S.H., Liao, Y.C., 2013. CISA: Contig Integrator for Sequence Assembly of Bacterial Genomes. PLoS One 8.
Luisi, N., De Cicco, V., Cutuli, G., Salerno, M., 1979. Ricerche su un metodo di studio della patogenicità del fungo del Mal secco degli Agrumi. Ann. Dell'Ist. Sper. Per L'Agrumic. Di Acireale 9, 167–173.
Luo, R., Lam, T.-W., Liu, B., Xie, Y., Li, Z., Huang, W., Yuan, J., He, G., Chen, Y., Pan, Q., Liu, Y., Tang, J., Wu, G., Zhang, H., Shi, Y., Yu, C., Wang, B., Lu, Y., Han, C., Cheung, D., Yiu, S., Peng, S., Xiaoqian, Z., Liu, G., Liao, X., Li, Y., Yang, H., Wang, J., 2012. SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. Gigascience 1, 1–6.
Magnano di San Lio, G., Perrotta, G., 1986. Variabilità in *Phoma tracheiphila*. In: Cavalloro, R., Di Martino, E. (Eds.), Integrated Pest Control in Citrus Groves. Balkema A.A, Rotterdam, The Netherlands, pp. 267–270.
Manni, M., Berkeley, M.R., Seppey, M., Simão, F.A., Zdobnov, E.M., 2021. BUSCO update: novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic, and viral genomes. Mol. Biol. Evol. 38, 4647–4654.

Martínez-García, P.M., López-Solanilla, E., Ramos, C., Rodríguez-Palenzuela, P., 2016. Prediction of bacterial associations with plants using a supervised machine-learning approach. Environ. Microbiol. 18, 4847–4861.
Matthijs, S., Brandt, N., Ongena, M., Achouak, W., Meyer, J.-M., Budzikiewicz, H., 2016. Pyoverdine and histicorrugatin-mediated iron acquisition in *Pseudomonas thivervalensis*. Biometals 29, 467–485.
Mazzola, M., Fujimoto, D.K., Thomashow, L.S., Cook, R.J., 1995. Variation in sensitivity of *Gaeumannomyces graminis* to antibiotics produced by fluorescent *Pseudomonas* spp. and effect on biological control of take-all of wheat. Appl. Environ. Microbiol 61, 2554–2559.
Meier-Kolthoff, J.P., Carbasse, J.S., Peinado-Olarte, R.L., Göker, M., 2022. TYGS and LPSN: a database tandem for fast and reliable genome-based classification and nomenclature of prokaryotes. Nucleic Acids Res 50, D801–D807.
Meng, J., Zan, F., Liu, Z., Zhang, Y., Qin, C., Hao, L., Wang, Z., Wang, L., Liu, D., Liang, S., 2024. Genomics analysis reveals the potential biocontrol mechanism of *Pseudomonas aeruginosa* QY43 against *Fusarium pseudograminearum*. J. Fungi 10, 298.
Migheli, Q., Cacciola, S.O., Balmas, V., Pane, A., Ezra, D., Magnano di San Lio, G., 2009. Mal secco disease caused by *Phoma tracheiphila*: a potential threat to lemon production worldwide. Plant Dis. 93, 852–867.
Mulet, M., Lalucat, J., García-Valdés, E., 2010. DNA sequence-based analysis of the *Pseudomonas* species. Environ. Microbiol. 12, 1513–1530.
Nachmias, A., Barash, I., Buchner, V., Solel, Z., Strobel, G.A., 1979. A phytotoxic glycopeptide from lemon leaves infected with *Phoma tracheiphila*. Physiol. Plant Pathol. 14, 135–140.
Nelkner, J., Torres Tejerizo, G., Hassa, J., Lin, T.W., Witte, J., Verwaaijen, B., Winkler, A., Bunk, B., Spröer, C., Overmann, J., 2019. Genetic potential of the biocontrol agent *Pseudomonas brassicacearum* (formerly *P. trivialis*) 3Re2-7 unraveled by genome sequencing and mining, comparative genomics and transcriptomics. Genes 10, 601.
Ni, H., Kong, W.-L., Zhang, Y., Wu, X.-Q., 2022. Effects of volatile organic compounds produced by *Pseudomonas aurantiaca* ST-TJ4 against *Verticillium dahliae*. J. Fungi 8, 697.
Nigro, F., Ippolito, A., Salerno, M.G., 2011. Mal secco disease of citrus: a journey through a century of research. J. Plant Pathol. 523–560.
Oliveri, C., Modica, G., Bella, P., Dimaria, G., Cirvilleri, G., Continella, A., Catara, V., 2022. Preliminary evaluation of a zinc-copper-citric acid biocomplex for the control of *Plenodomus tracheiphilus* causal agent of citrus mal secco disease. Acta Hortic. 1354, 231–236.
Oni, F.E., Esmaeel, Q., Onyeka, J.T., Adeleke, R., Jacquard, C., Clement, C., Gross, H., Ait Barka, E., Höfte, M., 2022. *Pseudomonas* lipopeptide-mediated biocontrol: chemotaxonomy and biological activity. Molecules 27, 372.
Overbeek, R., Olson, R., Pusch, G.D., Olsen, G.J., Davis, J.J., Disz, T., Edwards, R.A., Gerdes, S., Parrello, B., Shukla, M., 2014. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). Nucleic Acids Res 42, D206–D214.
Parisi, A., Piattelli, M., Tringali, C., San Lio, G.M. di, 1993. Identification of the phytotoxin mellein in culture fluids of *Phoma tracheiphila*. Phytochemistry 32, 865–867.
Patz, S., Gautam, A., Becker, M., Ruppel, S., Rodríguez-Palenzuela, P., Huson, D.H., 2021. PLaBAse: a comprehensive web resource for analyzing the plant growth-promoting potential of plant-associated bacteria. Biorxiv 2012–2021.
Patz, S., Rauh, M., Gautam, A., Huson, D.H., 2024. mgPGPT: Metagenomic analysis of plant growth-promoting traits. bioRxiv, pp. 2002–2024.
Perrotta, G., Graniti, A., 1988. *Phoma tracheiphila* (Petri) Kanchaveli and Ghikashvili. In: Smith, I.M., Dunez, J., Lelliott, R.A., Phillips, D.H., Archer, S.A. (Eds.), European handbook of plant diseases. Blackwell Scientific Publications, Oxford, UK, pp. 396–398.
Picard, C., Afonso, T., Benko-Beloglavec, A., Karadjova, O., Matthews-Berry, S., Paunovic, S.A., Pietsch, M., Reed, P., Van Der Gaag, D.J., Ward, M., 2018. Recommended regulated non-quarantine pests (RNQP s), associated thresholds and risk management measures in the European and Mediterranean region. EPPO Bull. 48, 552–568.
Potter, S.C., Luciani, A., Eddy, S.R., Park, Y., Lopez, R., Finn, R.D., 2018. HMMER web server: 2018 update. Nucleic Acids Res 46, W200–W204.
Pritchard, L., Glover, R.H., Humphris, S., Elphinstone, J.G., Toth, I.K., 2016. Genomics and taxonomy in diagnostics for food security: soft-rotting enterobacterial plant pathogens. Anal. Methods 8, 12–24.
Putri, R.E., Mubarik, N.R., Ambarsari, L., Wahyudi, A.T., 2021. Antagonistic activity of glucanolytic bacteria *Bacillus subtilis* W3. 15 against *Fusarium oxysporum* and its enzyme characterization. Biodiversitas J. Biol. Divers 22.
Raaijmakers, J.M., De Bruijn, I., Nybroe, O., Ongena, M., 2010. Natural functions of lipopeptides from *Bacillus* and *Pseudomonas*: more than surfactants and antibiotics. FEMS Microbiol. Rev. 34, 1037–1062.
Reis, R.F., De Almeida, T.F., Stuchi, E.S., De Goes, A., 2007. Susceptibility of citrus species to *Alternaria alternata*, the causal agent of the Alternaria brown spot. Sci. Hortic. 113, 336–342.
Risse, D., Beiderbeck, H., Taraz, K., Budzikiewicz, H., Gustine, D., 1998. Corrugatin, a lipopeptide siderophore from *Pseudomonas corrugata*. Z. F. üR. Naturforsch. C. 53, 295–304.
Romano, I., Ventorino, V., Pepe, O., 2020. Effectiveness of plant beneficial microbes: overview of the methodological approaches for the assessment of root colonization and persistence. Front. Plant Sci. 11, 487940.
Russo, R., Sicilia, A., Caruso, M., Arlotta, C., Di Silvestro, S., Gmitter, F.G., Nicolosi, E., Lo Piero, A.R., 2021. De novo transcriptome sequencing of rough lemon leaves (*Citrus jambhiri* Lush.) in response to *Plenodomus tracheiphilus* infection. Int. J. Mol. Sci. 22, 1–21.
Salerno, M., Catara, A., 1967. Ricerche sul "mal secco" degli Agrumi (*Deuterophoma tracheiphila* Petri). VI. Indagini sulla riproduzione sperimentale della malattia. Riv. di Patol. Veg. 3, 89–97.
Sallam, N., AbdElfatah, H.-A.S., Elfarash, A., Abo-Elyousr, K.A.M., Sallam, A., 2023. Exploring the mechanisms of endophytic bacteria for suppressing early blight disease in tomato (*Solanum lycopersicum* L.). Front. Microbiol. 14, 1184343.
Scaloni, A., Dalla Serra, M., Amodeo, P., Mannina, L., Vitale, R.M., Segre, A.L., Cruciani, O., Lodovichetti, F., Greco, M.L., Fiore, A., 2004. Structure, conformation and biological activity of a novel lipodepsipeptide from *Pseudomonas corrugata*: cormycin A. Biochem. J. 384, 25–36.
Schouten, A., van den Berg, G., Edel-Hermann, V., Steinberg, C., Gautheron, N., Alabouvette, C., De Vos, C.H., Lemanceau, P., Raaijmakers, J.M., 2004. Defense responses of *Fusarium oxysporum* to 2,4-diacetylphloroglucinol, a broad-spectrum antibiotic produced by *Pseudomonas fluorescens*. Mol. Plant-Microbe Interact. 17, 1201–1211.
Sharma, M., Manhas, R.K., 2022. Biocontrol potential of *Streptomyces* sp. M4 and salvianolic acid B produced by it against Alternaria black leaf spot. Microb. Pathog. 173, 105869.
Shi, Y., Liu, X., Fang, Y., Tian, Q., Jiang, H., Ma, H., 2018. 2, 3-Butanediol activated disease-resistance of creeping bentgrass by inducing phytohormone and antioxidant responses. Plant Physiol. Biochem. 129, 244–250.
Sicilia, A., Catara, V., Dimaria, G., Scialò, E., Russo, M., Gentile, A., Lo Piero, A.R., 2023. Transcriptome analysis of lemon leaves (*Citrus limon*) infected by *Plenodomus tracheiphilus* reveals the effectiveness of *Pseudomonas mediterranea* in priming the plant response to mal secco disease. J. Plant Inter. 18, 2243097.
Sicilia, A., Russo, R., Caruso, M., Arlotta, C., Di Silvestro, S., Gmitter, F.G., Gentile, A., Nicolosi, E., Lo Piero, A.R., 2022. Transcriptome analysis of *Plenodomus tracheiphilus* infecting rough lemon (*Citrus jambhiri* Lush.) indicates a multifaceted strategy during host pathogenesis. Biology 11.
Sicilia, A., Russo, R., Catara, V., Lo Piero, A.R., 2024. Hub Genes and Pathways Related to Lemon (*Citrus limon*) Leaf Response to *Plenodomus tracheiphilus* Infection and Influenced by *Pseudomonas mediterranea* Biocontrol Activity. Int. J. Mol. Sci. 25, 2391.
Simpson, J.T., Wong, K., Jackman, S.D., Schein, J.E., Jones, S.J.M., Birol, I., 2009. ABySS: a parallel assembler for short read sequence data. Genome Res 19, 1117–1123.
Strano, C.P., Bella, P., Licciardello, G., Caruso, A., Catara, V., 2017. Role of secondary metabolites in the biocontrol activity of *Pseudomonas corrugata* and *Pseudomonas mediterranea*. Eur. J. Plant Pathol. 149, 103–115.
Surico, G., Lavermicocca, P., Iacobellis, N.S., 1988. Produzione di siringomicina e di siringotossina in colture di *Pseudomonas syringae* pv. *syringae*. Phytopathol. Mediterr. 163–168.
Takeuchi, K., Someya, N., Morohoshi, T., 2023. Genome-mining approaches for the evaluation of *Pseudomonas protegens* and related strains isolated from the rhizosphere in Japan. Physiol. Mol. Plant Pathol. 125, 101981.
Trantas, E.A., Sarris, P.F., Pentari, M.G., Mpalantinaki, E.E., Ververidis, F.N., Goumas, D. E., 2015. Diversity among *Pseudomonas corrugata* and *Pseudomonas mediterranea* isolated from tomato and pepper showing symptoms of pith necrosis in Greece. Plant Pathol. 64, 307–318.
Tringali, C., Parisi, A., Piattelli, M., Magnano di San Lio, G., 1993. Phomenins A and B, bioactive polypropionate pyrones from culture fluids of *Phoma tracheiphila*. Nat. Prod. Lett. 3, 101–106.
Ullah, H., Yasmin, H., Mumtaz, S., Jabeen, Z., Naz, R., Nosheen, A., Hassan, M.N., 2020. Multitrait *Pseudomonas* spp. isolated from monocropped wheat (*Triticum aestivum*) suppress Fusarium root and crown rot. Phytopathology 110, 582–592.
Vincent, J.M., 1947. Distortion of fungal hyphae in the presence of certain inhibitors. Nature 159, 850.
Xu, D., Xue, M., Shen, Z., Jia, X., Hou, X., Lai, D., Zhou, L., 2021. Phytotoxic secondary metabolites from fungi. Toxins 13, 261.
Yu, J., Bhatnagar, D., Cleveland, T.E., 2004. Completed sequence of aflatoxin pathway gene cluster in *Aspergillus parasiticus*. FEBS Lett. 564, 126–130.
Zboralski, A., Biessy, A., Filion, M., 2023. Genome exploration and ecological competence are key to developing effective *Pseudomonas*-based biocontrol inoculants. Can. J. Plant Pathol. 45, 330–339.
Zhang, C.-M., Xu, M.-J., Li, X.-W., Gong, Y., Xing, K., Qin, S., 2021. Complete genome sequence of the biocontrol agent *Pseudomonas chlororaphis* subsp. *aureofaciens* SPS-41. Mol. Plant-Microbe Inter. 34, 839–841.
Zhao, P., Crous, P.W., Hou, L.W., Duan, W.J., Cai, L., Ma, Z.Y., Liu, F., 2021. Fungi of quarantine concern for China I: dothideomycetes. persoonia-molecular phylogeny evol. Fungi 47, 45–105.
Zhou, L., De Jong, A., Yi, Y., Kuipers, O.P., 2021. Identification, isolation, and characterization of medipeptins, antimicrobial peptides from *Pseudomonas mediterranea* EDOX. Front. Microbiol. 12, 732771.
Zhou, Y., Liang, Y., Lynch, K.H., Dennis, J.J., Wishart, D.S., 2011. PHAST: a fast phage search tool. Nucleic Acids Res 39, W347–W352.