Genome sequence of the desert endophyte Pseudomonas granadensis R4-79 reveals potential for plant-growth promotion and disease suppression

Linah Alghanmi
King Abdullah University of Sciences and Technology
Ahmed Elhady
King Abdullah University of Sciences and Technology
Sabiha Parween
King Abdullah University of Sciences and Technology
Heribert Hirt
heribert.hirt@kaust.edu.sa

King Abdullah University of Sciences and Technology

Research Article

Keywords: Genome, Pseudomonas, Desert agriculture, Phytopathogens, Biocontrol, Plant Pathogen Suppression

Posted Date: May 23rd, 2025

DOI: https://doi.org/10.21203/rs.3.rs-6620576/v1

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at BMC Microbiology on October 28th, 2025. See the published version at https://doi.org/10.1186/s12866-025-04431-4.

Abstract

Desert ecosystems are limited by resources and optimal climatic conditions to support cropping in different farming models. Recently, microbial applications have emerged as promising strategies to enhance plant survival and adaptation in such extreme environments. However, identifying beneficial microbes and understanding their functional roles and adaptation mechanisms remains underexplored. This study reports, for the first time, the isolation and characterization of the *Pseudomonas* sp. R4-79 strain from the arid environment of Wadi Rum, Jordan, associated with *Ifloga spicata*. The genome of *Pseudomonas* sp. R4-79, sequenced at 275× PacBio coverage, consists of a 6.18 Mbp chromosome encoding 5,445 proteins, including gene clusters for siderophores, phenazines, hydrogen cyanide, and phytohormones, as well as advanced secretion systems (T2SS, T4SS, T6SS, and TAT). Genomic and phenotypic analyses revealed that *Pseudomonas* sp. R4-79 belongs to the genus *P. granadensis* and exhibits plant growth-promoting attributes and substantial biocontrol potential. *Pseudomonas* sp. R4-79 effectively suppresses key phytopathogens, including the necrotrophic fungus *Botrytis cinerea in vitro*, as well as *Pseudomonas syringae* pv. tomato DC3000 and root-knot nematodes (*Meloidogyne incognita*) *in vivo* in Arabidopsis and tomato. This work suggests that *P. granadensis* R4-79 might be a good biocontrol agent to improve crop yield and ecological restoration in arid systems.

Introduction

Desert ecosystems are among the most fragile environments on Earth, where water is scarce and soils are not rich in nutrients (Coleine et al., 2024). These factors not only discourage ecological restoration but also create a limit to the scope of practicable models for sustainable farming (Rastgoo & Hasanfard, 2021). Conversely, while previously, water resources and minimum vegetation cover were sought to be the focus for the rejuvenation of the desert ecosystem, recent findings emphasize the significance of microbial communities in boosting the desert ecosystem (Alsharif et al., 2020; Islam et al., 2024). Microbes, particularly those adapted to arid conditions, hold substantial potential for enhancing soil fertility, promoting plant growth, and improving resilience to environmental stresses (Alsharif et al., 2020). However, identifying beneficial microbes, enhancing their growth, and expanding their community diversity continue to be major challenges. Moreover, investigating their functional traits and phylogenomic adaptations is necessary for advancement in ecological and agricultural applications.

As abiotic and biotic stresses exist, they make ecological revegetation and farming in arid deserts highly challenging. Plants and crops in these environments are highly susceptible to infestations by parasitic nematodes and insects, as well as infections caused by bacterial and fungal phytopathogens (Elhady et al., 2024; Sulaiman & Bello, 2024). *Meloidogyne incognita* is among the most destructive plant-parasitic nematodes, with nearly 100% occurrence in the collected soil samples, often exceeding the economic threshold, particularly in the Sahara and Arabian deserts (Elhady et al., 2024). Additionally, pathogenic fungal species, including *Fusarium* spp., *Alternaria* spp., *Pythium* spp., *Rhizoctonia solani*, *Phytophthora* spp., *Sclerotinia* spp., and *Verticillium* spp., are widely distributed and cause significant damage, with losses in agricultural production reaching 50–75% in some cases (Panth et al., 2020). The harsh abiotic

conditions, coupled with limited plant and microbial diversity, further exacerbate plant susceptibility to phytopathogens that hinder the establishment of resilient vegetation. Compared to other ecosystems, plant pathogens have a significantly greater impact on arid ecosystems, but efforts to control these pathogens have received minimal attention (Elhady et al., 2024; Jat et al., 2012).

Desert plants depend on mutualistic interactions with specialized microbial species to persist in extreme environments. These interactions are vital for the fitness and survival of both partners. For example, the legume shrub *Vachellia jacquemontii* in the Thar Desert associates with the nitrogen-fixing bacterium *Ensifer*, which provides bioavailable nitrogen to support the plant's nutritional requirements (Ardley, 2017). Similarly, despite the extreme aridity of the Atacama Desert, arbuscular mycorrhizal (AM) fungi have been reported (Santander et al., 2021) to potentially play significant roles in phosphorus solubilization, plant stress tolerance, and growth promotion.

The isolation and application of microbial strains from native desert plants has emerged as an effective strategy to enhance crop performance under harsh conditions. For instance, the halotolerant *Bacillus cabrialesii* from the Qatar desert improved seedling growth under salt stress and reduced tomato infections by gray mold disease (*Botrytis cinerea*) (Masmoudi et al., 2024). Similarly, two *Klebsiella* strains from *Parastrephia quadrangularis* in the Atacama Desert increased wheat seedling fresh weight by up to 60% under salinity stress (Acuña et al., 2019). *Serratia marcescens* from *Capparis decidua* in India's Thar Desert enhanced wheat tolerance to salinity stress and reduced fungal disease severity caused by *Fusarium graminearum* (Singh & Jha, 2016). Additionally, *Pseudomonas argentinensis* SA190 from *Indigofera argentea* in Saudi Arabia's Jizan region improved drought tolerance in *Arabidopsis thaliana* seedlings (Alwutayd et al., 2023).

In an attempt to identify beneficial bacterial strains from the Arabian desert to support arid farming and ecological restoration, *Pseudomonas* sp. R4-79 was tested for promoting plant growth and suppressing various phytopathogens. This strain was isolated from the desert plant *Ifloga spicata* in Wadi Rum, Jordan. In this work, we characterize the phenotypic properties of *Pseudomonas* sp. R4-79 which inhibited several phytopathogens such as the bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000, the root-knot nematode (*Meloidogyne incognita*) and the fungal pathogen *Botrytis cinerea*. Whole-genome sequencing was conducted to determine its taxonomic classification and explore its biochemical potential to promote plant growth and inhibit pathogens.

Materials and Methods

Root collection and bacterial strain isolation

Root samples of the desert plant *Ifloga spicata* were collected from Wadi Rum, Jordan. To study the root endophytic bacteria, the root tissues were washed to remove soil debris and surface-sterilized by immersing them in 70% ethanol for 30 seconds, followed by treatment with 2% sodium hypochlorite for five minutes and thorough rinsing with sterile distilled water. The sterilized roots were macerated in a

solution containing 0.8% saline and subjected to serial dilutions ranging from 10^{-2} to 10^{-5}. Each dilution was plated in duplicate on different culture media, including Tryptone-Yeast (TY), R2A, LB agar, and TSA. Bacterial colonies were selected based on distinctive morphological characteristics and pigmentation, followed by further purification to obtain individual strains. The purified bacterial strains were suspended in a 25% glycerol-LB solution and stored at − 80°C to serve as reference stocks.

Genomic DNA extraction and strains identification

To identify the bacterial strains, cells were streaked from 25% glycerol stock onto LB agar and incubated at 28°C overnight. A single bacterial colony was then inoculated into 10 mL of LB broth in a 50 mL Falcon tube and incubated at 28°C with shaking at 220 rpm. Genomic DNA was extracted using the Genelute Bacterial Genomic DNA Kit (Sigma-Aldrich). The 16S rRNA gene was amplified using Taq DNA Polymerase PCR Master Mix (Promega, Madison, WI, USA) and universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3'). The amplified PCR products were purified using ExoSAP-IT (Affymetrix, Santa Clara, CA, USA) and sequenced via Sanger sequencing at the Core Labs, KAUST, Saudi Arabia. Strain identification was performed by comparing the sequences to NCBI's GenBank database using BLAST. Strains were sorted and grouped into collections based on their genus-level taxonomy, including *Pseudomonas spp.*, *Bacillus spp.*, *Enterobacter spp.*, rhizobial strains, and others.

Genome sequencing and annotation of Pseudomonas sp. R4-79

After screening 86 microbial strains for plant growth promotion, the *Pseudomonas* sp. R4-79 strain was selected for its high effectiveness in promoting plant growth. The strain was further analyzed to characterize its genetic features and beneficial potential using *in silico*, *in vitro*, and *in vivo* approaches. To analyze the genome of the *Pseudomonas* sp. R4-79 strain, the total genomic was shipped to Novogen Bioinformatics Technology Co., Ltd. (Singapore). Genomic sequencing and assembly were performed using Single-Molecule Real-Time (SMRT®) sequencing on the PacBio Sequel II/IIe platform. The whole genome assembly was performed using the Hierarchical Genome Assembly Process (HGAP). Genome polishing and circularization were carried out using Arrow (v2.3.3) and Circlator (v1.5.5), respectively. To assess the quality of the genome assembly, quantitative measurements were obtained using BUSCO (v4.0.2) (Benchmarking Universal Single-Copy Orthologs, https://busco.ezlab.org). Genome annotation was performed for coding genes, repetitive sequences, non-coding RNAs, and pseudogenes using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP). PGAP identifies structural annotations by comparing open reading frames (ORFs) against libraries containing protein hidden Markov models (HMMs), representative RefSeq proteins, and proteins from well-characterized reference genomes. For genomic regions lacking HMM or protein evidence, GeneMarkS-2 + was used to generate ab initio coding region predictions and to determine start sites for ORFs based on available HMM evidence.

Phylogenetic analysis

The genome sequence data were analyzed using the Type (Strain) Genome Server (TYGS), a free bioinformatics platform available at https://tygs.dsmz.de, for whole-genome-based taxonomic identification. Two methods were employed to identify the closest type strain genomes. First, the *Pseudomonas* sp. R4-79 strain genome was compared to all type strain genomes in the TYGS database using the MASH algorithm, which approximates intergenomic relatedness. The 10 closest type strains with the smallest MASH distances were selected. Second, 16S rDNA gene sequences were extracted from the *Pseudomonas* sp. R4-79 strain genome using RNAmmer, and each sequence was BLASTed against the 16S rDNA gene sequences of 18,357 type strains in the TYGS database. The 50 best-matching type strains were identified, and precise distances were calculated using the Genome BLAST Distance Phylogeny (GBDP) approach with the “coverage” algorithm and distance formula d5. These distances were used to determine the 10 closest type strain genomes to the *Pseudomonas* sp. R4-79 strain genome.

For phylogenomic analysis, pairwise comparisons among the selected genomes were performed using GBDP, with intergenomic distances inferred using the ‘trimming’ algorithm and distance formula d5. One hundred distance replicates were calculated for each comparison. Digital DNA-DNA hybridization (dDDH) values and confidence intervals were computed using GGDC 3.0. The intergenomic distances were used to infer a balanced minimum evolution tree with branch support via FASTME 2.1.6.1, including SPR postprocessing and 100 pseudo-bootstrap replicates. The trees were rooted at the midpoint and visualized with PhyD3. Species clustering was based on a 70% digital DDH threshold, while subspecies clustering was done using a 79% dDDH threshold.

Sequence accession number

The WGS has been submitted to NCBI under Bioproject-PRJNA708169, Biosample-SAMN18220753, Genome accession-CP071650-CP071651.

Phenotypic and biochemical properties of Pseudomonas sp. R4-79

Strain culturing. Cells of *Pseudomonas* sp. R4-79 strain were revived from a 25% glycerol stock stored at − 80°C by streaking onto King’s B agar (Sigma-Aldrich) and incubating at 28°C overnight. Then, a single pure colony was inoculated into 10 mL of sterilized LB broth (Invitrogen) in a 50 mL Falcon tube (Fisher Scientific) and incubated overnight on an orbital shaker (Innova 42, New Brunswick) at 28°C /220 rpm. A fresh culture was prepared by adding 5 ml of LB broth (Lennox L Broth Base, Invitrogen) to a 15 ml tube (Fisher Scientific), followed by the addition of 500 µl of bacterial culture. The mixture was incubated at 28°C with shaking at 220 rpm on an orbital shaker for 45 minutes to allow the culture to reach the exponential phase. The optical density at 600 nm (OD_{600}) was then measured and adjusted to 0.2 using a spectrophotometer (BioPhotometer, Eppendorf).

Electron microscopy. The morphology of the bacterial strain *Pseudomonas* sp. R4-79 was visualized using the transmission electron microscopy (TEM). A 5-µl of the bacterial cells OD_{600} = 0.2 was

deposited onto carbon/Formvar-coated TEM grids (Electron Microscopy Sciences) and incubated for 2 minutes to allow adhesion. The grids were subsequently washed with distilled water and stained with 1% uranyl acetate for 1 minute. Imaging was performed using a Titan ST (Thermo Fisher Scientific) transmission electron microscope operated at an accelerating voltage of 300 kV. For scanning electron microscope (SEM), bacterial samples were fixed overnight at 4°C in 2.5% glutaraldehyde prepared in 0.1 M sodium cacodylate buffer (pH 7.4). After fixation, the samples were washed three times in 0.1 M sodium cacodylate buffer (pH 7.4) to remove residual fixative. Post-fixation was carried out using 1% osmium tetroxide in water for 1 hour at room temperature, followed by three washes with distilled water. The samples were dehydrated through a graded ethanol series (25%, 50%, 75%, 95%, and 100%) with a 10-minute incubation in each concentration. Critical point drying was performed using a Leica Microsystems critical point dryer to preserve structural integrity. The dried samples were sputter-coated with a 6 nm layer of platinum to enhance conductivity and imaged using a Zeiss Merlin Gemini II field emission scanning electron microscope (FE-SEM) operated at an accelerating voltage of 3 kV and a beam current of 30 pA.

Motility assays. To assess the motility of the *Pseudomonas* sp. R4-79 strain, a motility test was conducted using Motility Test Media (SIGMA-ALDRICH) in solid agar form. A 10 µL aliquot of an overnight bacterial culture OD_{600} = 0.2 was carefully inoculated onto the center of the motility agar plate. The plate was then incubated at 28°C for 48 hours to allow bacterial growth and potential motility. After the incubation period, the motility of the *Pseudomonas* sp. R4-79 strain was assessed by observing the spread of bacterial growth from the point of inoculation. A clear zone of growth extending beyond the initial inoculation point would indicate bacterial motility, whereas the absence of such spread would suggest the strain is non-motile. The results were recorded based on the extent of the growth diffusion from the inoculation site.

Siderophore production. To assess the siderophore production potential of the *Pseudomonas* sp. R4-79 strain, the assay was performed using the method described by (Brian et al.,1990) with blue agar CAS media. A 10 µL aliquot of the overnight bacterial culture was inoculated onto the CAS agar plate, which contains a blue iron complex. The plate was incubated at 28°C for 48 hours. Siderophore production was indicated by a yellow-orange halo around the bacterial growth, caused by the chelation of iron from the CAS complex, resulting in a color change in the surrounding agar.

Biofilm formation. 200 µl of diluted overnight bacterial culture was added to each well of a 96-well plate and incubated overnight at 28°C. The liquid culture was removed by inverting the plate, followed by two washes with water to eliminate excess liquid. Each well was then treated with 125 µl of 0.1% crystal violet solution and incubated for 15 minutes. After rinsing the plate 3–4 times with water, it was inverted and left to dry overnight on a paper towel. The following day, 125 µl of 30% acetic acid was added to each well and incubated for 15 minutes. Biofilm quantification was performed using a plate reader (Infinite M200 PRO, TECAN).

Effect of Pseudomonas sp. R4-79 against Pseudomonas syringae (Pst) DC3000 infection of Arabidopsis thaliana

Pst DC3000 seed germination assays. We used *Arabidopsis thaliana* Col-0 (wild type) to quantify the survival percentage affected by *Pseudomonas* sp. R4-79 and *P*st DC3000. Seeds of *A. thaliana* were surface sterilized with 70% ethanol containing 0.05% Triton X-100 and shaken for 10 minutes, followed by three washes with absolute ethanol. The sterilized seeds were placed in a 2 ml Eppendorf tube containing 1 ml sterilized water (H_2O) and kept at 4°C for two days to ensure uniform germination. In square Petri dishes (12 × 12 cm), 50 ml Murashige and Skoog (MS) Basal Salt Mixture containing 0.9% agar (½ MS agar) at pH 5.8 (Murashige and Skoog, 1962; M5524, Sigma Aldrich, Germany) were mixed with 100 µl of *Pseudomonas* sp. R4-79 cells (OD_{600} = 0.2), 100 µl of DC3000 cells (OD_{600} = 0.2), 100 µl of *Pseudomonas* sp. R4-79 and DC3000 cells together (OD_{600} = 0.2), and ½ MS agar media alone as a mock. *A. thaliana* seeds were spotted individually onto the ½ MS agar plates, with approximately 36 seeds per plate. The plates were placed in growth chambers (Percival Scientific Inc., USA) vertically (~ 75° angle to the horizontal) and incubated for 16 days at 23°C with a 16/8 h (light/dark) photoperiod. After 16 days, the number of *Arabidopsis* seedlings was counted, and the survival percentage was measured using this formula:

$$Survival\ percentage = number\ of\ Arabidopsis\ seedlings \div number\ of\ seeds\ (36) \times\ 100$$

Pst DC3000 seedling growth assays. We further investigated the inhibitory effect of the *Pseudomonas* sp. R4-79 strain at different concentrations of the pathogenic *P*st DC3000 bacteria. Surface-sterilized seeds of *A. thaliana* were sown on ½ MS agar mixed with 100 µl of *Pseudomonas* sp. R4-79 strain (OD_{600} = 0.2), sealed with micropore tape, covered with aluminum foil, and stratified for two days at 4°C. After stratification, the plates were placed vertically (~ 75° angle to the horizontal) in a growth chamber for five days at 23°C with a 16/8 h (light/dark) photoperiod to allow germination. After five days, ½ MS agar plates without or with *P*st DC3000 were prepared by mixing them with 100 µl of different CFU concentrations (2 × 10^8 to 1 × 10^9). Six seedlings colonized and non-colonized with root lengths of ~ 1–1.5 cm were transferred to the prepared plates. The plates were incubated under the same growth conditions for 16 days. At the end of the experiment, the fresh weight of the plants was measured. Three biological replicates were performed (Fig. 1).

Suppressive effect of Pseudomonas sp. R4-79 against Meloidogyne incognita infection of tomato

The inhibitory effect of the *Pseudomonas* sp. R4-79 strain was tested against various phytopathogens to assess its potential to control disease complexes. We evaluated its effects both *in vitro* and *in vivo* on the root-knot nematode (*Meloidogyne incognita*, RKN), the fungal pathogen *Botrytis cinerea*, and the bacterial pathogen *Pseudomonas syringae* pv. *Tomato* (*Pst*, DC3000 strain). In a greenhouse experiment, two-week-old tomato seedlings (Moneymaker cultivar) were grown in ½-liter pots filled with sand supplemented with 1 gram of Osmocote per liter. The plants were treated with *Pseudomonas* sp. R4-79 both via soil drenching or foliar spray at an OD_{600} of 0.2. Control plants, which received no bacterial application, were also prepared. As positive controls, plants were treated with *Pseudomonas fluorescens*

strain SBW25 or the nematicide Velum® Prime (Fluopyram 41.5% active ingredient; Bayer CropScience). Each pot was drenched once with 10 µL of Velum® Prime diluted in 50 mL of water. After 5 days of bacterial treatment, the pots were infected with 300 second-stage juvenile RKN (J2s) to assess the bacterial strain's potential to inhibit RKN. Three weeks post-infection, the plants were sampled, and the roots were washed to remove soil. Root galls were counted, and plant biomass was measured. The experiment was repeated twice with a total of n = 21 – 16 replicates. In an analogous experiment, tomato seedlings were grown in 2-liter pots filled with peat moss supplemented with 1 gram of Osmocote per liter. The bacterial-treated and untreated plants followed the same procedure but were not infected with any pathogens. This setup allowed for the quantification of the plant growth-promoting effects of the *Pseudomonas* sp. R4-79 strain on tomato plants. The plants were maintained under greenhouse conditions (25°C, 16-hour photoperiod) and watered every 5 days. The experiment was done once with 30 to 40 replicates.

Growth inhibitory effect of Pseudomonas sp. R4-79 strain on the pathogenic fungus Botrytis cinerea

To assess the antifungal activity of the *Pseudomonas* sp. R4-79 strain against *Botrytis cinerea*, a dual-culture assay was conducted. A 1 cm² plug of *B. cinerea* mycelium, grown on Potato Dextrose Agar (PDA), was placed at the center of a fresh PDA plate. Three individual spots of *Pseudomonas* sp. R4-79 culture were inoculated on the same plate; each positioned 1.5 cm away from the fungal plug. The plates were incubated at 25°C for 7 days, after which inhibition of fungal growth was assessed and visualized. The experiment was performed with three biological replicates.

Results

Genome assembly and functional annotation

The genome analysis of the *Pseudomonas* sp. R4-79 strain using PacBio technology revealed a genome size with 6,175,612 base pairs chromosome and 5473 genes and gene clusters with no plasmids. The annotation pipeline generated 5445 protein-coding sequences (CDS), 96 exons, 73 tRNAs, 67 pseudo genes, 19 rRNAs, 9 riboswitches, and 1 ncRNA **(**Table 1**).**

Genome functional analysis of the *Pseudomonas* sp. R4-79 strain was performed using the Blast KOALA platform. Genome mining revealed the presence of several pathways related to metabolism, genetic and environmental information processing, cellular processes, and organismal systems. In addition, the *Pseudomonas* sp. R4-79 strain uses different systems, such as ATP-binding cassette (ABC) transporters, phosphotransferase system (PTS), and bacterial secretion systems for membrane transport. The ABC transporter can transport minerals and organic ions, oligosaccharides, polyols, lipids, monosaccharides, phosphate and amino acids, peptides and nickel, metallic cation, iron-siderophore, and vitamin B12. The PTS includes N-acetyl-D-glucosamine, trehalose, fructose, and nitrogen regulation. The *Pseudomonas* sp. R4-79 strain has three secretion systems (type 2, type 4, and type 6), twin arginine targeting (TAT), and Sec-SRP.

The *Pseudomonas* sp. R4-79 strain produces secondary metabolites, such as hydrogen cyanide (HCN), which can confer several benefits in plant-microbe interactions (Sehrawat et al., 2022), and phenazine, which showed wide spectrum activity against numerous plant pathogenic bacteria and fungi (Karmegham et al., 2020).

Phylogenetic analysis based on a partial 16S rRNA gene sequence or whole-genome sequence revealed that *Pseudomonas* sp. R4-79 forms a well-supported monophyletic group with *Pseudomonas granadensis* LMG 27940 (Fig. 2A-B). *P. granadensis* LMG 27940 was isolated from soil samples collected in the Tejeda, Almijara, and Alhama Natural Park in Granada, Spain.

Table 1
Summary of Pseudomonas sp. R4-79 genome features.

Feature	Chromosome
Genome size (bp)	6175612
Genome coverage	275x
Genes	5473
CDS	5445
Exon	96
tRNA	73
Pseudo genes	67
rRNA	19
Riboswitch	9
tmRNA	1
SRP_RNA	1
Rnase_P_RNA	1
ncRNA	1

Bacterial and biochemical features of Pseudomonas sp. R4-79

Pseudomonas sp. R4-79 is a gram-negative, rod-shaped bacterium equipped with lophotrichous flagella (Fig. 4B-C). The colonies exhibit a mucoid and glossy appearance that suggests the production of extracellular polysaccharides (EPS) or biofilm formation (Fig. 4A). Additionally, the colonies display spreading with irregular edges, which indicates notable motility. This observation was further supported by a motility assay, which demonstrated swarming behavior on agar plates (Fig. 4D). A biofilm formation assay confirmed the capability of the *Pseudomonas* sp. R4-79 strain to produce biofilm (Fig. 4E). Furthermore, the strain exhibited a fluorescent yellow-green color on King B agar, which suggests the

potential production of the siderophore pyoverdine. To confirm this, a chrome azurol sulfonate (CAS) agar assay was performed. The *Pseudomonas* sp. R4-79 strain showed yellowish growth on CAS agar, further indicating siderophore production (Fig. 4F).

In the bacterial strain *Pseudomonas sp.* R4-79, antiSMASH analysis revealed the presence of siderophore-producing gene clusters, which are crucial for iron acquisition in iron-limited environments, as well as BGCs for potential antimicrobial compounds such as hydrogen cyanide (HCN), fragin, and lokisin (Supplementary Table.1). These metabolites not only enhance bacterial survival but also contribute to plant growth promotion and protection by inhibiting pathogenic microbes, highlighting their ecological and biotechnological significance. The *Pseudomonas* sp. R4-79 strain can produce quorum-sensing molecules such as toxoflavin and riboflavin. Additionally, genome functional analysis reveals that *Pseudomonas* sp. R4-79 possesses the same biofilm-associated genes found in *Pseudomonas aeruginosa*.

Arabidopsis bacterial Pst DC3000 phytopathogen suppression by Pseudomonas sp. R4-79

Pst DC3000 seed germination assays. The *Pseudomonas* sp. *R4-79* strain demonstrated a strong inhibitory effect against the pathogenic bacterium *Pseudomonas syringae* pv. *tomato* DC3000 (*P*st) in *Arabidopsis thaliana*. Plant survival percentage varied based on whether *Arabidopsis* was treated with the *Pseudomonas* sp. R4-79 strain, *P*st DC3000, or a combination of both. Whereas mock-inoculated plants showed a survival percentage of 83.34%, plants pre-colonized with the *Pseudomonas* sp. R4-79 strain showed full survival (100%). In contrast, infection with *P*st DC3000 completely inhibited the growth of *A. thaliana* immediately after germination, and no plants survived. Notably, *A. thaliana* plants infected with *P*st DC3000 but treated with the *Pseudomonas* sp. R4-79 strain afterward exhibited a high survival rate of 86.11% (Fig. 5A).

Pst DC3000 seedling growth assays. We further investigated whether the inhibitory effect of the *Pseudomonas* sp. R4-79 strain depended on the pathogen load of *P*st DC3000. The *Pseudomonas* sp. R4-79 strain maintained its ability to suppress *P*st DC3000 across bacterial concentrations ranging from 2×10^8 to 1×10^9 CFU, which confirmed its capacity to reduce disease severity even at higher pathogen loads (Fig. 5B-C).

Pseudomonas sp. R4-79 promotes tomato growth and suppresses the root-knot nematode M. incognita

We investigated the efficacy of *Pseudomonas* sp. R4-79 in promoting plant growth and suppressing phytopathogens. In greenhouse experiments, tomato plants colonized by *Pseudomonas sp.* R4-79 exhibited a significant increase in fresh weight, with a 14% improvement compared to the control group (t-test: $P < 0.0001$) under normal conditions. Additionally, these plants demonstrated greater shoot length relative to the control (Fig. 6). Meanwhile, we evaluated the ability of the *Pseudomonas* sp. R4-79 strain to suppress the root-knot nematode *M. incognita* in tomato plants. Tomato plants colonized with *Pseudomonas* sp. R4-79 under greenhouse conditions exhibited a significant reduction up to 59.4% in gall numbers on the root system compared to control non-colonized tomato plants ($P < 0.0001$) (Fig. 7A).

Compared to *Pseudomonas fluorescens* SBW25, the *Pseudomonas* sp. R4-79 strain exhibited a comparable suppressive effect on *M. incognita* (Fig. 7A). In contrast, the chemical commercial product Velum® Prime completely impeded gall formation on tomato roots (Fig. 7A). The reduction in gall numbers by *Pseudomonas* sp. R4-79 was accompanied by a slight, non-significant increase (26%) in tomato fresh weight compared to control plants (Fig. 7B).

Pseudomonas granadensis, R4-79, inhibits the gray mold fungus, Botrytis cinerea

To evaluate the suppressive and biocontrol potential of *P. granadensis* R4-79 against other phytopathogens, we examined its antagonistic activity toward the widespread necrotrophic fungus *Botrytis cinerea.* The dual culture assay revealed that the *Pseudomonas* sp. R4-79 strain effectively inhibits the growth of *Botrytis cinerea*. A clear zone of inhibition was observed around the site of bacterial inoculation, which demonstrates its ability to limit fungal proliferation. Within this inhibition zone, fungal growth exhibited a marked reduction or was entirely suppressed, whereas colonies situated beyond this zone displayed normal growth patterns (Fig. 8). These observations confirm that *P. granadensis* R4-79 effectively limits *B. cinerea* proliferation in vitro.

Discussion

Dual capability of Pseudomonas granadensis in growth promotion and disease suppression

The application of beneficial microbes from arid environments, such as the Arabian desert, can significantly enhance sustainable farming practices and ecological restoration in water-limited regions. *Pseudomonas* sp. R4-79 demonstrated remarkable effectiveness in enhancing plant growth and pathogen suppression. Here, we provide a detailed analysis of the strain's phenotypic and genomic features along with its potential effect of suppressing plant diseases. The phylogenetic analysis showed that the genome of the *Pseudomonas* sp. R4-79 strain is very similar to that of *Pseudomonas granadensis* strain LMG 27940, which was previously isolated from soil (Cea-Torrescassana et al., 2024; Pascual et al., 2015). This study identifies *Pseudomonas* sp. R4-79 as the third strain reported under the *P. granadensis* species and the first to be isolated from an arid environment, specifically in Wadi Rum, Jordan. In addition to *Pseudomonas granadensis* strain LMG 27940, strain CT364—phylogenetically clustered in the same clade as LMG 27940 and R4-79 based on 16S rRNA analysis—exhibited notable plant growth-promoting traits (Supplementary Fig. 1). Specifically, it enhanced rooting in olive semi-hardwood cuttings more effectively than the synthetic auxin indole-3-butyric acid (IBA) (Cea-Torrescassana et al., 2024). CT364 is also a phosphorus-solubilizing and siderophore-producing. All these parameters led to a greater root length of canola or mung bean seeds and mung bean cuttings, respectively, in gnotobiotic conditions. Such observations delineate the plant growth-promoting activities of *P. granadensis* (Cea-Torrescassana et al., 2024). In this context, *Pseudomonas* sp. R4-79 exhibited comparable plant growth-promoting activity as demonstrated by the larger fresh weight and shoot length of tomato plants colonized by this strain as compared to non-colonized tomato plants. Furthermore, this study provides the first evidence of a *P. granadensis* strain with the ability to suppress phytopathogens.

Targeting in particular, *Pseudomonas* sp. R4-79 inhibited root-knot nematode *M. incognita* in tomato and yielded the same inhibitory result as *P. fluorescens* SBW25. In addition to its nematode-suppressive properties, *Pseudomonas* sp. R4-79 demonstrated robust antagonistic activity against the bacterial pathogen *P. syringae* pv. tomato DC3000 (*P*st) in Arabidopsis. The colonization of the *Pseudomonas* sp. R4-79 strain conferred plant survival under pathogen attack. The strain suppressed *P*st across varying concentrations, showing consistent efficacy in reducing disease severity. In addition, *Pseudomonas* sp. R4-79 also exhibited antifungal activity against *B. cinerea*, a fungal pathogen, in dual culture assays. These results suggest that *Pseudomonas* sp. R4-79 might represent a plant-growth-promoting and biocontrol option to control a broad range of phytopathogens attacking crops grown in arid environments and farm fields.

Genetic features related to host-microbe interactions and pathogen suppression

Exploring the genetic features of beneficial bacteria is essential for understanding their role in plant-microbe interactions. KEGG-based functional annotation of the *Pseudomonas* sp. R4-79 genome revealed key traits linked to plant growth promotion, stress tolerance, and pathogen suppression. For instance, Bacterial membrane transporters, such as ATP-binding cassette (ABC) transporters, phosphotransferase (PTS) systems, and bacterial secretion systems, mediate cellular homeostasis and host-microbe interactions. *Pseudomonas* sp. R4-79 strain uses these systems for the acquisition of nutrients, ion transport, and secretion of molecules relevant to its ecological interactions. For instance, in *P. fluorescens* Pf-5, ABC transporters are implicated in the secretion of antimicrobial metabolites, such as Pyoluteorin, which exhibit biocontrol activity against soilborne pathogens and improve rhizosphere colonization. In particular, pltI and pltJ genes that promote pyoluteorin export are both necessary for pyoluteorin production and efficient secretion (Brodhagen et al., 2005). Moreover, the *Pseudomonas* sp. R4-79 possesses a PTS, which serves as a sugar transport system that uptake and phosphorylation of sugars, such as glucose and fructose, maintains bacterial viability by tuning the fluxes of carbons. In *P. putida*, the system not only enables metabolic plasticity but also plays important roles in both bioremediation and metabolic engineering (Kremling et al., 2012). On the other hand, the KEGG analysis by BlastKOALA showed that *Pseudomonas* sp. R4-79 possess various secretion systems, including Type II (T2SS), Type IV (T4SS), and Type VI (T6SS), and arginine targeting (TAT). Such secretion systems allow the translocation of proteins, toxins, DNA, and small molecules. In *Dyella japonica*, T2SS was crucial in suppressing MAMP-triggered immunity (MTI) in plants, thus promoting root colonization. For example, mutations in key T2SS components, GspD and GspE, in *Dyella japonica* MF79 led to the loss of the ability to inhibit MTI (Teixeira et al., 2021). On the other hand, T4SS is involved in DNA conjugation systems and the secretion of protein complexes that help in bacterial competition and host manipulation. For example, T4SS in *P. putida* IsoF protects tomato plants against *Ralstonia solanacearum* by competitive exclusion and by disruption of biofilm (Purtschert-Montenegro et al., 2022). T6SS injects effector proteins into both prokaryotic and eukaryotic cells and is involved in competition and pathogen suppression processes. For instance, in *P. putida* KT2440, the isogenic triple mutant (ΔT6SS) inhibited

its ability to suppress the pathogenic bacteria *Xanthomonas campestris* compared to the *P. putida* KT2440 wild-type (Bernal et al., 2017; Bernal et al., 2018). Along with that, the *Pseudomonas* sp. R4-79 strains are producing secondary metabolites, such as hydrogen cyanide (HCN), that can provide various advantages in plant-microbe interactions, especially in the suppression of a broad spectrum of phytopathogens (Sehrawat et al., 2022). For example, HCN produced by *Pseudomonas chlororaphis* O6 has been shown to have suppressive activity against a root-knot nematode, *Meloidogyne hapla* (Lee et al., 2011). Further, HCN has also demonstrated the ability to suppress mycelial growth of *Phytophthora infestans* fungal pathogen with inhibition rates of 57–80% (Anand et al., 2020). Another secondary metabolite that *Pseudomonas* sp. R4-79 strain showed capability to produce is phenazine. *Pseudomonas sp.* LBUM223 produces phenazine-1-carboxylic acid (PCA), which plays a significant role in controlling potato common scab caused by *Streptomyces scabies* (Arseneault et al., 2013).

Conclusion

Pseudomonas granadensis R4-79 has high activity not only as a plant growth-promoting bacterium (PGPB) but also as a biocontrol agent. Its varied genetic makeup, such as membrane transporters, secretion systems, and secondary metabolites production, is attributed to its ability to promote plant growth and inhibit phytopathogens. Isolated from an arid area, the strain's robustness in an extreme environment sheds light on its significance for use in farming in water-deficit areas. Genome functional analysis also shows advantageous properties, including biosynthetic gene clusters of siderophores, antimicrobial substances, and phytohormones, making *P. granadensis* R4-79 a valuable potential agent for increasing plant immunity and ecological restoration in dry ecosystems. Future studies should address its effect on abiotic stresses and optimize its application in field conditions to fully harness its capabilities.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Funding

The work was supported by grant BAS/1/1062-01-01 from the University of Science and Technology to HH.

Availability of data and materials

The WGS has been submitted to NCBI under Bioproject-PRJNA708169, Biosample-SAMN18220753, Genome accession-CP071650-CP071651.

Author contributions

H.H. and A.E. conceptualized the project. L.A. extracted the R4-79 genomic DNA and carried out all plant- and pathogen-related work. S.P. annotated, did the taxonomy and functional analysis of the R4-79 genome. L.A., A.E. and H.H. wrote the paper and all authors reviewed and corrected the final version.

Acknowledgements

The work was supported by KAUST grant BAS/01/1062-01-01 to HH. We are also grateful to the Hirt lab members for technical assistance and fruitful discussions.

References

1. Acuña, J. J., Campos, M., Mora, M. d. l. L., Jaisi, D. P., & Jorquera, M. A. (2019, 2019/04/01/). ACCD-producing rhizobacteria from an Andean Altiplano native plant (Parastrephia quadrangularis) and their potential to alleviate salt stress in wheat seedlings. *Applied Soil Ecology, 136*, 184-190. https://doi.org/https://doi.org/10.1016/j.apsoil.2019.01.005
2. Alsharif, W., Saad, M. M., & Hirt, H. (2020, 2020-July-22). Desert Microbes for Boosting Sustainable Agriculture in Extreme Environments [Review]. *Frontiers in Microbiology, 11*. https://doi.org/10.3389/fmicb.2020.01666
3. Alwutayd, K. M., Rawat, A. A., Sheikh, A. H., Almeida-Trapp, M., Veluchamy, A., Jalal, R., Karampelias, M., Froehlich, K., Alzaed, W., Tabassum, N., Schley, T. R., Schäffner, A. R., Daur, I., Saad, M. M., & Hirt, H. (2023). Microbe‐induced drought tolerance by ABA‐mediated root architecture and epigenetic reprogramming. *EMBO reports, 24*(8), e56754. https://doi.org/https://doi.org/10.15252/embr.202256754
4. Anand, A., Chinchilla, D., Tan, C., Mène-Saffrané, L., L'Haridon, F., & Weisskopf, L. (2020, Jul 28). Contribution of Hydrogen Cyanide to the Antagonistic Activity of Pseudomonas Strains Against Phytophthora infestans. *Microorganisms, 8*(8). https://doi.org/10.3390/microorganisms8081144
5. Ardley, J. (2017, 2017/01/01). Legumes of the Thar desert and their nitrogen fixing Ensifer symbionts. *Plant and Soil, 410*(1), 517-520. https://doi.org/10.1007/s11104-016-3125-5
6. Arseneault, T., Goyer, C., & Filion, M. (2013). Phenazine Production by Pseudomonas sp. LBUM223 Contributes to the Biological Control of Potato Common Scab. *Phytopathology®, 103*(10), 995-1000. https://doi.org/10.1094/phyto-01-13-0022-r
7. Bernal, P., Allsopp, L. P., Filloux, A., & Llamas, M. A. (2017). The Pseudomonas putida T6SS is a plant warden against phytopathogens. *The ISME Journal, 11*(4), 972-987. https://doi.org/10.1038/ismej.2016.169

8. Bernal, P., Llamas, M. A., & Filloux, A. (2018, Jan). Type VI secretion systems in plant-associated bacteria. *Environ Microbiol, 20*(1), 1-15. https://doi.org/10.1111/1462-2920.13956
9. Brodhagen, M., Paulsen, I., & Loper, J. E. (2005, Nov). Reciprocal regulation of pyoluteorin production with membrane transporter gene expression in Pseudomonas fluorescens Pf-5. *Appl Environ Microbiol, 71*(11), 6900-6909. https://doi.org/10.1128/aem.71.11.6900-6909.2005
10. Cea-Torrescassana, E., Howard Thomas, P., Stach, J., & Montero-Calasanz Maria del, C. (2024). Whole-genome sequence of the plant-associated bacterium Pseudomonas granadensis CT364 isolated in Seville, Spain. *Microbiology Resource Announcements, 13*(6), e00735-00723. https://doi.org/10.1128/mra.00735-23
11. Coleine, C., Delgado-Baquerizo, M., DiRuggiero, J., Guirado, E., Harfouche, A. L., Perez-Fernandez, C., Singh, B. K., Selbmann, L., & Egidi, E. (2024). Dryland microbiomes reveal community adaptations to desertification and climate change. *The ISME Journal, 18*(1). https://doi.org/10.1093/ismejo/wrae056
12. Elhady, A., Alghanmi, L., Abd-Elgawad, M. M. M., Heuer, H., Saad, M. M., & Hirt, H. (2024, 2024-July-22). Plant-parasitic nematode research in the arid desert landscape: a systematic review of challenges and bridging interventions [Systematic Review]. *Frontiers in Plant Science, 15*. https://doi.org/10.3389/fpls.2024.1432311
13. Islam, W., Zeng, F., Alotaibi, M. O., & Khan, K. A. (2024, 2024/05/01/). Unlocking the potential of soil microbes for sustainable desertification management. *Earth-Science Reviews, 252*, 104738. https://doi.org/https://doi.org/10.1016/j.earscirev.2024.104738
14. Jat, R. A., Wani, S. P., & Sahrawat, K. L. (2012). Chapter Four - Conservation Agriculture in the Semi-Arid Tropics: Prospects and Problems. In D. L. Sparks (Ed.), *Advances in agronomy* (Vol. 117, pp. 191-273). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-394278-4.00004-0
15. Karmegham, N., Vellasamy, S., Natesan, B., Sharma, M. P., Al Farraj, D. A., & Elshikh, M. S. (2020, Dec). Characterization of antifungal metabolite phenazine from rice rhizosphere fluorescent pseudomonads (FPs) and their effect on sheath blight of rice. *Saudi J Biol Sci, 27*(12), 3313-3326. https://doi.org/10.1016/j.sjbs.2020.10.007
16. Kremling, A., Pflüger-Grau, K., Chavarría, M., Puchalka, J., dos Santos, V., & Lorenzo, V. (2012, 2012/12/06). Modeling and analysis of flux distributions in the two branches of the phosphotransferase system in Pseudomonas putida. *BMC Systems Biology, 6*(1), 149. https://doi.org/10.1186/1752-0509-6-149
17. Lee, J. H., Ma, K. C., Ko, S. J., Kang, B. R., Kim, I. S., & Kim, Y. C. (2011, 2011/03/01). Nematicidal Activity of a Nonpathogenic Biocontrol Bacterium, Pseudomonas chlororaphis O6. *Current Microbiology, 62*(3), 746-751. https://doi.org/10.1007/s00284-010-9779-y
18. Masmoudi, F., Al Naimi, L., Trigui, M., Al Safran, M., Tounsi, S., & Saadaoui, I. (2024, 2024/08/25). Novel Thermo-Halotolerant Bacteria Bacillus cabrialesii Native to Qatar Desert: Enhancing Seedlings' Growth, Halotolerance, and Antifungal Defense in Tomato. *Journal of Plant Growth Regulation*. https://doi.org/10.1007/s00344-024-11460-2

19. Panth, M., Hassler, S. C., & Baysal-Gurel, F. (2020). Methods for Management of Soilborne Diseases in Crop Production. *Agriculture, 10*(1), 16. https://www.mdpi.com/2077-0472/10/1/16
20. Pascual, J., García-López, M., Bills, G. F., & Genilloud, O. (2015, Feb). Pseudomonas granadensis sp. nov., a new bacterial species isolated from the Tejeda, Almijara and Alhama Natural Park, Granada, Spain. *Int J Syst Evol Microbiol, 65*(Pt 2), 625-632. https://doi.org/10.1099/ijs.0.069260-0
21. Purtschert-Montenegro, G., Cárcamo-Oyarce, G., Pinto-Carbó, M., Agnoli, K., Bailly, A., & Eberl, L. (2022, 2022/10/01). Pseudomonas putida mediates bacterial killing, biofilm invasion and biocontrol with a type IVB secretion system. *Nature Microbiology, 7*(10), 1547-1557. https://doi.org/10.1038/s41564-022-01209-6
22. Rastgoo, M., & Hasanfard, A. (2021). Desertification in Agricultural Lands: Approaches to Mitigation. In. https://doi.org/10.5772/intechopen.98795
23. Santander, C., García, S., Moreira, J., Aponte, H., Araneda, P., Olave, J., Vidal, G., & Cornejo, P. (2021, 2021/06/01/). Arbuscular mycorrhizal fungal abundance in elevation belts of the hyperarid Atacama Desert. *Fungal Ecology, 51*, 101060. https://doi.org/https://doi.org/10.1016/j.funeco.2021.101060
24. Sehrawat, A., Sindhu, S. S., & Glick, B. R. (2022, 2022/02/01/). Hydrogen cyanide production by soil bacteria: Biological control of pests and promotion of plant growth in sustainable agriculture. *Pedosphere, 32*(1), 15-38. https://doi.org/https://doi.org/10.1016/S1002-0160(21)60058-9
25. Singh, R. P., & Jha, P. N. (2016). The Multifarious PGPR Serratia marcescens CDP-13 Augments Induced Systemic Resistance and Enhanced Salinity Tolerance of Wheat (Triticum aestivum L.). *PLOS ONE, 11*(6), e0155026. https://doi.org/10.1371/journal.pone.0155026
26. Sulaiman, M. A., & Bello, S. K. (2024, 2024/04/01). Biological control of soil-borne pathogens in arid lands: a review. *Journal of Plant Diseases and Protection, 131*(2), 293-313. https://doi.org/10.1007/s41348-023-00824-7
27. Teixeira, P. J. P. L., Colaianni, N. R., Law, T. F., Conway, J. M., Gilbert, S., Li, H., Salas-González, I., Panda, D., Del Risco, N. M., Finkel, O. M., Castrillo, G., Mieczkowski, P., Jones, C. D., & Dangl, J. L. (2021). Specific modulation of the root immune system by a community of commensal bacteria. *Proceedings of the National Academy of Sciences, 118*(16), e2100678118. https://doi.org/doi:10.1073/pnas.2100678118

Figures

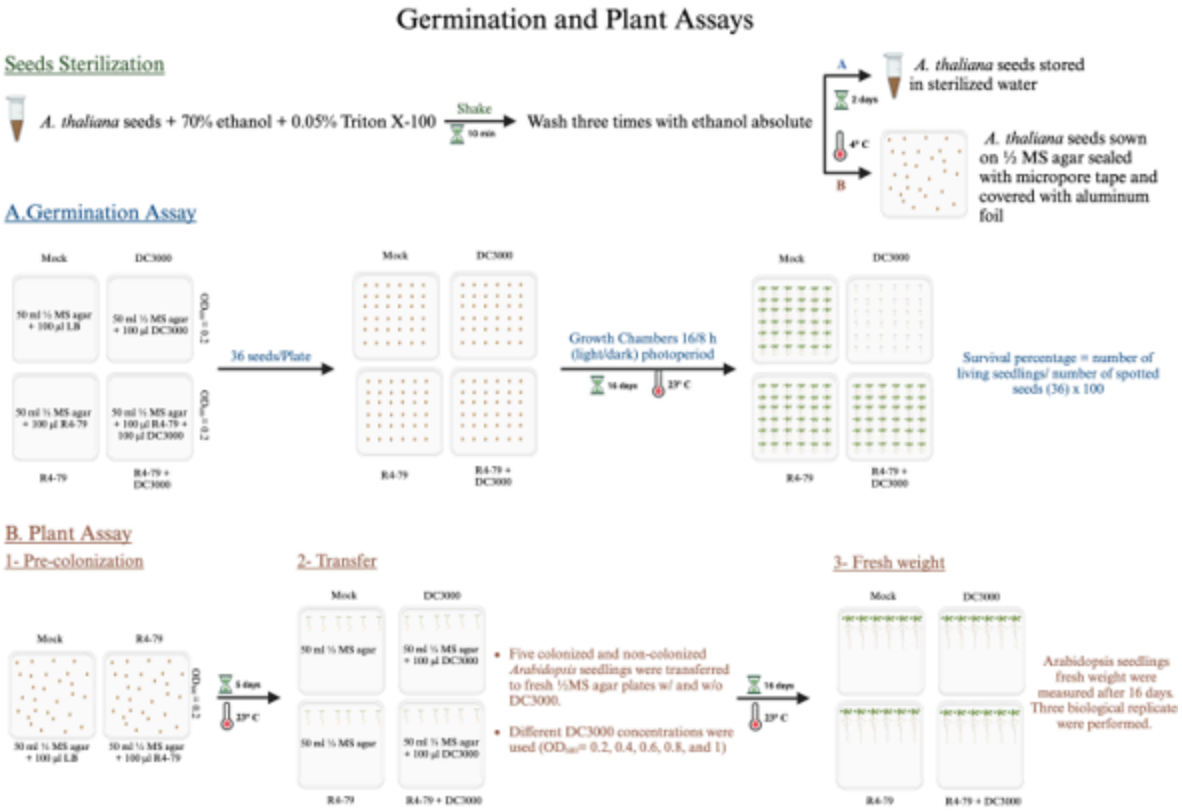

Figure 1

Schematic representation of germination and plant growth assays using *Arabidopsis thaliana* in response to bacterial treatments. A) Germination Assay: seeds were sown on ½ MS agar with and without *Pseudomonas* sp. R4-79 strain under DC3000 stress. Survival rates were quantified. B) Plant Assay: 5-day-old pre-colonized seeds with *Pseudomonas* sp. R4-79 strain were transferred to ½ MS agar mixed with DC3000. After 16 days, the fresh weight was measured.

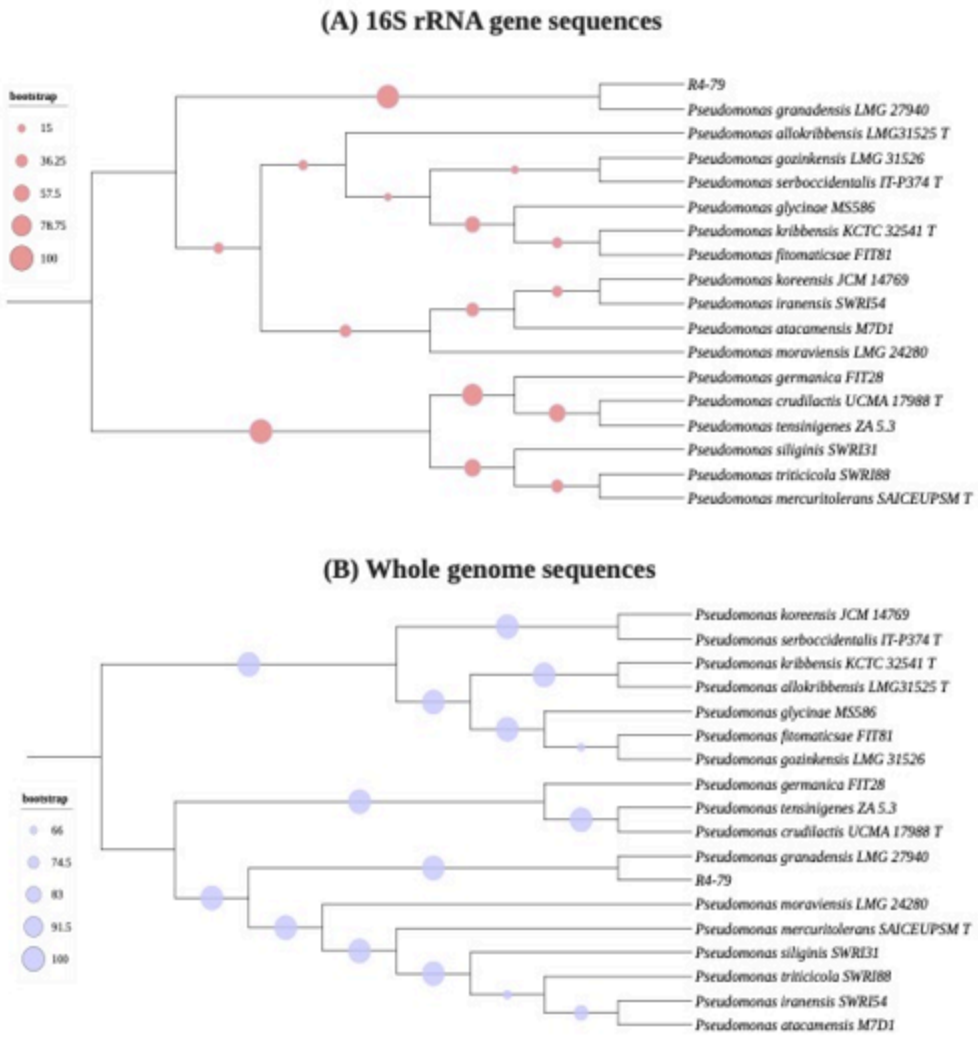

Figure 2

Phylogenetic tree shows that strain *Pseudomonas* sp. R4-79 forms a monophyletic group with *Pseudomonas granadensis* LMG 27940 based on A) partial 16S rRNA gene sequences and B) whole-genome sequence comparison. The scale bars represent the number of substitutions per site. Gradient blue and red squares denote GC% and Δ statistics and illustrate genomic and compositional variations between the *Pseudomonas* sp. R4-79 strain and other *Pseudomonas*species within the phylogenetic tree.

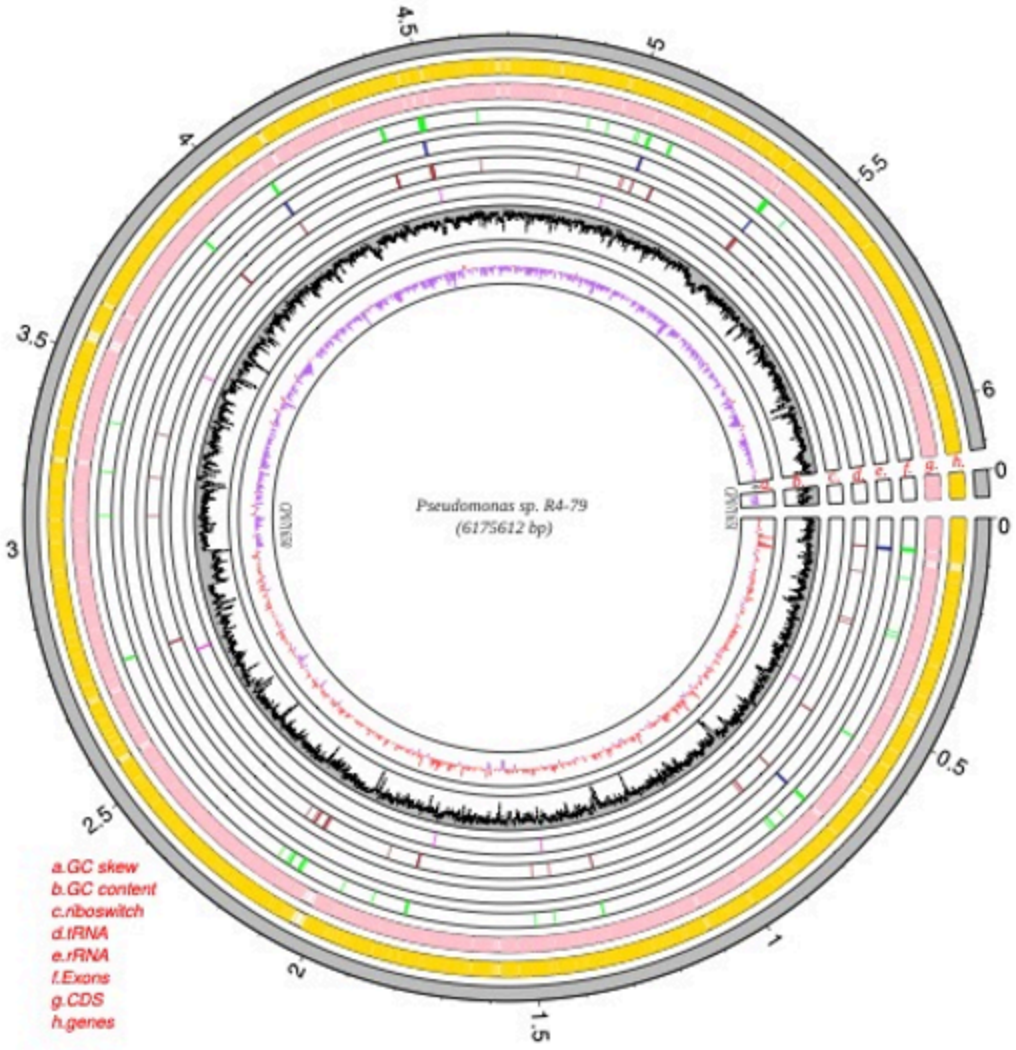

Figure 3

Circular genome map of the Pseudomonas sp. R4-79 chromosome. The tracks from inside to outside: GC skew [(G−C/ (G+C)] positive (red) and negative (purple), % GC content (black), Arrows on the GC skews indicate the origin of replication (oriC) and replication termination (terC) regions where the shifts occur.

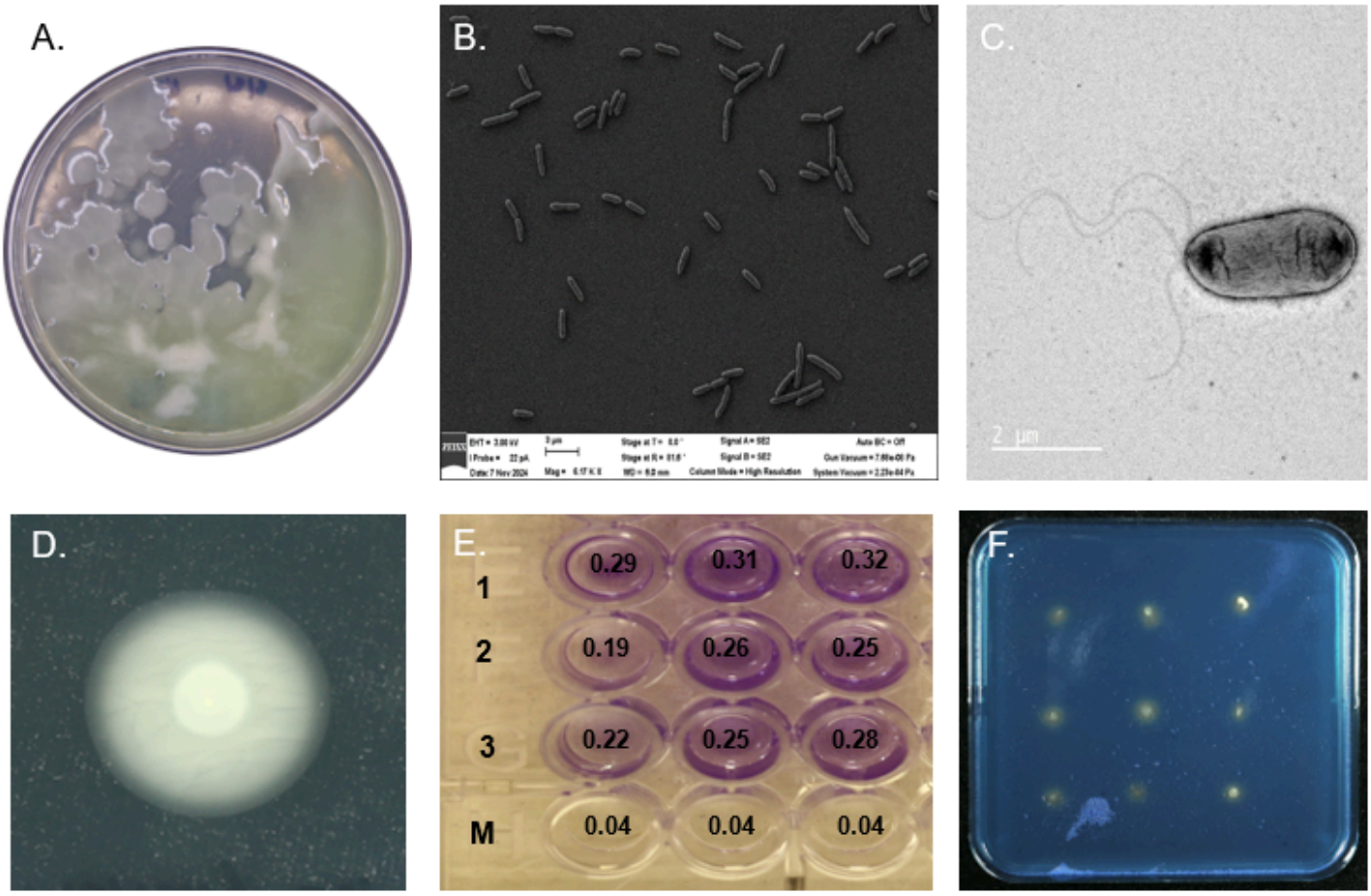

Figure 4

Morphological features of Pseudomonas granadensis sp. R4-79. A) Colony formation of the Pseudomonas sp. R4-79 strain on a Petri dish, with slimy colonies on King's B medium. B) Scanning Electron Microscope (SEM) and C) Transmission Electron Microscope (TEM) shows rod-shaped bacterium equipped with lophotrichous flagella. D) The Pseudomonas sp. R4-79 strain motility on motility test agar media. E) Biofilm formation of Pseudomonas sp. R4-79 strain with absorbance quantification biofilms using a plate reader at 630 nm. 1,2 and 3 are Pseudomonas sp. R4-79 strain replicates, and M is the mock. F) Siderophore production test of Pseudomonas sp. R4-79 strain on blue agar CAS assay.

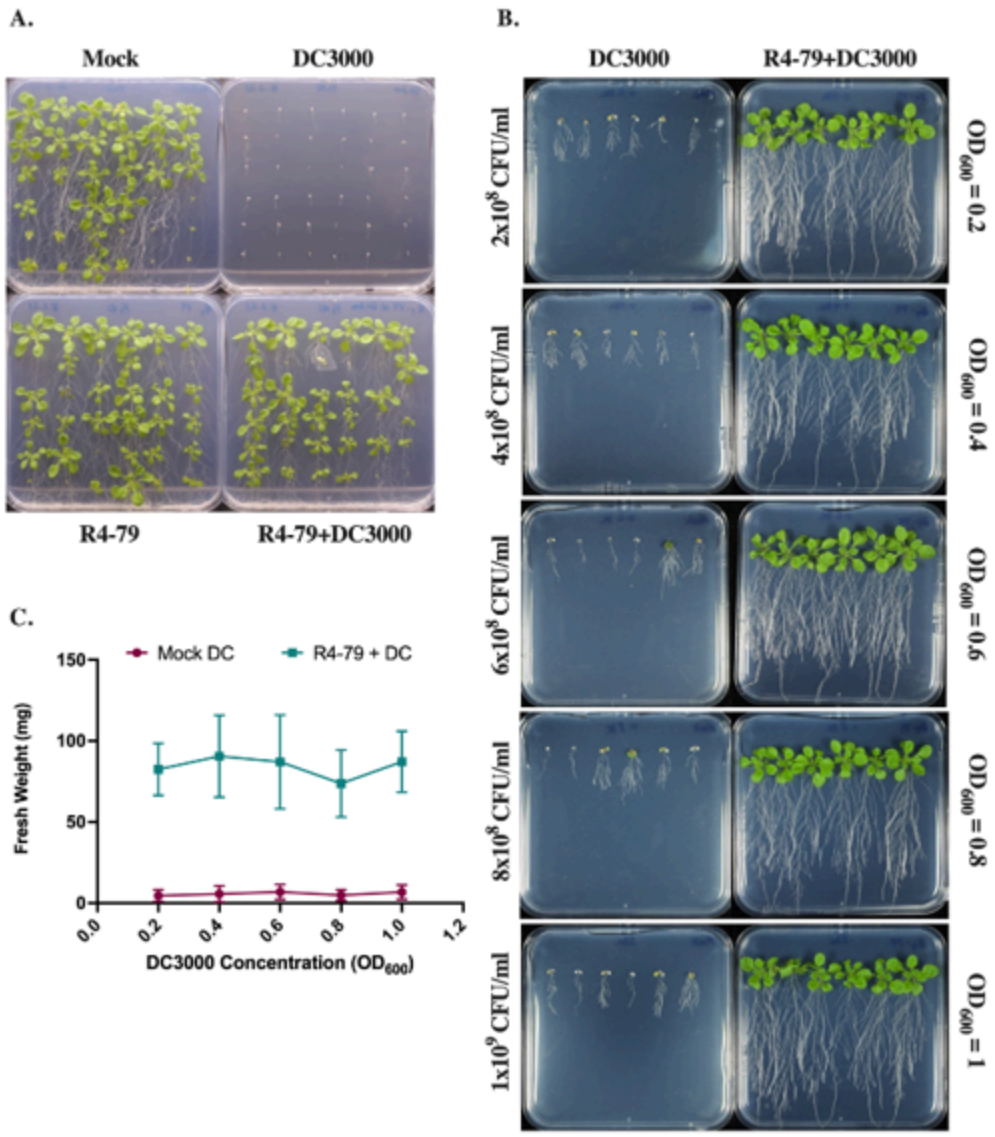

Figure 5

Effect of *Pseudomonas*sp. R4-79 on the pathogenicity of *Pseudomonas syringae* pv. tomato DC3000 (*P*st) in *Arabidopsis thaliana*. A) Survival rates of *A. thaliana* plants infected with Pst DC3000 after pre-treatment with *Pseudomonas*sp. R4-79. Controls include mock plants, plants infected with *P*st DC3000 without *Pseudomonas* sp. R4-79 pre-treatment, and plants pre-colonized with *Pseudomonas* sp. R4-79 alone. (B-C) Effect of varying concentrations of Pst DC3000 (2×10^8 to 1×10^9 CFU) on the fresh weight of *A. thaliana* plants pre-treated with *Pseudomonas*sp. R4-79 compared to untreated controls (n = 18). Error bars indicate standard deviation.

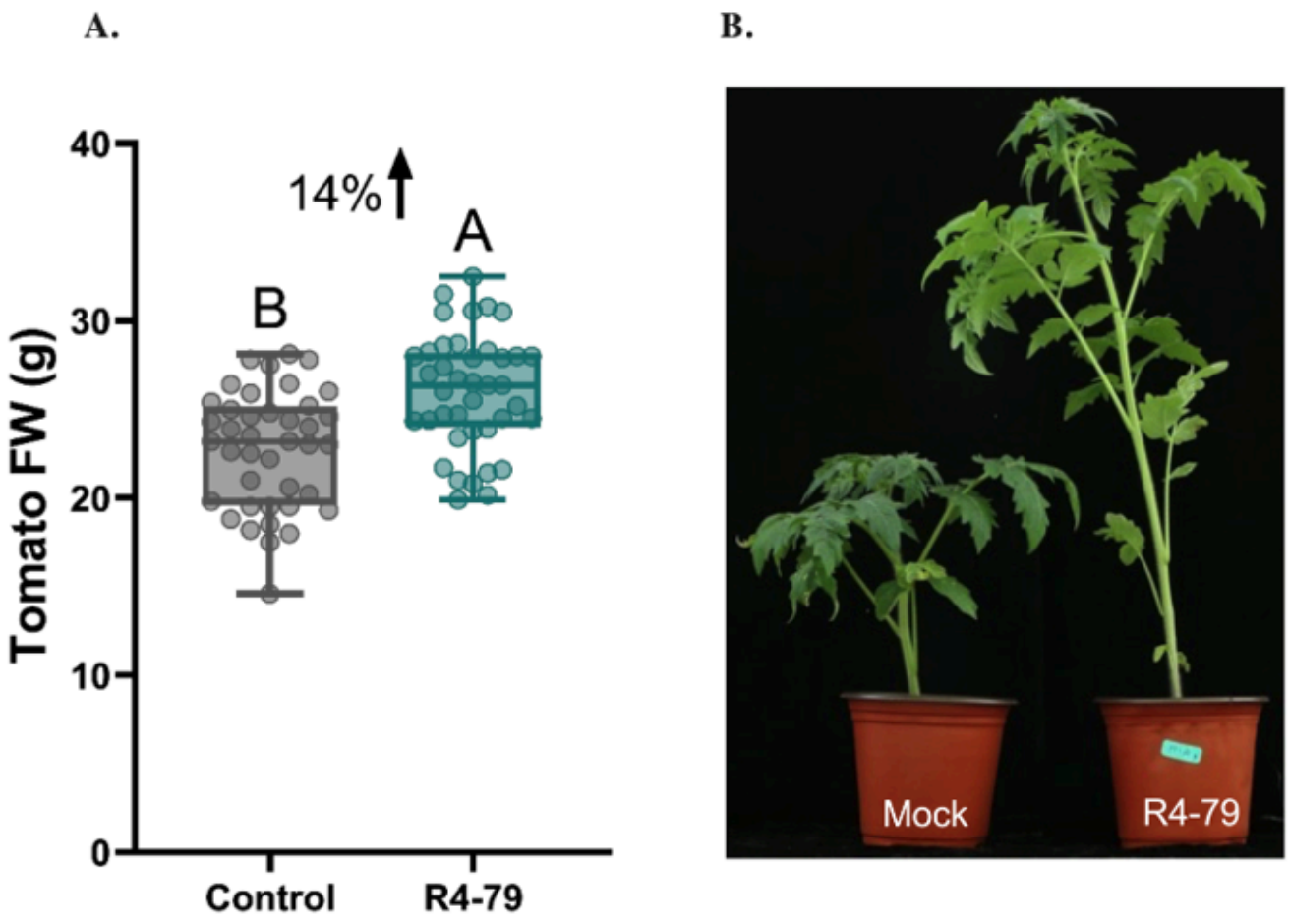

Figure 6

Tomato growth promotion by *Pseudomonas* sp. R4-79. A) Boxplot illustrates the effect of the *Pseudomonas*sp. R4-79 strain on tomato fresh weight in comparison to untreated control plants. B) A picture of tomato plants treated with the *Pseudomonas* sp. R4-79 strain versus untreated plants. The experiment was conducted in a greenhouse under a randomized complete block design (RCBD) with 40 replicates.

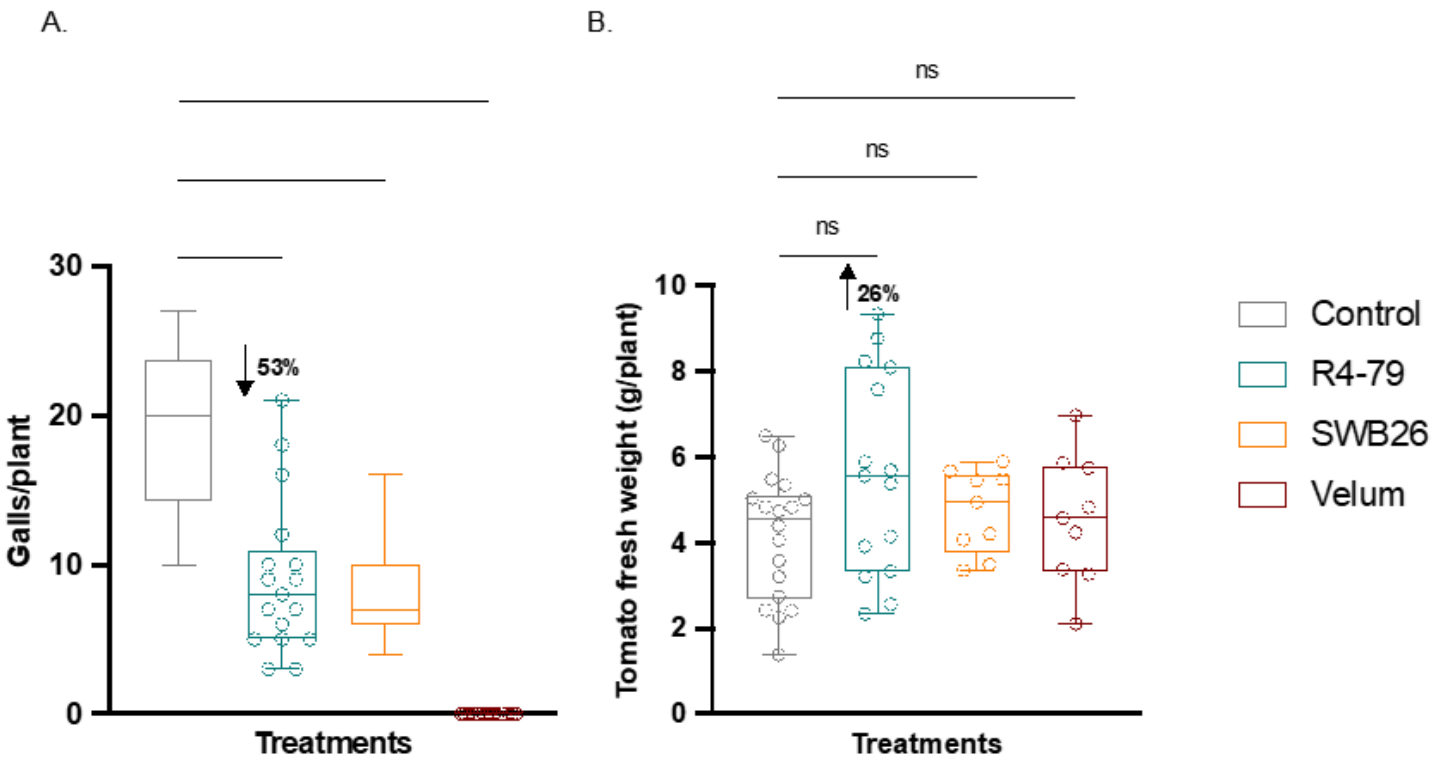

Figure 7

Effect of *Pseudomonas* sp. R4-79 on tomato growth and infection by the root-knot nematode (*Meloidogyne incognita*). (A) Plant growth effect of *Pseudomonas* sp. R4-79 strain on infected plants. (B) Suppressive effect of *Pseudomonas* sp. R4-79 strain against *M. incognita* on tomato plants. Boxplots show root gall counts and plant biomass across treatments (n = 21–16). Tomato seedlings were grown in sand with Osmocote and treated with *Pseudomonas* sp. R4-79 strain (OD_{600} = 0.2), *Pseudomonas fluorescens* SBW25, Velum® Prime, or left untreated. Plants were inoculated with 300 nematode juveniles after 5 days, and data were collected 3 weeks later. Each boxplot displays the median, interquartile range, and data points.

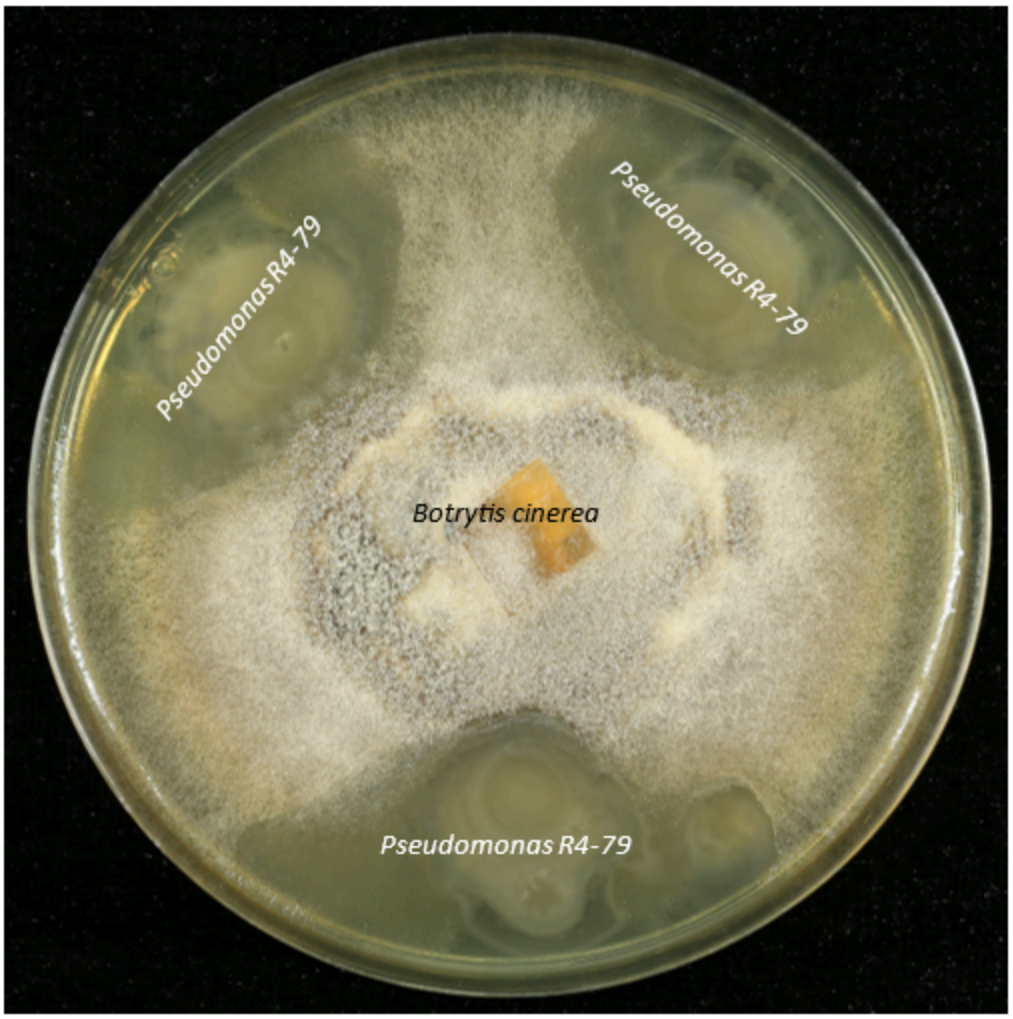

Figure 8

Antifungal activity of *Pseudomonas* sp. R4-79 against *Botrytis cinerea* on Potato Dextrose Agar medium (PDA). A dual-culture assay was carried out to observe the antifungal activity of the *Pseudomonas* sp. R4-79 strain against *B. cinerea*. The assay was replicated three times (n=3).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementary.pptx