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Scientific Article

In vitro evaluation of the antagonistic potential of *Bacillus zhangzhouensis* JJR198 against *Phytophthora capsici*

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ABSTRACT

Background/Objective. *Phytophthora capsici* represents one of the most significant threats to pepper production worldwide, and its control relies predominantly on fungicide applications, which entail considerable environmental concerns. Therefore, exploring biological control strategies has become a relevant alternative. This study aimed to assess the potential of a bacterial isolate as a biocontrol agent against six *P. capsici* and one *P. cinnamomi* isolates.

Materials and Methods. Biocontrol effects were assessed *in vitro* using dual-culture assays between the bacterial isolate JJR198 and *Phytophthora* isolates to evaluate inhibition of mycelial growth, sporangia formation, and zoospore release, with additional observations by scanning electron microscopy. The efficacy of JJR198 in suppressing Phytophthora root rot was evaluated in pepper plants under greenhouse conditions by measuring disease severity in inoculated plants. In addition, salt tolerance and persistence of the bacterium in the rhizosphere and rhizoplane were determined. All data were subjected to ANOVA followed by Tukey's test ($p \leq 0.05$).

Results. Identified as *Bacillus zhangzhouensis* through 16S rRNA sequencing, the bacterial isolate JJR198, exhibited antagonistic activity. *In vitro* assays showed that strain JJR198 reduced mycelial growth by 42.0 to 52.7% across six tested *Phytophthora* isolates. The biocontrol activity depended on the initial bacterial concentration, as sporangia formation and zoospore release in the tested *Phytophthora capsici* isolates were completely suppressed at 1.0×10^8 CFU mL^{-1}, whereas no effect was observed at lower concentrations (1.0×10^4 and 1.0×10^6 CFU mL^{-1}). Using optical and electron microscopy, it was observed that the hyphae and mycelia were covered by bacterial cells. However, in the *in vivo* evaluation, pretreatment of pepper plants with JJR198 (1.0×10^8 CFU mL^{-1}) at 7, 3, or 0 days before oomycete inoculation did not reduce disease severity caused by *Phytophthora capsici*. JJR198 also showed the ability to grow under high-salinity conditions, as it developed on NA medium containing 7.5% NaCl after 48 h of incubation at 30 °C. Additionally, the presence of the bacterial inoculum was confirmed in pepper roots: colonies with morphological characteristics identical to JJR198 were recovered from rhizosphere and rhizoplane samples, with counts of 1.6×10^6 and 0.19×10^6 CFU mL^{-1}, respectively.

Conclusion. The results indicate that *Bacillus zhangzhouensis* JJR198 exhibits *in vitro* antagonistic activity against multiple *Phytophthora* isolates, achieving mycelial growth inhibition ranging from 42.0 to 52.7%, as well as inhibition of sporangia formation and zoospore release at a concentration of 1.0×10^8 CFU mL^{-1}. However, its effectiveness *in planta* remained limited under the conditions tested.

Keywords: Biological control, Pepper plant, Plant–pathogen interaction, Soil bacterium, Zoospore inhibition

INTRODUCTION

Chili peppers (*Capsicum* spp.) are among the most important crops worldwide, and in Mexico, where in 2024 production reached 3.2 million tons of green chili peppers, ranking fourth in global production and representing the second highest production value nationwide (FAO, 2023; SIAP, 2024). Phytophthora root rot, caused by the oomycete *Phytophthora capsici*, is the main limiting factor in pepper production, causing severe economic losses (Silva-Rojas *et al*., 2009). Its presence has reduced the cultivated area in important regions and displaced susceptible crops. This pathogen causes damping-off, wilting, leaf blight and the rotting of roots, stems, and fruits. The control methods for *P. capsici* are based on integrated management practices, including cultural practices, fungicide applications, and the cultivation of resistant varieties. However, fungicide use remains predominant, leading to considerable environmental and human health concerns and contributing to pathogen resistance to fungicides (Hausbeck and Lamour, 2004; Sánchez-Gurrola *et al*., 2019). Together, the high impact of the disease on pepper production and the limitations and risks associated with current control practices highlight the need for alternative and more sustainable management strategies.

Mexico is the center of origin and domestication of *Capsicum annuum*, the most economically important of all five domesticated species of the genus *Capsicum* (*C. annuum, C. chinense, C. frutescens, C. baccatum* and *C. pubescens*). A large diversity of peppers is cultivated across Mexico; therefore, the country harbors a high genetic diversity of *P. capsici*, which is associated with its high host-pathogen coevolutionary rate, rapid spread, and adaptability to new hosts and environments. These factors make this pathogen economically significant and a potential global threat to food security (Retes-Manjarrez *et al*., 2020; Reyes-Tena *et al*., 2020; Sánchez-Gurrola *et al*., 2019). The sporangia and zoospores of *P. capsici* provide high reproductive potential under wet conditions, facilitating rapid spread via water, root penetration, and successive infection cycles within a single growing season (Granke *et al*., 2012). Thus, it is essential to explore alternative approaches for the control of Phytophthora blight and root rot, such as biological control.

Various bacterial genera have been assessed *in vitro* and *in vivo* as antagonists of *P. capsici*, demonstrating promising potential (Akgül and Mirik, 2008; Lee *et al*., 2008; Ley-López *et al*., 2018; Xu *et al*., 2020). However, due to the high aggressiveness of this pathogen and the variability in reported outcomes, it is important to continue screening for new antagonists and to further assess their interactions (Lee *et al*., 2013; Xu and Kim, 2016).

The genus *Bacillus* comprises ubiquitous bacteria inhabiting diverse environments, including soil, water, air, the rhizosphere, and many extreme environments. This genus is among the most commonly used bacterial groups for the biological control of phytopathogenic fungi and oomycetes, such as *P. capsici*, mainly due to its ability to produce a wide array of secondary metabolites with antimicrobial activity and plant growth-promoting properties (Ferreira *et al*., 1991; Fira *et al*., 2018; Zhang *et al*., 2010).

First described in 2016 after being isolated from shrimp farm water in Zhangzhou, China (Ruginescu *et al*., 2018), *Bacillus zhangzhouensis* has attracted increasing attention in recent years as a promising halophyte plant growth-promoting bacterium (Alp *et al*., 2024; Bezerra *et al*., 2022; Emami *et al*., 2019). Regarding biological control traits, several studies have reported the antimicrobial potential of *B. zhangzhouensis* strains against animal, human, and plant pathogens, underscoring their broad-spectrum antagonistic activity (Anil *et al*., 2019; Aytenov *et al*., 2024; Molina-Garza *et al*., 2021; Soliman *et al*.,

2022). However, to date, no studies have evaluated the biocontrol performance of *Bacillus zhangzhouensis* against Mexican isolates of *P. capsici*, nor its effectiveness in pepper plants, which is particularly relevant given the high variability of this pathogen. The objective of this study was to evaluate the biocontrol capacity of a bacterial isolate as a biocontrol agent against six *P. capsici* and one *P. cinnamomi* isolates. Biocontrol effects were assessed based on mycelial growth, sporangia and zoospore release, light and electron microscopy, as well as severity of Phytophthora root rot in pepper plants.

MATERIALS AND METHODS

Microbial culture conditions. Bacterial isolate JJR198 was provided by the Laboratory of Bioremediation, Universidad Autónoma de Aguascalientes. The isolate was cultured by streaking on LB agar plates and incubated for 24–48 h at 30 °C. A single colony was transferred to liquid LB medium and grown at 30 °C for 24 h on a rotary shaker at 160 rpm. After incubation, the bacterial suspension was adjusted with sterile distilled water (SDW) to final concentrations of 1.0×10^4, 1.0×10^6, and 1.0×10^8 CFU mL^{-1} for subsequent evaluations, with the highest concentration corresponding to an optical density of 0.1 at 600 nm (Kim *et al*., 2009; Xu *et al*., 2020).

Six isolates of *Phytophthora capsici* (Pc1, Pc2aIT, Pc43M, PcAR-2, Pc57, and PcAR-14) and one isolate of *P. cinnamomi* (Pcn5-1) were kindly provided by the Plant Pathology Laboratory, Instituto de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolás de Hidalgo. Information on host, mating type, and geographic origin was available for *P. capsici* isolates Pc2aIT, Pc43M, PcAR-2, Pc57, PcAR-14 and Pcn5-1. Specifically, PcAR-2 was isolated from poblano pepper mating type (MT) A1 in Copándaro de Galeana, Michoacán, Mexico; PcAR-14 from serrano pepper (MT A1) in Cuitzillo el Grande, Tarímbaro, Michoacán, Mexico; Pc57 from pepper (MT A2) in Guanajuato, Mexico; Pc43M from pepper in Guanajuato, Mexico; and Pc2aIT from poblano pepper (MT A2) in Yurécuaro, Michoacán, Mexico and Pcn5-1 from avocado in Michoacán, Mexico. All isolates were grown on PDA, and medium disks containing 7-day-old mycelium were transferred to 15 mL tubes containing sterile distilled water (SDW) and stored at 4 °C until use.

Identification of the bacterial isolate. Phenotypic tests, including cell morphology, Gram staining, and catalase and oxidase assays, were performed. The bacterial isolate was identified through 16S rRNA gene sequencing carried out by the Laboratory of Genomic Services at CINVESTAV (Irapuato, Mexico). The axenic isolate was shipped, and the service consisted of DNA extraction and PCR amplification of the 16S rRNA gene were performed using universal primers, according to the provider's standard protocol. The exact length of the amplified fragment and primer sequences were not specified by the service provider. Sequencing was performed using the Sanger terminal di-deoxy method (Sanger *et al*., 1977). Homologous sequences of the amplified regions were searched using the Basic Local Alignment Search Tool (BLASTn) from the National Center for Biotechnology Information (NCBI) database. Multiple sequence alignment was performed using the MUSCLE tool. Phylogenetic analysis was conducted using MEGA 11 (Molecular Evolutionary Genetics Analysis version 11), applying the average-distance method with 1,000 bootstrap replicates (Tamura *et al*., 2021)

Inhibition of mycelial growth. The inhibitory effect of JJR198 was evaluated using a dual-culture method (Chávez-Díaz *et al*., 2016). Six of the *Phytophthora* isolates described above were included in this assay and cultured on PDA at 25 °C for four days. Mycelial plugs (8 mm in diameter) of each isolate were excised and placed at the center of PDA plates. A sterile cotton swab was used to inoculate the bacterial suspension (1.0×10^8 CFU mL^{-1}) at four equidistant edges, 2.7 cm from the center, toward the outer margin, forming an uninoculated square area around the mycelial plug. Plates of each isolate without bacterial inoculation served as controls. After five days of incubation at 25 °C, the mycelial growth diameter was measured, and the inhibition rate was calculated based on mycelial growth of the control. The experiment followed a completely randomized design with two treatments (with and without bacterial inoculation), six replicates per *Phytophthora* isolate, and individual Petri dishes as experimental units.

Effect of bacteria inoculum concentrations on sporangia formation. To evaluate the inhibitory effect of the bacterial isolate on sporangia formation, two *P. capsici* isolates: PcAR-2 and Pc57 selected for their high and stable sporangia production, were cultured on V8 juice agar (200 mL L^{-1} V8 juice, 800 mL L^{-1} distilled water, 3 g L^{-1} $CaCO_3$, 15 g L^{-1} agar, pH 7.2) at 25 °C for four days. Three mycelial plugs (8 mm in diameter) were placed in each 80 mm x 10 mm Petri dish and flooded with 25 mL of bacterial suspensions at three concentrations (1.0×10^4, 1.0×10^6, and 1.0×10^8 CFU mL^{-1}). The plates were incubated at 25 °C for 48 hours to induce sporangia formation. Petri plates with only sterile distilled water (SDW) served as a control. Sporangia formed on the mycelial plugs were observed *in situ* for each concentration by placing a Petri dish on a Zeiss Primo Star light microscope with a 4x objective lens; for each plug, sporangia were counted within 2 mm^2 observation areas using ImageJ software. The experiment was performed with four replicates (Petri plates) per concentration treatment.

Scanning electron microscopy. Mycelial plugs of PcAR-2 with sporangia, either untreated or treated with JJR198 (1.0×10^8 CFU mL^{-1}), were fixed by immersion in 2.5% (v/v) glutaraldehyde and washed several times with 1X PBS. The samples were dehydrated through a graded ethanol series (60, 70, 80, 90, 96, and 100%), for 20 minutes at each concentration. After dehydration in 100% ethanol, the samples were critical-point dried using a Samdri critical point dryer (Tousimis Research Corporation), mounted on stubs, gold-coated for 3 minutes using a Denton Vacuum Desk II sputter coater, and examined with a JEOL JSM-5900LV scanning electron microscope (Hayat, 1981, 1989; JEOL, 2000).

Effect of bacteria inoculum concentrations on zoospore release. To assess the effect of JJR198 on zoospore release, three V8 agar mycelial plugs (8 mm in diameter) with four-day-old mycelium of the Pc57 isolate (the most prolific sporangia producer) were placed in the center of a Petri dish (80 mm x 10 mm). The mycelial plugs were flooded with 25 mL of SDW and incubated for 48 h at 25 °C to induce sporangia formation. Afterward, the water was removed with a pipette and replaced with 25 mL of bacterial suspensions at three concentrations (1.0×10^4, 1.0×10^6 and 1.0×10^8 CFU mL^{-1}). To induce zoospore release, the Petri dishes were incubated at 10 °C for 30 minutes followed by 30 min at 25 °C. After incubation, the number of zoospores per mL were estimated for each Petri plate using a hemocytometer. Each concentration treatment was replicated four times, with SDW serving as a control.

Evaluation of disease suppression in potted pepper plants. Pepper seedlings of a susceptible *Guajillo* landrace were produced in a pest and disease-free commercial greenhouse. After eight weeks individual seedlings were transplanted into plastic trays with six-cell cavities of 195 cm^3 each. Cell cavities were prefilled with sterilized Berger BM2 substrate (70-80% peat moss, perlite, vermiculite, and wetting agent) and supplemented with 13 beads of controlled-release fertilizer (Osmocote 14-14-14). All seedlings were maintained in a greenhouse for two weeks at 11 to 30 °C; irrigation was provided every two days or as needed. To evaluate the protective effect of bacterial isolate JJR198 against *P. capsici*, plants were preinoculated with JJR198 at 7, 3, or 0 days before the oomycete inoculation

All bacterial treatments were performed by applying 5 mL (1.0×10^8 CFU mL^{-1}) suspensions to the substrate at the plant base above the roots. Plants free of bacteria and *P. capsici*, plants infected only with the oomycete, and plants treated only with bacteria were used as controls. Each of the four treatments consisted of six plants and was replicated twice. Prior to inoculate *P. capsici* isolate AR-14 all plant trays were placed in water-filled containers to ensure root saturation. A zoospore suspension was prepared and adjusted as described above for *P. capsici* inoculations. Inoculated plants with the oomycete, received 10 000 zoospores per cell. All plant trays were maintained under flooded conditions for 48 hours; afterward excess water was removed. The severity of root rot per plant was based on aerial parts of the plant at 7 and 14 days after inoculation (DAI) with *P. capsici* using a modified scale of severity (0 to 5) based on Ristaino (1990), where: 0 = no visible symptoms; 1 = slight leaf wilting with initial brown lesions on the stem; 2 = 30–50% of the plant showing disease symptoms; 3 = 50–70% of the plant affected; 4 = 70–90% of the plant affected; 5 = dead plant.

Salt Tolerance Assay. Following molecular identification of the bacterial isolate and considering the physiological characteristics previously reported in the literature for phylogenetically related species, its salt resistance was evaluated by assessing growth on nutrient agar (NA) medium with increasing NaCl concentrations (5, 7.5, 10, and 15%). Plates without NaCl served as controls. All assays were performed in triplicate, and plates were incubated at 30 °C for 48–72 h before measuring bacterial colony growth.

Presence of bacterial inoculum in pepper roots. Bacterial isolate JJR198 was recovered from the rhizoplane and rhizosphere of pepper plants two weeks after inoculation using the dilution plating technique (Shankar *et al*., 2011; Shahid *et al*., 2012). Samples of bacterial inoculated pepper root balls were shaken to remove excess substrate. For the rhizosphere bacterial population, one gram of adhering substrate was suspended in 9 mL of SDW in 25 mL glass tubes. For the rhizoplane bacterial population, roots were washed ten times with SDW, and then one gram of roots was vigorously shaken with glass beads in 9 mL of SDW, also in 25 mL glass tubes, to dislodge root-adhering bacteria. All tubes were subsequently heated in a water bath at 80 °C for 15 minutes to eliminate vegetative cells. Serial 10-fold dilutions were prepared up to 1.0×10^4, and 0.1 mL of each dilution was plated onto NA plates supplemented with 6% NaCl. Colony-forming units (CFU) were counted, and Gram staining was performed after 48 hours of incubation at 30 °C. Uninoculated plants served as controls. Three replicates were performed in this assay.

Statistical analysis. Data of each experiment was organized in a specific database and analyzed with STATISTICA software 8.0 (StatSoft Inc. USA). For each experiment, statistical analyses included analysis of variance (ANOVA), followed by mean separation

using Tukey's test at $p \leq 0.05$. Assumptions of normality and homogeneity of variances were also evaluated.

RESULTS

Identification of the bacterial isolate. The JJR198 strain was Gram-positive, rod shaped catalase and oxidase positive. JJR198 sequence was 1513 nt. The search for homologous genes in NCBI showed 99.6% similarity with *Bacillus zhangzhouensis* strain MCCC 1A08372 and *Bacillus pumilus*. The phylogenetic tree showed that JJR198 is in the clade together with *B. zhangzhouensis* and *B. pumilus* (Figure 1). The 16S rRNA gene sequence was deposited in the GeneBank under accession number PV826766.

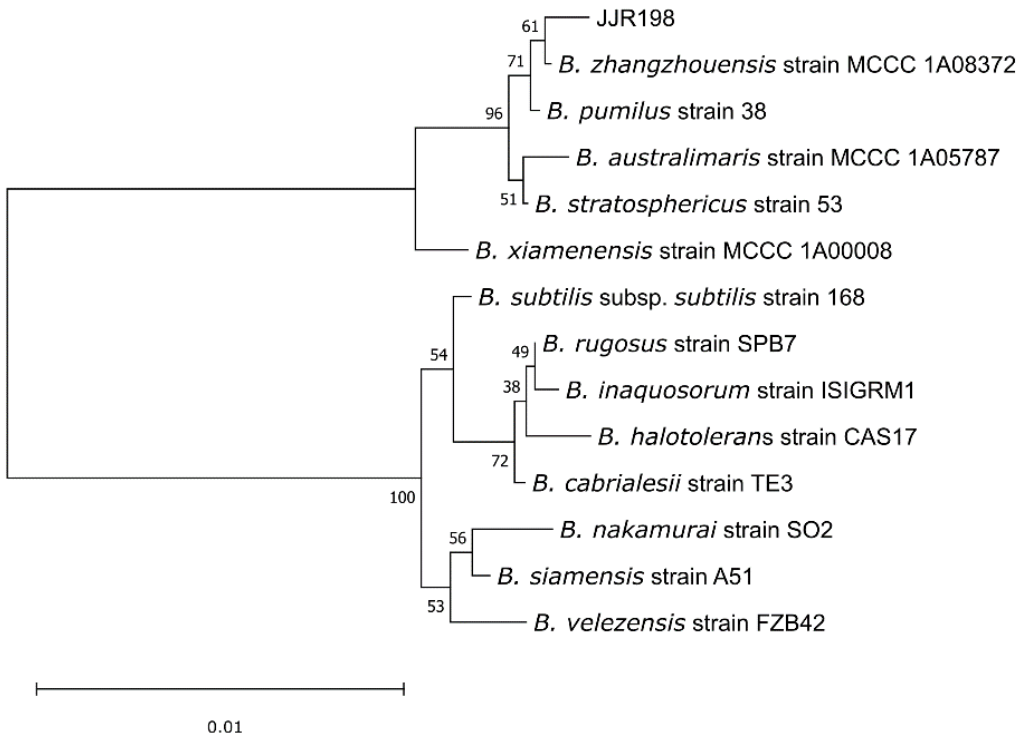

Figure 1. Phylogenetic tree of *Bacillus zhangzhouensis* (JJR198) with another related taxa based on 16S gene sequences. The tree shows two distinct branches where JJR198 is in a clade together with *Bacillus zhangzhouensis* and *Bacillus pumilus*.

Inhibition of mycelial growth. The JJR198 bacterial strain (1.0×10^8 CFU mL^{-1}) exhibited similar inhibitory activity against the mycelial growth of the five *P. capsici* isolates and one isolate of *P. cinnamomi* (Figures 2, and 3) Inhibition rates ranged from 42. to 52.7%. The highest inhibition was observed in isolate PcAR-2 (52.7%), which differed significantly from Pc43M (42.1%). Pc2aIT (51.6%) also showed higher inhibition than Pc43M, while Pc1 (42.0%), Pc57 (47.0%), and Pcn5-1 (46.6%) exhibited intermediate values without significant differences among them (Tukey, $p \leq 0.05$).

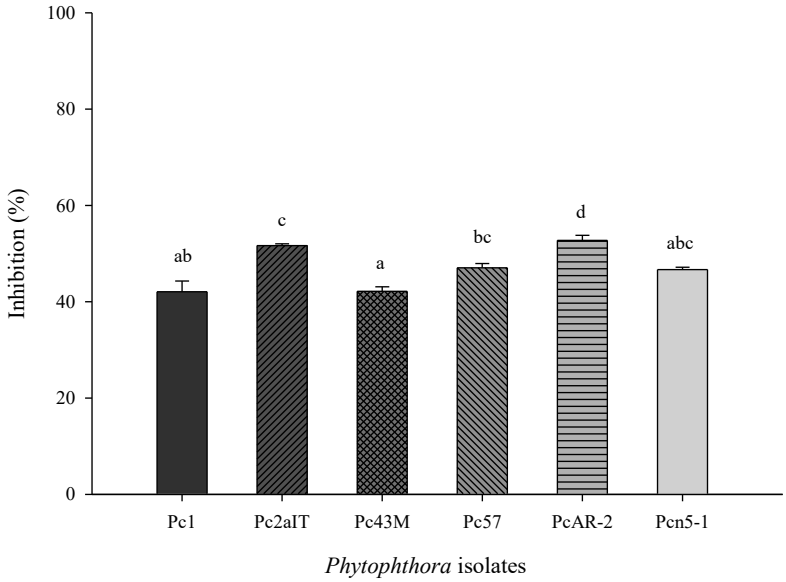

Figure 2. Inhibitory effect of *Bacillus zhangzhouensis* JJR198 (1.0×10^8 CFU mL^{-1}) on the mycelial growth of five isolates of *Phytophthora capsici* (Pc) and one isolate of *Phytophthora cinnamomi* (Pcn) after five days. Bars and vertical lines represent the means and standard errors of six replications. Columns with the same letters indicate no significant differences (Tukey's test, $p \leq 0.05$).

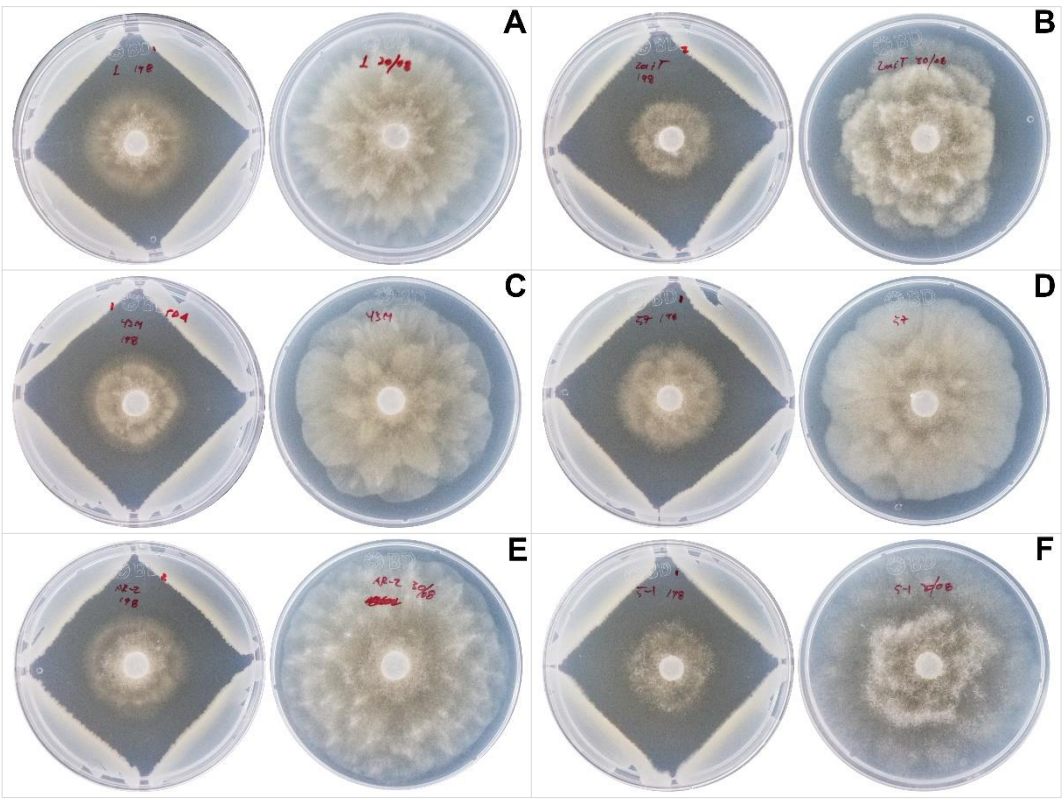

Figure 3. Mycelial growth inhibition (left) of five *Phytophthora capsici* isolates (Pc) and one *Phytophthora cinnamomi* isolate (Pcn) by *Bacillus zhangzhouensis* JJR198 (1.0×10^8 CFU mL^{-1}), compared to controls (right). **A**, Pc1; **B**, Pc2aIT; **C**, Pc43M, **D**, Pc57; **E**, PcAR-2; **F**, Pcn5-1.

Effect of bacteria inoculum concentrations on sporangium formation. Under the sporangia-inducing conditions (48 h at 25 °C), the JJR198 bacterial strain did not exhibit a significant antagonistic effect against *P. capsici* isolates PcAR-2 and Pc57 at low and medium concentrations (1.0×10^4 and 1.0×10^6 CFU mL^{-1}). However, sporangia formation was completely inhibited at 1.0×10^8 CFU mL^{-1} (Table 1). At the highest bacterial inoculum, the morphology of the *P. capsici* isolates appeared altered, suggesting modifications of the mycelium and hyphae, along with the presence of bacterial aggregates (Figure 4).

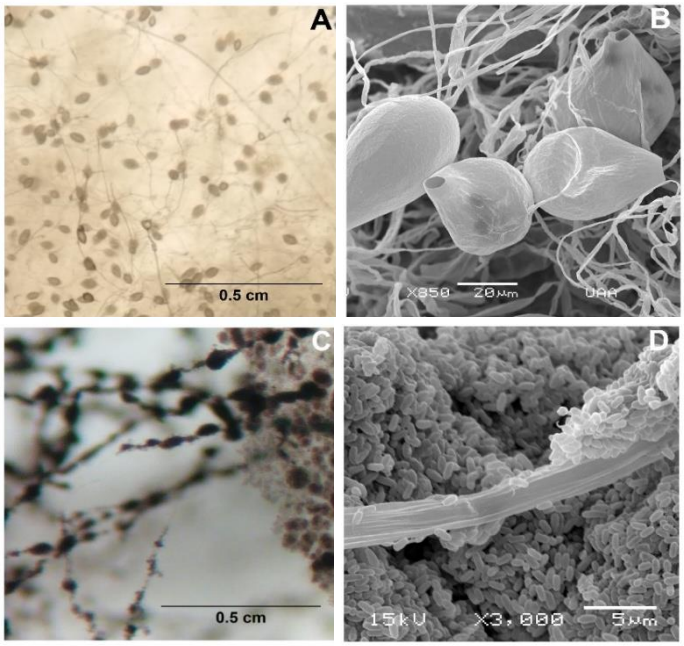

Figure 4. Colonization of *Phytophthora capsici* mycelia by *Bacillus zhangzhouensis* strain JJR198 observed by light and scanning electron microscopy. **A-B**, unexposed control showing well-developed mycelium, hyphae, and sporangia. **C-D**, exposure to JJR198 (1.0×10^8 CFU mL^{-1}), where bacterial cells are observed adhering to and covering hyphae and sporangia; sporangial development appears inhibited.

Effect of bacteria inoculum concentrations on zoospore release. Zoospore release from Pc57 sporangia was not inhibited by 1.0×10^4 or 1.0×10^6 CFU mL^{-1} suspensions of strain JJR198, showing no significant difference ($p > 0.05$) compared to the control. However, a significant reduction was observed at a concentration of 1.0×10^8 CFU mL^{-1}, with almost no spore release detected (Table 1).

Table 1. Effect of inoculum concentration of *Bacillus zhangzhouensis* JJR198 on sporangia formation and zoospore release in two isolates of *Phytophthora capsici*.

JJR198 inoculum density (CFU mL^{-1})	No. of sporangia/VA*		No. of zoospores (1.0×10^3 mL^{-1})
	PcAR-2	Pc57	Pc57
SDW¤ (control)	60.1 ± 6.2 a	234.2 ± 12.7 a	25.9 ± 4.9 a
1.0×10^4	41.0 ± 4.9 a	207.2 ± 15.3 a	26.1 ± 2.6 a
1.0×10^6	50.1 ± 10.7 a	205.5 ± 31.4 a	20.6 ± 3.3 a
1.0×10^8	0.0 ± 0.0 b	0.0 ± 0.0 b	0.2 ± 0.1 b

*VA, viewing area of 2 mm^2 under light microscope. ¤SDW, sterile distilled water. Results are means ± standard errors of four replications. Same letters in each column denote no significant difference (Tukey, $p \leq 0.05$).

Evaluation of disease suppression in potted pepper plants. The bacterial isolate JJR198 did not significantly reduced root rot severity in treated plants regardless of bacterial application at 7, 3 or 0 days prior to *P. capsici* inoculation. Root rot severity increased gradually with DAI in all four treatments. No statistically significant differences were observed between the bacterial treatments and the uninoculated control (Figure 5).

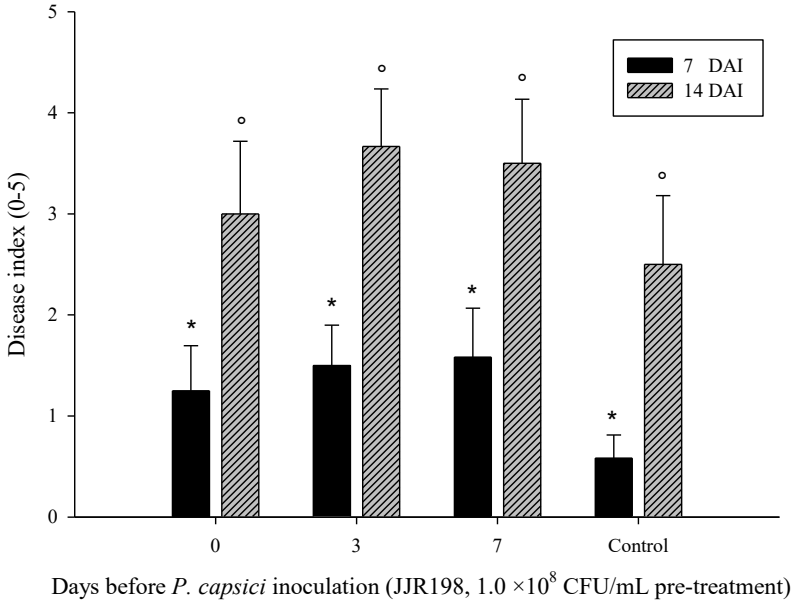

Figure 5. Effect of JJR198 applied to pepper plants at 7 and 14 days after inoculation (DAI) with *Phytophthora capsici*. Pepper plants were pre-treated with JJR198 (1×10^8 CFU mL^{-1}) 7, 3, and 0 days before *Phytophthora capsici* inoculation. Plants treated only with the oomycete served as controls. Vertical bars and lines are means and standard errors of 12 replications. Statistical comparisons were performed separately within each DAI. Bars sharing the same symbol within the same DAI are not significantly different (Tukey, $p \leq 0.05$).

Salt Tolerance Assay. JJR198 was assessed for its capacity to grow at high NaCl concentrations. JJR198 was able to grow in NA media after 48 hours of incubation at 30 °C with 7.5% NaCl.

Presence of bacterial inoculum in pepper roots. In rhizosphere and rhizoplane samples from two-week inoculated pepper roots, bacterial colonies with the same macro- and micro-morphological characteristics as the JJR198 isolate were observed, including irregular, medium-sized colonies with irregular texture, cream coloration, and Gram-positive rods. No other colonies grew on NA medium supplemented with 6% NaCl. The bacterial count was 1.6×10^6 CFU mL^{-1} in rhizosphere and 0.19×10^6 CFU mL^{-1} in rhizoplane samples.

DISCUSSION

In previous studies, Liu *et al*. (2016) isolated a strain from a shrimp aquaculture farm in Zhangzhou City, China and they called as DW5-4. The Molecular identification of DW5-4 was performed using 16S and gyr genes, and the search for homologous genes showed an identity with *Bacillus pumilus.* However, a detailed bioinformatic analysis demonstrated that it was a different strain, which was named *Bacillus zhangzhouensis*. Thus, JJR198 has a very high similarity to both strains, as demonstrated in the phylogenetic tree. The

molecular identification of JJR198 using 16S is consistent with other strains of *B. zhangzhouensis* and its close similarity with *B. pumilus* (Anil *et al*., 2019; Liu *et al*., 2016; Soliman *et al*., 2022).

Strain JJR198 exhibited *in vitro* antagonistic activity against mycelial growth of five *P. capsici* isolates and one *P. cinnamomi* isolate. Although JJR198 achieved a maximum mycelial inhibition of 52.7%, it showed a comparable effect across all *Phytophthora* isolates, suggesting a relevant potential broad-spectrum protective role. This is particularly relevant considering the biological differences in growth rate, sporangia production, and pathogenicity between *P. capsici* isolates (Li *et al*., 2007). A similar effect of *B. zhangzhouensis* on mycelial growth was reported by Anil *et al*. (2019), with 62% inhibition of mulberry (*Morus* spp.) leaf spot. Likewise, Aytenov *et al*. (2024) found that 47 strains of *B. zhangzhouensis* obtained from the rhizosphere and phyllosphere of plants in a hypersaline environment displayed broad-spectrum inhibition of fungal mycelial growth against 13 pathogenic fungi.

In the evaluation against sporangia and zoospores of *P. capsici*, JJR198 completely restricted sporangia formation and halted zoospore release at a bacterial concentration of 1.0×10^8 CFU mL^{-1} but showed no effect on either at 1.0×10^4 and 1.0×10^6 CFU mL^{-1}. Similar results were described by Kim *et al*. (2009) and Kim *et al*. (2010), who studied various *Paenibacillus polymyxa* strains, observing little to no effect on sporangia formation and zoospore release at low bacterial inoculum concentrations, but complete inhibition at 1.0×10^7 and 1.0×10^8 CFU mL^{-1}, where higher inoculum levels also altered sporangial morphology.

Soliman *et al*. (2022) determined through mass spectrometric analysis that phenol, 2,4-bis(1,1-dimethylethyl), is the bioactive compound responsible for the antimicrobial activity of *B. zhangzhouensis*, which they also evaluated as a broad-spectrum biological control agent exhibiting high activity against different pathogens, including fungi, Gram-positive, and Gram-negative bacteria. This compound has been previously reported for its anti-oomycete properties against *P. capsici* and *P. cinnamomi* (Rangel-Sánchez *et al*., 2014; Romero-Correa *et al*., 2014; Sang *et al*., 2011; Sang and Kim, 2012).

In vivo evaluations of JJR198 comprised two complementary approaches. First, the bacterium was successfully re-isolated from the rhizoplane and rhizosphere of inoculated pepper plants, confirming its ability to persist in the root environment. Second, and critically, its antagonistic performance against *P. capsici* was assessed in potted pepper plants, where no reduction in disease severity was observed. This lack of disease suppression, despite effective root colonization, underscores an important limitation in the *in planta* performance of JJR198 and highlights the discrepancy between root colonization, in vitro antagonistic traits, and effective disease control. Although other *B. zhangzhouensis* isolates have been reported as rhizosphere-associated or endophytic bacteria with promising plant growth–promoting and biocontrol traits in potted plant and field studies (Bhutani *et al*., 2021; Carreiras *et al*., 2023; Karagoz *et al*., 2018; Maheshwari *et al*., 2022).

Strain JJR198 was found to tolerate up to 7.5% NaCl. Several studies have described *B. zhangzhouensis* strains as halotolerant bacteria with promising agricultural potential due to their plant growth-promoting and antagonistic properties in saline soils (Aytenov *et al*., 2024). This is particularly relevant given that soil salinization is a major and increasingly prevalent problem in agricultural systems (Kramer and Mau, 2023). Primarily isolated from marine samples, hypersaline coastal lakes, and the rhizosphere and phyllosphere of plants in saline environments, this species has also been reported to exhibit significant enzymatic

activities, including cellulolytic, protease and keratinase (Moridshahi *et al*., 2020; Niu-Niu *et al*., 2023; Ruginescu *et al*., 2018), with proteases being among the main enzymes involved in the degradation of the cell walls of fungal and oomycete pathogens (Latijnhouwers *et al.,* 2003; Carmona-Hernandez *et al*., 2019).

The results of this study demonstrate that, under *in vitro* conditions, *B. zhangzhouensis* JJR198 exhibits potential as a biocontrol agent against *P. capsici* due to its inhibitory effects on mycelial growth, sporangia formation, and zoospore release. Nevertheless, since it did not show antagonistic activity against Phytophthora root rot in pepper plants under greenhouse conditions, factors limiting its in planta performance, including environmental conditions, rhizosphere dynamics, and host-mediated responses, should be addressed in future studies, while its *in vitro* activity suggests that this strain could be further explored as a source of antimicrobial bioactive compounds.

CONCLUSIONS

This study demonstrated that *Bacillus zhangzhouensis* JJR198 exhibited *in vitro* antagonistic activity against several *Phytophthora capsici* isolates. JJR198 bacterial isolate inhibited mycelial growth by 42.0 to 52.7% across five *P. capsici* isolates and one *P. cinnamomi* isolate. The biocontrol response was strongly dependent on the initial bacterial concentration, as sporangia formation and zoospore release were completely suppressed at 1.0×10^8 CFU mL^{-1}, whereas no inhibitory effect was detected at lower concentrations.

Microscopic observations revealed that hyphae and mycelia were covered by bacterial cells, suggesting a possible association between bacterial aggregation and alterations in sporangial development. Additionally, the presence of the bacterial inoculum was confirmed in the rhizosphere and rhizoplane of inoculated pepper plants. However, pretreatment of pepper plants with JJR198 at different times prior to the oomycete inoculation (7, 3, or 0 days) did not reduce disease severity caused by *P. capsici* under the conditions evaluated.

These findings highlight the *in vitro* biocontrol potential of *Bacillus zhangzhouensis* JJR198 against multiple *Phytophthora* isolates; however, its effectiveness as a live inoculant in pepper plants remained limited under the conditions evaluated. Consequently, further studies are needed to identify the factors restricting the *in planta* performance of JJR198.

Conflict of interest. The authors declare that they have no conflict of interest in the publication.

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