Article

Biological Control of Streptomyces sp. PR69 Against Phytophthora capsici and Its Growth-Promoting Effects on Plants

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Biological Control of *Streptomyces sp. PR69* Against *Phytophthora capsici* and Its Growth-Promoting Effects on Plants

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Abstract: Actinomycetes are a group of bacteria that have been reported as potential controllers of several plant pathogens and as plant growth promoting agents. This study focused on the isolation of 60 actinobacterial strains from the Cuatro Cienegas Basin, Coahuila, Mexico, with an emphasis on evaluating their potential as biocontrol agents against plant pathogens. Among the strains tested, the isolate PR69 exhibited significant *in vitro* antagonistic activity against eight plant pathogens, with inhibition rates ranging from 44% to 73%, including *Phytophthora capsici*. The genome of PR69 was sequenced and assembled, confirming its classification as a member of the genus *Streptomyces* through a phylogenetic analysis based on concatenated multiple sequence alignments of 81 core bacterial genes. Additionally, volatile compounds produced by PR69 enhanced the growth of *Arabidopsis thaliana* seedlings *in vitro*, increasing seedling weight, primary root length, and the number of secondary roots. Furthermore, the soil treated with *Streptomyces* sp. PR69 effectively controlled the infection caused by the pathogen *P. capsici* in bell pepper plants, reducing disease symptoms by 50% compared to plants inoculated solely with the pathogen. PR69-treated plants also showed an increase in fresh weight compared to untreated controls. These findings suggest that *Streptomyces* sp. PR69 holds promise as a bioinoculant for promoting pepper plant growth and controlling *P. capsici* populations.

Keywords: antifungal activity; phytopathogen; biocontrol; streptomyces; *Capsicum annum*; plant growth promotion

1. Introduction

Phytophthora capsici is an oomycete pathogen that affects numerous economically important crops worldwide, particularly members of the Solanaceae and Cucurbitaceae families. It is considered one of the most significant pathogens of bell pepper. Disease management primarily relies on fungicide application; however, under environmental conditions favorable to the pathogen, no currently available fungicide achieves complete disease control [1].

Over the years, numerous fungicides have been developed to prevent and control pepper blight, with metalaxyl being one of the most widely used. However, *Phytophthora* spp. is highly susceptible to mutations and has developed resistance to such fungicides, which typically have a single site of action on pathogens [2]. Cases of resistance have also been reported with other fungicides, such as mefenoxam, fluopicolide, cyazofamid, and oxathiapiprolin, particularly in regions where intensive, fungicide-based disease management programs are practiced [3].

Pesticide resistance is one of the primary causes driving the increased use of chemical pesticides. Extensive pesticide application has been associated to various human diseases and health disorders, as well as environmental damage. Consequently, environmentally friendly pest control methods, such as biological control, have been promoted to minimize chemical pesticide usage [4].

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The use of living organisms for pest control is considered a more successful alternative for pest management. Recent studies have investigated antagonistic bacteria for the control of fungal and bacterial pathogens, with particular attention to species from the genera *Pseudomonas* spp., *Bacillus* spp, and *Streptomyces* spp, some of which are already available as commercial products [5].

Streptomyces are an important group of soil-dwelling bacteria within the Actinomycetaceae family, distinguished by their high G+C content, Gram-positive nature, and filamentous structure similar to that of fungi. These bacteria produce approximately 75% of currently used antibiotics and various bioactive metabolites with antifungal, antiviral, immunosuppressive, anticancer, and antioxidant activities [6].

Streptomyces strains from unexplored environments are considered potential sources of novel natural compounds, particularly those from desert habitats. Microorganisms in these extreme environments endure desiccation, extreme temperatures, intense radiation, low nutrient levels, low water availability, and high salinity, which may enhance their production of unique bioactive compounds [7].

The Cuatro Cienegas Basin, an oasis in the middle of the Chihuahuan Desert, contains a network of pools with exceptional microbial diversity and endemism. Its highly oligotrophic conditions make it a promising area for the discovery of microbial agents with antagonistic potential [8].

The objectives of this study were to (i) identify and characterize a strain isolated from Cuatro Cienegas, (ii) evaluate the antifungal activity of selected isolates against soil-borne fungal pathogens *in vitro*, and (iii) determine the efficacy of the selected isolate as a growth promoter and biological control agent against *P. capsici* in bell pepper (*Capsicum annuum*) plants.

2. Materials and Methods

2.1. Bacterial Strain, Fungal Plant Pathogens and Seeds

Sixty strains of actinomycetes used in this study (Table S1) were isolated in a previous investigation at different points in Cuatro Cienegas, Coahuila, Mexico and were reactivated and grown at 28°C/10 days on International *Streptomyces* Project 2 (ISP2) culture medium [8].

Phytopathogens used in this study (Phytophthora capsici, Fusarium solani, Fusarium oxysporum, Fusarium lateritium, Macrophomina phaseolina, Colletotrichum coccodes, Botryosphaeria rhodina, Botrytis cinerea and Sclerotium rolfsii), were obtained from culture collections from Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) General Terán; Universidad Autónoma de Querétaro (UAQ), and Centro de Investigación en Alimentación y Desarrollo (CIAD) Cuauhtémoc, Chihuahua. All pathogens were cultured at 28°C in Petri dishes with potato dextrose agar (PDA) for 10 days.

Bell pepper seeds (*Capsicum annuum* cv. California Wonder) from the 'Rancho Los Molinos' brand, or *Arabidopsis thaliana* ecotype Columbia, were used for all growth promotion and biocontrol assays.

2.2. Screening of Actinobacterias with Antagonistic Properties

Sixty strains of actinobacteria were evaluated for their ability to inhibit the growth of seven phytopathogens using the dual culture technique. A mycelial disk (7 mm diameter) of each pathogen was placed at the center of a Petri dish with ISP4 solid medium and a different actinobacteria strain was inoculated at four points equidistant from the fungal inoculum. Control plates contained only the pathogen inoculum. After 10 days of incubation at 28°C, the actinobacteria that showed inhibition halos against the pathogen were recorded. The actinobacteria with the highest number of antagonisms against phytopathogens was selected for a second round of selection using the dual culture technique against 9 phytopathogens.

2.4. Antifungal Activity In Vitro

Spores of the selected strain PR69 were streaked in two parallel lines using a bacteriological loop, each line placed 1.5 cm from the edge of a Petri dish containing ISP4 agar medium, and incubated for

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3 days at 28°C. Then, a mycelial disk from a pathogen was placed in the center of a Petri dish containing ISP4 medium, between the parallel lines of actinomycetes. Control plates contained only the pathogen inoculum. After incubation for 10 days at 28°C, the radial fungal growth of each pathogen was measured and the reduction in colony radius was calculated and compared to control samples using the formula: % Inhibition = [(D-d)x100]/D, where D is the mycelial diameter (mm) in control plates and d is the mycelial diameter grown opposite to actinobacteria [9]. The experiment was conducted in triplicate.

2.5. VOCs-Mediated Antifungal Activity

The effect of PR69 volatile organic compounds (VOCs) on pathogens was investigated using the sealed plate method as previously described [10]. Strain PR69 was streaked by covering the entire ISP4 medium in a Petri dish. Then, a 7mm mycelial disk from a pathogen was placed in the center of another Petri dish with ISP4 medium. The two plates (without lids) were sealed using parafilm to obtain a double-plate chamber where the plate with phytopathogen was above and the plate with actinomycete below. Double plates were incubated at 27°C for 10 days. As a control, the Petri dish containing pathogens was exposed to a Petri dish containing only ISP4. The experiment was performed in triplicate and the radial growth of the pathogen was measured to calculate the percentage of inhibition.

2.3. Morphological and Culture Characterization

Strain PR69 was stained with Gram staining to determine the thickness of its peptidoglycan wall and then was grown in 11 different liquid and solid media and incubated at 27°C for 14 days: **ISP1** (5 g hydrolyzed casein, 3 g yeast extract); **ISP2** (25 g Kent marine reef salt mix, 10 g malt extract, 5 g yeast extract, 4 g dextrose,); **ISP3** (20 g oat meal, 0.001 g $MgCl_2$, 0.001 g $ZnSO_4$, 0.001 g $Fe_2(SO_4)_3$); **ISP4** (10 g starch, 2 g $CaCO_3$, 2 g $(NH_4)_2SO_4$, 1 g NaCl, 1 g K_2HPO_4, 1 g $MgSO_4{\cdot}7H_2O$, 0.001 g $ZnSO_4$, 0.001 g $MgCl_2$, 0.001 g $FeSO_4{\cdot}7H_2O$); **ISP5** (1 g L-asparagine, 1 g K_2HPO_4, 0.001 g $FeSO_4$, 0.001 g $MnCl_2$, 0.001 g $ZnSO_4$ and 10 mL glycerol); **ISP6** (15 g peptone, 5 g protease peptone, 1 g yeast extract, 0.5 g $C_6H_{11}FeNO_7$, 1 g K_2HPO_4, 0.08 g $Na_2S_2O_3$); **ISP7** (1 g L-Asparagine, 0.05 g L-Tyrosine, 0.05 g NaCl, 0.05 g K_2HPO_4, 0.05 g $MgSO_4{\cdot}7H_2O$, 2.850 mg H_3BO_3, 1.8 mg $MgCl_2{\cdot}4H_2O$, 1.77 mg $C_4H_4Na_2O_6$, 1.360 mg $FeSO_4{\cdot}7H_2O$, 0.027 mg $CuCl_2{\cdot}2H_2O$, 0.04 mg $CoCl_2$, 0.02 mg $ZnCl_2$, 0.025 mg Na2MoO4·2H2O, 15 mL Glycerol 100%); **SFM** (20 g mannitol, 20 g soy flour, 25 g Kent marine reef salt mix); **M1** (50 mL salt stock, 5 g mannitol, 5 g glycerol); **M3** (8 g peptone, 1 g starch) and **Chitin** (25 g Kent marine reef salt mix and 10 g chitin). Microscopic observations were performed to record the color of aerial and substrate mycelium and the capabilities to produce diffusible pigments and spore production.

2.6. Genome Sequencing, Assembly, and Bioinformatics Analysis

Genomic DNA was extracted using a modified phenol/chloroform method described previously [8]. The Actinobacteria mycelium was scraped from agar plates and transferred into Eppendorf tubes. The mycelium was then washed with 1 mL of 10% sucrose solution and resuspended in 400 µL of QTP extraction buffer, containing 4% Triton X-100, 20% SDS, 5 M NaCl, 2 M Tris-HCl (pH 8), and 500 mM EDTA (pH 8). Following resuspension, 400 µL of a 1:1 phenol/chloroform solution was added. To facilitate lysis, 0.1 mm glass beads were included, and the mixture was mechanically disrupted for 2 minutes. The lysates were centrifuged at 12,000 rpm for 15 minutes, and DNA in the aqueous phase was precipitated with 2 volumes of ethanol and 1/10 volume of 3 M sodium acetate. After incubation at -20°C, the DNA was pelleted by centrifugation at 12,000 rpm for 10 minutes at 4°C. The pellet was washed twice with 70% ethanol and then resuspended in Milli-Q water containing RNase. Sequencing was carried out by whole genome sequencing using Illumina Mi-Seq 2*300 at CINVESTAV-LANGEBIO (Irapuato, Mexico).

Resulting sequences were trimmed to remove low-quality reads using Trimgalore v0.6.6 (version 0.12.5), then assembled with Unicycler v0.4. After assembly, partial 16S rDNA was compared to the NCBI GenBank database using BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to determine

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the genus. Phylogenetic analysis was performed based on 81 core genes from genomic sequences using the UBCG2 tool [11]. Comparison of isolate PR69 with the most closely related strain was performed using average nucleotide identity (ANI) using EzBioCloud ANI calculator (https://www.ezbiocloud.net/tools/ani) [12] and Kostas Lab ANI (http://enve-omics.ce.gatech.edu/ani/index) [13]. DSMZ GGDC Genome-to-Genome Distance Calculator 3.0 (https://ggdc.dsmz.de/ggdc.php#) was used for digital DNA-DNA hybridization (dDDH) [14]. The assembled genome was annotated with RAST v2.0 (Rapid Annotation using Subsystem Technology) (https://rast.nmpdr.org/rast.cgi) [15]. Genome mining for potential secondary metabolites was performed using antiSMASH bacterial version 7.0 (https://antismash.secondarymetabolites.org/#!/start) [16].

2.7. Production of Extracellular Enzymes and Biochemical Characterization

The bacterial isolates were qualitatively screened for the production of enzymes such as cellulase, protease, lipase, and chitinase. Additionally, their growth-promoting characteristics, including siderophore production, phosphate solubilization, and nitrogen fixation, were evaluated.

Cellulase: Carboxymethyl cellulose agar (CMC) (10 g CMC carboxymethylcellulose, 2 g K_2HPO_4, 0.35 g $(NH_4)_2SO_4$, 0.0748 g urea, 25 g Kent marine reef salt mix) with pH 6.8 to 7.0. Cellulolytic activity was evidenced by qualitative evaluation with the 1% Congo Red reagent (w/v).

Lipase: Rhodamine B agar (250 µL Tween 20%, 30 mL vegetable oil (extra virgin), 20 mL Rhodamine B 0.02% w/v). The production of lipase enzymes was observed by UV light (350 nm) [17].

Protease: Nutrient Agar supplemented with 20% (w/v) non-fat milk. The stock solution of 20% (w/v) of the milk was mixed with nutrient agar to a final concentration of 2% (w/v). The results were visualized by a clear halo around the colony [18].

Chitin: Colloidal chitin medium (10 g chitin with 25 g Kent marine reef salt mix). Colloidal chitin substrate was prepared using the method reported previously [19] modified by adding H_3PO_4 to dissolve the chitin and allowed to stir for 24 hours. Subsequently, cold 98% ethanol was added and centrifuged, followed by washing with distilled water until reaching pH 7.0. A clear halo around the colonies showed the capacity for chitinase production.

Siderophore Production: Chrome-Azurol S (CAS) agar was used to determine siderophore production according to the method reported previously [20]. Chrome Azurol S solution was prepared with 60.5 mg chrome azurol S, 72.9 mg HDTMA, 30.29 g PIPES and agarose 0.9% filled with MiliQ water. After incubation for 1 hour at room temperature in the dark, the change of color around the colony (from blue to orange) indicated the siderophore production.

Phosphate Solubilization: Pikovskaya's agar (PVK) was used to measure phosphate solubilizing ($Ca_3(PO_4)_2$) activity. Solubilization was observed by the appearance of a clear zone around the colony [21].

Nitrogen fixation: Nitrogen fixation capacity was determined by the presence or absence of bacterial colony growth in NFB medium (Nitrogen-Free medium: 15 g sucrose, 5 g malic acid, 0.2 g $MgSO_4 \cdot H_2O$, 0.5 g K_2HPO_4, 0.1 g NaCl, 0.2 g $CaCl_2 \cdot H_2O$, 0.4 g KOH, 4 mL $FeCl_3 \cdot 7H_2O$, 2 mL bromothymol blue 1%, 2 mL macronutrient solution, 1 mL vitamin solution, 15 g bacteriological agar). The macronutrient solution contains 0.2 g $Na_2MoO_4 \cdot 2H_2O$, 0.28 g H_3BO_3, 0.235 g $MnSO_4$, 0.008 g $CuSO_4 \cdot 5H_2O$ and the vitamin solution was prepared with 0.1 g biotin, 0.2 g pyridoxal-HCl. Each colony was inoculated onto NFB agar medium and then incubated at 27°C for 10 days.

2.8. VOC-Mediated Plant Growth Promotion

Arabidopsis thaliana seeds were surface-sterilized using 70% ethanol for 1 minute, followed by 20% sodium hypochlorite for 2 minutes, and then rinsed three times with sterile, double-distilled water. Eight seeds were sown in 90x15 mm Petri dishes containing 50% MS medium, composed of 2.21 g of MS salts, 15 g of sucrose, and 15 g of bacteriological agar. After 4 days of growth, the seedlings were exposed to either bacterial volatiles or an agar medium control.

The experimental setup involved placing a 90x15 mm Petri dish with the *A. thaliana* seedlings (without its lid) inside a larger 150x20 mm glass Petri dish. Below it, a small 35x10 mm Petri dish

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containing a 7-day-old culture of *Streptomyces* sp. PR69 grown in ISP4 medium (or an agar medium for the negative control) was positioned, also without a lid. The glass plate was sealed with plastic wrap to ensure the volatile compounds from the *Streptomyces* isolate could interact directly with the plants. The seedlings were then allowed to grow for 7 days at 23°C, under a 16-hour light/8-hour dark cycle. The effects of the treatment were assessed by measuring the number of secondary roots, the root length and the fresh weight.

2.9. Inoculation of Bell Pepper Plants with Streptomyces sp. PR69 and Phytophthora capsici

Streptomyces sp. PR69 was grown in SFM medium with shaking at 125 rpm for 10 days at 30°C. The biomass around the flask was collected and resuspended in sterile distilled water (sdw) to prepare an inoculum with 10^6 cfu/mL. Bell pepper seeds were sterilized with 97% alcohol, 15% sodium hypochlorite and several washes with sdw. The seeds were sown in seedling trays filled with a soil mix (Sunshine mix 3) inoculated with 1 mL of spore suspension of *Streptomyces sp*. PR69. Germinated seeds were sown in pots (9cm x 6.5cm x 6.5cm) containing soil treated with 10 mL of *Streptomyces sp.* PR69 suspension and kept at 27–30 °C with a relative humidity level of 40–60%. One week after inoculation, each pot was inoculated with 10 mL zoospore suspension (approximately $1x10^6$ zoospores/mL) of *P. capsici*. The zoospore suspension was prepared using cultures of *P. capsici* grown on potato dextrose agar(PDA). Sterile, bidistilled water was added to the culture, which were then left at room temperature for 24 hours. Following this, the cultures were refrigerated at 4 °C for 1 hour and subsequently incubated at 30°C for 1 hour to induce zoospore release. The supernatant was collected from the plates, and the zoospore concentration was adjusted to $1×10^6$ zoospores/mL using a hemocytometer. One week after pathogen inoculation, each pot was additionally inoculated with 10 mL of *Streptomyces sp*. PR69 suspension. Each treatment, consisting of 10 plants, was replicated three times and observed daily for 20 days post-inoculation. Seedlings inoculated only with *P. capsici* or only with *Streptomyces sp.* PR69 served as controls.

2.10. Statistical Analysis

All experiments were performed in triplicate, and statistical significance was set at $p < 0.05$. The results were analyzed using a Student's t-test or ANOVA, with means compared using Tukey's test.

3. Results

3.1. Antifungal Activity In Vitro

A total of 60 actinomycete isolates were tested for their inhibitory effects against various phytopathogens. Of these, 29 strains demonstrated inhibitory activity against at least one plant pathogen (Figure S1). Notably, strain PR69 exhibited significant growth inhibition in direct confrontation assays against several fungal pathogens, including the soilborne pathogens *Phytophthora capsici*, *Colletotrichum coccodes*, *Macrophomina phaseolina*, *Fusarium lateritium*, *Fusarium solani*, and *Fusarium oxysporum*, as well as foliar and floral pathogens *Botryosphaeria rhodina* and *Botrytis cinerea* (Figure 1). The strongest inhibitory effect was observed against *C. coccodes* (Table 1). However, isolate PR69 did not inhibit pathogen growth through volatile compound-mediated mechanisms (Figure S2)

Table 1. Antifungal activity of *Streptomyces sp.* PR69 against different phytopathogens.

	% Inhibition							
	Pc[1]	**Cc**[2]	**Mp**[3]	**Fl**[4]	**Br**[5]	**Fs**[6]	**Bc**[7]	**Fo**[8]
Isolate **PR69**	71.09±0.18	73.63±0.16	63.92±0.19	52.59±0.12	65.46±0.10	50.43±0.31	69.76±0.13	44.85±0.29

Data are expressed as means ± standard errors of three replicates. [1]*Phytophthora capsici*, [2]*Colletotrichum coccodes*, [3]*Macrophomina phaseolina*, [4]*Fusarium lateritium*, [5]*Botryosphaeria rhodina*, [6]*Fusarium solani*, [7]*Botrytis cinerea*, and [8]*Fusarium oxysporum*.

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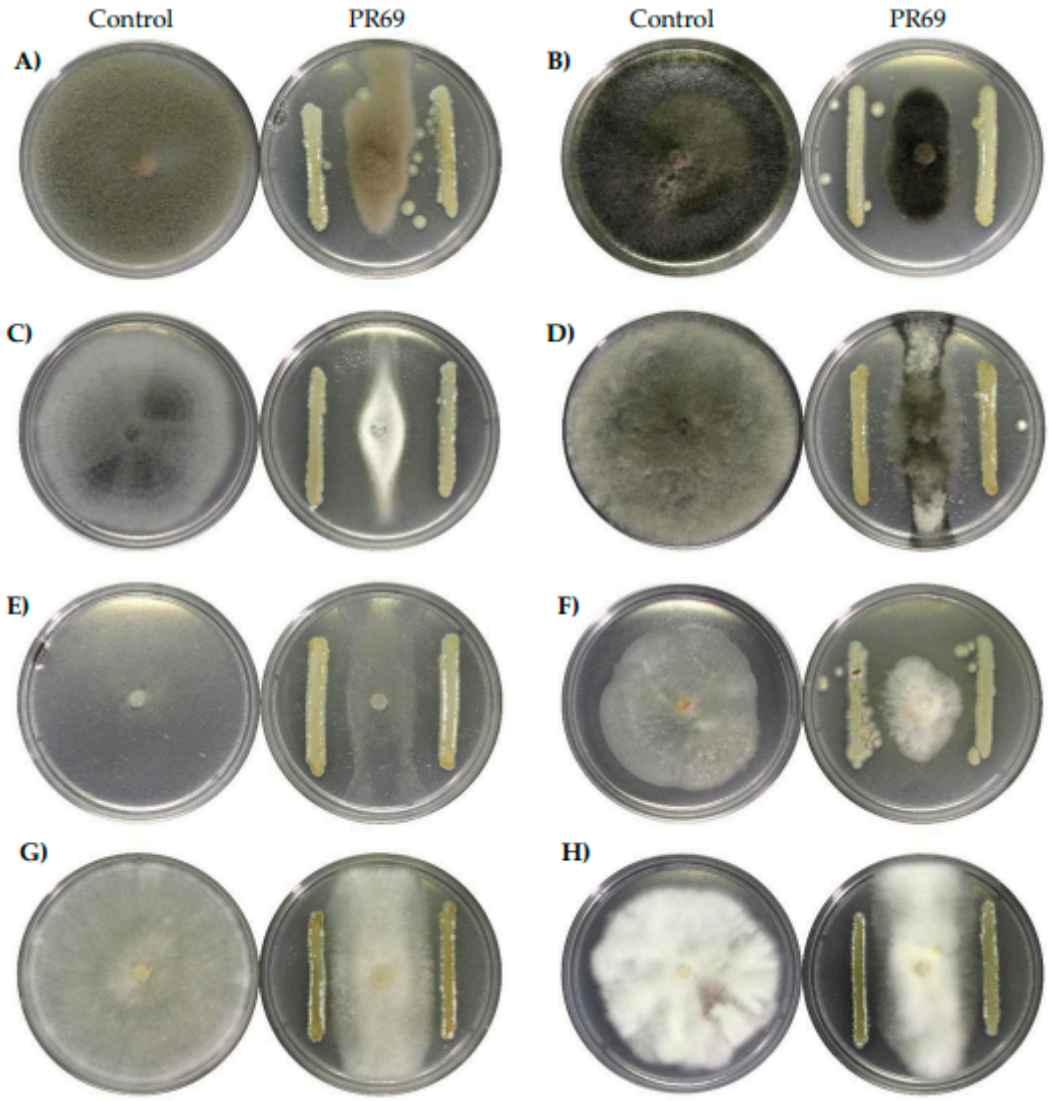

Figure 1. Antifungal activity of Streptomyces sp. PR69 against different phytopathogens **A)** *Botrytis cinerea*, **B)** *Botryosphaeria rhodina*, **C)** *Colletotrichum coccodes*, **D)** *Macrophomina phaseolina*, **E)** *Phytophthora capsici*, **F)** *Fusarium lateritium* **G)** *Fusarium solani*, and **H)** *Fusarium oxysporum*.

3.2. Media-Dependent Growth and Biomass Optimization of Strain PR69

The strain PR69 was preliminarily identified as an actinomycete by Gram staining (Figure S3), and then was incubated in 11 different media (Figure S4). In solid media the strain was able to grow in the 11 types of culture media tested (ISP1 to ISP7, M1, M2, SFM and Chitin). Colonies were circular, raised, and irregular, it was also observed that the morphology of the colony does not differ with the medium (Table S2). In liquid it was observed that they excrete dark brown pigments, melanin or melanoid, in ISP1, ISP6, ISP7 and M3, light brown in ISP2 and SFM, also yellow pigment in Chitin media. In the SFM medium it showed greater production of biomass and spores for which this medium was selected for future tests (Table S3).

3.3. Genome Sequencing, Assembly, and Bioinformatics Analysis

The genome of *Streptomyces* sp. PR69 was successfully sequenced and assembled. The final assembly consisted of 36 contigs, encompassing a total genomic length of 6,533,639 base pairs (bp). The N50 value of the assembly was calculated to be 6,393,861, indicating a high quality of genome assembly. The genome displayed a high G+C content of 71.52%. In terms of genomic features, the *Streptomyces* sp. PR69 genome contains 5,909 protein coding sequences (CDS), alongside 63 transfer

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RNA (tRNA) genes, and 4 ribosomal RNA (rRNA) genes (Table 2). No plasmid sequences were found. The genomic sequence of *Streptomyces* sp. PR69 was deposited in the NCBI GenBank database under BioProject ID PRJNA889045, with BioSample accession number SAMN31231225. The genome assembly is available by searching the NCBI database as GCA_026420845 to facilitate future research and comparative analysis with other microbial genomes.

Table 2. Genome assembly and annotation data *Streptomyces sp.* PR69.

Feature	
Contigs	105
Genome lenght	6,570,163bp
G+C %	71.51
Contig L50	12
ContigN50	186,895
CDS	5,956
tRNA	63
rRNA	4
Protein with functional assignments	3,898
Antibiotic Resistance (source CARD, NDARO,PATRIC)	43

The 16S rRNA gene sequence comparison using the BLASTn tool confirmed that PR69 belongs to the *Streptomyces* genus, showing the highest sequence identity of 98.97% with *Streptomyces* sp. strain HNM0663. In the phylogenetic tree constructed with 95 *Streptomyces* genomes (Table S4), *Streptomyces sp.* PR69 forms a clade with *Streptomyces spongiicola* HNM0071 and *Streptomyces tirandamycinicus* HNM0039 (Figure 2). To further clarify the phylogenetic relationship, digital DNA-DNA hybridization (dDDH) and Average Nucleotide Identity (ANI) values were calculated. The dDDH value between *Streptomyces sp. PR69* and *S. tirandamycinicus* HNM0039 was 24.70%, which is below the 70% threshold generally used for species delineation. Similarly, the ANI values, calculated using ANI calculator and OrthoANIu tool, were 82.79% and 80.45% respectively, both below the standard species delimitation threshold of 95-96% (Table S5). The genome was further analyzed for secondary metabolite biosynthetic potential using the antiSMASH version 7.0, revealing a total of 28 gene clusters associated with secondary metabolite biosynthesis. These clusters corresponded to seven different types of secondary metabolites. Remarkably, seven clusters showed 100% similarity to known clusters, while two clusters did not show similarity to any entries in the current database (Table 3).

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Figure 2. Phylogenetic tree inferred by the maximum likelihood method based on 81 core genes obtained with UBCG2, showing the taxonomic position of the strain *Streptmomyces sp.* PR69. Phylogenetic analysis used the GTR+GAMMA+I model. The circles indicate that the value supports 100%.

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Table 3. Potential Biosynthetic Gene clusters regions found using antiSMASH (Version 7.0).

Type	From-To (location)	Most similar known cluster		Similarity %
		Component	Type	
Ectoine	324,766 - 335,164	Ectoine	Other	100
Melanin	85,744 - 96,241	Melanin	Other	100
Terpene	223,129 - 244,059	Geosmin	Terpene	100
T3PKS	1 - 39,280	Naringenin	Polyketide:Type III polyketide	100
Lanthipeptide-class-iii	28,816 - 51,518	SapB	RiPP:Lanthipeptide	100
Terpene	1 - 16,602	Pristinol	Terpene	100
NRPS-like,NRPS	1 - 28,694	Antipain	NRP	83
NRP-metallophore,NRPS,redox-cofactor	278,062 - 337,959	Mirubactin	NRP	78
Terpene	263,573 - 290,198	Hopene	Terpene	76
Melanin	101,546 - 111,953	Grixazone A	Terpene	61
NRP-metallophore,NRPS,T1PKS	160,886 - 250,379	Peucechelin	NRP	55
T1PKS,T2PKS,RiPP-like	160,514 - 249,283	Xantholipin	Polyketide	51
NRPS	1 - 37,748	Netropsin	NRP	40
Phenazine	20,316 - 40,780	Endophenazine A/endophenazine B	Other:Phenazine	33
Melanin	170,575 - 180,985	Melanin	Other	28
T3PKS,NRPS	79,735 - 109,902	Totopotensamide A/totopotensamide B	NRP+Polyketide	28
NRPS,NRPS-like	1 - 36,960	Disgocidine/distamycin/congocidine	NRP	28
NRPS-like	12,279 - 53,110	Lipstatin	NRP	21
Terpene	24,797 - 46,026	Legonindolizidine A6	NRP+Alkaloid	12
NI-siderophore	132,640 - 147,396	Synechobactin C9/ C11/ 13/ 14/ 16/ A/ B/ C	Other	9
NRPS-like	43,673 - 85,050	Chejuenolide A/chejuenolide B	Polyketide	7
RiPP-like	151,678 - 160,095	Hexacosalactone A	Other	4
NRPS-like	109,671 - 132,257	Sanglifehrin A	NRP+Polyketide	4
Thioamitides	1 - 13,147	Prejadomycin/rabelomycin/gauDimycin C/gaudimycin D/UWM6/gaudimycin A	Polyketide:Type II polyketide+Saccharide:Hybrid/tailoring saccharide	4
Other	90,844 - 113,876	A-503083 A/A-503083 B/A-503083 E/A-503083 F	NRP	3
Terpene	54,693 - 71,392	Bombyxamycin A/bombyxamycin B	Polyketide	3

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CDPS	66,910 - 87,659
Indole	1 -19,239

NRPS, Nonribosomal peptide synthetase cluster; PKS, polyketide synthase cluster; T3PKS, Type 3 polyketide synthase; RiPP, ribosomally synthesized and post-translational modified peptides.

3.4. Production of Extracellular Enzymes and Biochemical Characterization

To assess the potential of *Streptomyces sp.* PR69 as a biological control agent, a series of qualitative enzymatic tests were conducted (Table 4). These tests revealed notable lipolytic activity, indicating the strain's ability to hydrolyze lipids (Figure S5).

Further investigations were carried out to evaluate the plant growth-promoting attributes of *Streptomyces sp*. PR69. The strain was tested for several key attributes, including phosphate solubilization, siderophore production and *in vitro* nitrogen fixation. Using a colorimetric method demonstrated the ability to produce siderophores. Additionally, the PR69 strain confirmed the ability to fix nitrogen. However, when tested on Pikovskaya agar it did not show phosphate solubilization activity (Figure S6).

Table 4. Characteristics of extracellular enzymes and growth promotion.

Characteristics	***Streptomyces sp.* PR69**
Cellulase	-
Protease	-
Chitinase	-
Lipase	+
Siderophores	+
Nitrogen fixation	+
Phosphate solubilization	-

+ positive, − negative

3.5. Effect of Streptomyces sp. PR69 on Arabidopsis thaliana Growth and Root Development

The influence of volatile organic compounds (VOCs) emitted by *Streptomyces sp.* PR69 on the growth of *Arabidopsis thaliana* was evaluated *in vitro*. Notably, *A. thaliana* plants exposed to the VOCs from *Streptomyces sp*. PR69 exhibited significant growth enhancement compared to the control group. These plants were observed to be larger with more developed secondary root systems. The results of the Student t-test confirmed that the differences in fresh weight, root length and secondary root development between the treated and control groups were statistically significant. This suggests that exposure to *Streptomyces sp.* PR69 VOCs positively influences the growth and root development of *A. thaliana* under the conditions tested (Figure 3).

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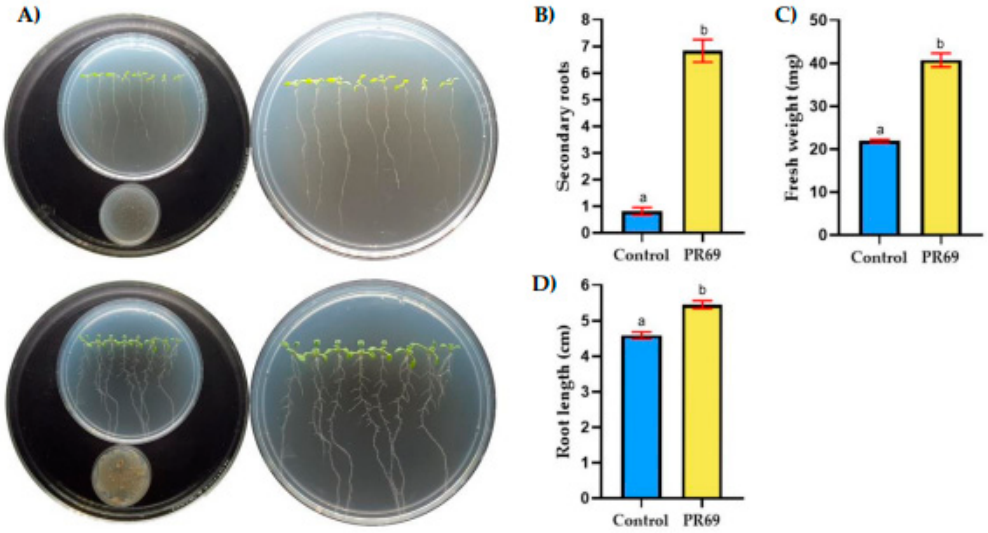

Figure 3. *Arabidopsis thaliana* plants in an atmosphere shared set with *Streptomyces sp*. PR69 **A)** Control plants without actinomycete and plants exposed to *Streptomyces sp.* PR69 VOCs. Comparison of **B)** Secondary roots**, C)** Fresh weight and **D)** Root length. Error bars indicated standard errors of the means from three repeated experiments. Different letters indicate a statistical difference compared to controls using Student's t-test ($p < 0.05$).

3.6. Biocontrol of P. capsici in Bell Pepper Plants by Streptomyces sp. PR69

To evaluate the potential of PR69 to control *P. capsici* infection in bell pepper plants, an experiment was conducted with four plant groups: an untreated control group (light blue bar), a group treated only with PR69 (yellow bar), a group treated with both PR69 spore suspension and the pathogen *P. capsici* (dark blue bar), and a group inoculated solely with the pathogen (red bar). After inoculating the bell pepper plants, the progression of disease symptoms was monitored. Stem rot symptoms, attributable to *P. capsici* infection, began to appear around day 10 post-inoculation. By day 20, plants treated with *Streptomyces* sp. PR69 exhibited a significant reduction in infection incidence compared to plants treated only with the pathogen (Figure 4). At the end of the experiment, approximately 40% of the bell pepper plants treated only with the pathogen (red bar) survived, whereas those treated with both the pathogen and PR69 (dark blue bar) reached a 67% survival rate. This result demonstrated that treatment with spore suspension of *Streptomyces* sp. PR69 increased bell pepper plant survival by 40% when grown in soil inoculated with a *P. capsici* suspension (Figure 5). Additionally, a significant difference was observed in the fresh weight of bell pepper plants between the treatment group that received only *Streptomyces* sp. PR69 and the untreated control group (Figure 6). This finding suggests that, beyond its role in disease suppression, *Streptomyces* sp. PR69 may positively influence plant growth parameters.

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Figure 4. Bell pepper plants (*Capsicum annuum*) were treated as follows: A) inoculation with *Streptomyces* sp. PR69 and *Phytophthora capsici* suspension, and B) inoculation with only *P. capsici* suspension (pathogen).

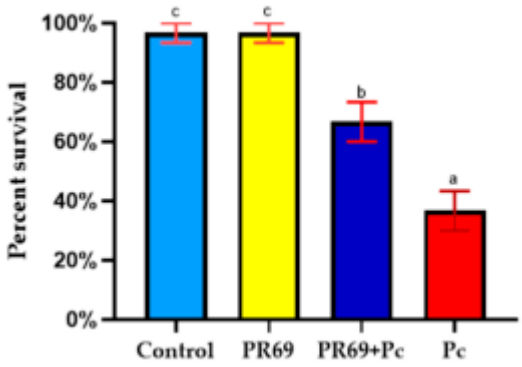

Figure 5. Survival percentage of bell pepper plants for the four treatments. Control, untreated plants. Pc, *Phytophthora capsici*. Means ± standard errors for each treatment labeled with different letters indicate significant differences determined by ANOVA and a Tukey´s test.

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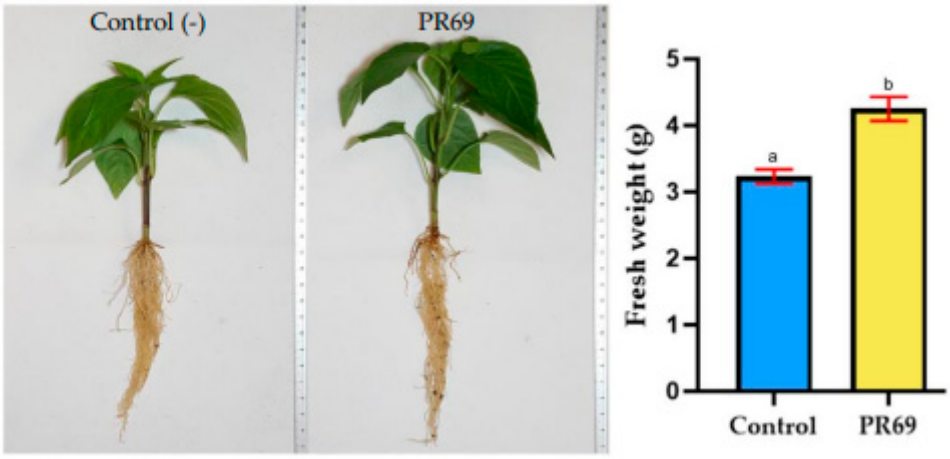

Figure 6. Effect of *Streptomyces* sp. PR69 on the fresh weight of bell pepper plants. Error bars represent the standard error of the mean from three independent experiments. Different letters denote statistically significant differences, as determined by the Student's t-test.

4. Discussion

Actinobacteria represent the largest group of microorganisms that produce antibiotics and other bioactive substances. Recent research has focused on discovering new organisms in extreme and underexplored environments, aiming to identify microorganisms with novel secondary metabolic pathways, thereby increasing the likelihood of uncovering new microbial products. These environments—such as deep oceans, ice sheets, deserts, and volcanoes—often harbor unique organisms that have evolved specialized survival mechanisms. One such environment is Cuatro Ciénegas, known for its exceptional nutrient conditions, which resemble those of the Precambrian Ocean, making it a distinctive and scientifically valuable site [22].

The Cuatro Ciénegas Valley, located in the Chihuahuan Desert, is one of Mexico's most important wetlands and has been internationally recognized as a RAMSAR site (Wetlands of International Importance). A recent study conducted in this area isolated and identified strains of Actinobacteria, with *Streptomyces* emerging as the most abundant genus [8]. The researchers also identified potential new species, highlighting the high diversity and endemism of the site. These findings increase the biotechnological interest in this region due to the potential industrial applications of the unique microorganisms found there.

The objective of this study was to evaluate the strain *Streptomyces* sp. PR69, isolated from Cuatro Ciénegas, Coahuila, as a biofungicide against the phytopathogen *Phytophthora capsici* in bell pepper plants. In preliminary *in vitro* confrontation tests, 50% of the actinomycetes isolated from Cuatro Ciénegas exhibited antifungal activity against at least one of the seven pathogens tested (Figure S1). Among the actinomycetes evaluated, the strain *Streptomyces* sp. PR69 demonstrated significant antifungal activity, inhibiting the growth of eight of the nine phytopathogens tested: *Botrytis cinerea*, *Botryosphaeria rhodina*, *Colletotrichum coccodes*, *Macrophomina phaseolina*, *Phytophthora capsici*, *Fusarium lateritium*, *Fusarium solani*, and *Fusarium oxysporum*.

The percentage of inhibition *in vitro* against *Phytophthora capsici* was 71.09%, which is higher than previously reported values for other *Streptomyces* strains. For instance, *Streptomyces griseus* H7602 showed 53.33% inhibition of *P. capsici* growth [23], while *Streptomyces rochei* IT20 and *Streptomyces vinaceusdrappus* SS14 demonstrated 69% and 63% inhibition, respectively [24]. Additionally, *Streptomyces* sp. ABV38 and ABV45 exhibited inhibition rates of 51% and 50%, respectively, against *P. capsici* CH11[25].

Due to its *in vitro* antagonistic capacity, the strain PR69 was selected for further morphological and genomic analysis. PR69 has a linear genome of 6,570,163 base pairs, with a G+C content of 71.51%. The genome size obtained is smaller compared to the average size of another *Streptomyces*, which

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ranges between 6.7 and 10.1 Mbp. However, it is much larger than *Streptomyces xiamenensis* 318 (isolated from mangrove soil), whose genome length is 5.96 Mb, being the smallest found so far [26],[27]. *Streptomyces albus* which is another of the smallest *Streptomyces* genomes, with 6.8Mb, but with the highest known G+C content 73.3% [28]. *Streptomyces* genomes typically have a high G+C content (70% to 74%) and many repetitive sequences, which influence their genetic stability and the production of secondary metabolites but makes it difficult to fully assemble them from short reads. Consequently, most available genomes are only in draft status [29]. High genomic G+C content is associated with certain environments such as soil and aerobic environments, which induce relatively high rates of DNA damage in the form of double-strand breaks (DSBs) requiring repair. This suggests that DNA damage may be a fundamental factor of G+C content and that it may be a selective adaptation to particular habitats [30]. Despite limited studies on marine adaptations, recent comparative genomics has revealed that *Streptomyces* of marine origin generally have smaller genome sizes and slightly higher G+C content compared to *Streptomyces* from other environments. Marine *Streptomyces* isolates have been shown to produce unique bioactive compounds not found in their terrestrial counterparts, highlighting their metabolic capabilities in marine environments [31]. After analyzing the 16S rRNA sequence and considering the morphological characteristics, strain PR69 was identified as a member of the *Streptomyces* genus. Further phylogenetic analysis using the complete genome showed that *Streptomyces sp*. PR69 is closely related to *Streptomyces tirandamycinicus* HNM0039. This strain of actinobacteria was isolated from a marine sponge, and its crude extract demonstrated strong antibacterial activity against *Streptococcus agalactiae* [32]. It is also related to *Streptomyces spongiicola* HNM0071, another actinobacteria isolated from a marine sponge that produces antitumor metabolites such as staurosporine and echinomycin [33]. *Streptomyces* sp. PR69 when compared with related species showed an ANI of 80% and a dDDH of 20%, indicating that it is a distinct strain. The relationship of strain PR69 to marine bacteria is not surprising given its source of isolation. Cuatro Cienegas is recognized for its unique aquatic ecosystems, with unusual and extreme stoichiometric ratios. These conditions mimic those found in the ancient seas of the late Precambrian, which host diverse microbial communities. Previous research on bacterial biodiversity in Cuatro Cienegas has reported the isolation of genomes of some *Bacillus* [22] and Actinobacteria [8] closely related to strains of marine origin, indicating their ecological importance in these ecosystems.

Once the complete genome of *Streptomyces sp.* PR69 was available, 28 putative biosynthetic gene clusters (BGC) were identified. In actinomycetes, the average BGC covers 1.64 Mbp and encodes 35 secondary metabolites. Certain strains, such as *Kutzneria albida*, *Streptomyces bingchenggensis*, and *Streptomyces rapamycinicus*, are found to encode the greatest number of natural products, dedicating 2.5 to 3.09 Mbp to encode between 48 and 53 secondary metabolites [34]. In the strain *Streptomyces* sp. PR69, 28 potential Biosynthetic Gene Clusters (BGCs) were identified (Table 3). Among these, 7 BGCs exhibit a similarity above 80%, while the remaining 21 BGCs show a very low similarity, indicating a small percentage of matching genes with the closest known compounds. Unfortunately, even though *Streptomyces* harbors numerous gene clusters responsible for producing natural products, most of these BGCs do not get expressed under laboratory conditions [35]. Although the presence of 28 gene clusters encoding secondary metabolites was identified, only 6 clusters could be identified that contained 100% of the known cluster genes for the biosynthetic clusters of ectoine, melanin, geosmin, naringenin, SapB and pristinol.

Ectoine (1,4,5,6-tetrahydro-2-methyl-4-pyrimidinecarboxylic acid) is a natural compound found in halophilic bacteria such as *Ectothiorhodospira halochloris*. It assists microorganisms in maintaining osmotic balance within cells without disrupting cellular metabolic processes. Due to its robust stabilizing ability, it often functions as a thermal protector against extreme freezing, drying, and heating conditions [36]. *Streptomyces* commonly produces ectoines in response to osmotic stress conditions [37]. Ectoine may be a key compound that helps *Streptomyces* sp. PR69 survive under conditions of extremely imbalanced nutrient stoichiometry, sulfur and magnesium minerals such as those found in Cuatro Cienegas [22]. Another compound that may help *Streptomyces* sp. PR69 in its survival in extreme environments is melanin. Melanin protects microorganisms from environmental stressors such as ultraviolet radiation, toxic heavy metals, and oxidative stress. They can also

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influence bacterial interactions with other organisms and are crucial for bacterial survival and pathogenesis in diverse environments [38]. In growth tests in different culture media, *Streptomyces* sp. PR69 showed great pigment production. The diverse properties of melanins have driven many applications and recent efforts have been made to produce the pigment on a biotechnologically relevant scale. One more metabolite found in the genome is SapB which is a hydrophobic peptide that plays a crucial role in the process of aerial hyphae formation and sporulation in *Streptomyces* species. It is thought to act as a surfactant during aerial morphogenesis, reducing surface tension and allowing hyphae to grow upwards [39]. These developmental processes are key in *Streptomyces* that led to the production of spores, to colonize new habitats and survive adverse conditions. A common metabolite found in *Streptomyces* is geosmin. Geosmin is a volatile compound known for its distinct earthy odor. It is produced by various microorganisms, including most *Streptomyces*, as well as several species of cyanobacteria, myxobacteria, and fungi [40]. The gene responsible for producing geosmin is present in almost all sequenced *Streptomyces* genomes. Geosmin plays a crucial role in mediating interactions between microorganisms and their environment, particularly in influencing the composition and dynamics of the soil microbial community. This, in turn, can impact nutrient cycling, soil structure, and resource availability for surrounding organisms [41]. Some studies suggest that the production of geosmin and other volatile organic compounds may be linked to defense or offense mechanisms against other microorganisms. The widespread conservation of the gene cluster layout suggests its significant role in regulating environmental adaptation. However, the precise function of geosmin and the benefits of this group of genes in bacteria are still not fully understood [42]. In *in vitro* assays, it was found that *Streptomyces* sp. PR69, which produces the earthy smell geosmin typical of most *Streptomyces*, also releases other volatile compounds. When tested with *A. thaliana*, this strain of *Streptomyces* sp. PR69 significantly boosted the total fresh weight of the seedlings by 186%, mainly due to an increase in the number of secondary roots. These findings align with previous studies show that *Streptomyces* can produce volatile compounds that enhance secondary root growth in *A. thaliana*. For example, Pérez-Corra *et al*. [43] demonstrated significant improvement in the *in vitro* growth of *A. thaliana* seedlings with their *Streptomyces* strains, particularly increasing fresh root weight by 94-300%. Additionally, Cordovez *et al.*[10] reported an increase in the fresh weight of *A. thaliana* seedlings exposed to VOCs from *Streptomyces lividans* 1326, mentioning acetoin (3-hydroxy-2-butanone) as a possible responsible. Another study showed that *Arabidopsis thaliana* Col-0 seedlings exposed to volatile compounds emitted by *Streptomyces coelicolor* M145 experienced significant morphological changes. In that experiment, it was identified that the volatile bacterial 3-octanone was the bioactive component responsible for this effect. Specifically, the weight of both roots (65%) and shoots (63%) significantly increased with the highest exposure to the volatile component [44]. The growth of secondary roots may play an important role in acquiring and transporting soil resources, interacting with soil organisms, and competing with other plants under stressful environmental conditions [45].

Besides producing volatile compounds, growth-promoting bacteria have multiple mechanisms to enhance plant growth, including producing phytohormones (auxins), improving nutrient acquisition (nitrogen fixation), and suppressing plant diseases (siderophores) [46]. The strain *Streptomyces* sp. PR69 demonstrated the ability to grow in a nitrogen-free medium, indicating its capability to obtain nitrogen from alternative sources. Nitrogen is essential for the formation of amino acids and proteins and plays a crucial role in all living organisms. Actinobacteria are recognized as plant growth-promoting bacteria (PGPB) because they can help metabolize nitrogen, converting it into compounds that plants can absorb [47]. Plants do not have the machinery to obtain nitrogen from the air on their own, but instead rely primarily on free-living bacteria in the soil or bacteria that live symbiotically in nodules on the roots that supply the combined nitrogen in the form of ammonia or nitrates, resulting from nitrogen fixation [48]. In addition to nitrogen fixation, *Streptomyces* sp. PR69 showed siderophore production activity in the Chrome Azurol S (CAS) assay, where the presence of siderophores is indicated by a color change from blue to orange. The chromium azurol S (CAS) assay is a universal colorimetric method that detects siderophores regardless of their structure [49]. Siderophore compounds produced by bacteria can promote plant growth and suppress diseases.

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Numerous studies have demonstrated that siderophore-producing bacteria can increase the yield of various plant species by enhancing iron uptake and act as antagonists by preventing iron availability to pathogens [50]. In experiments using bell pepper plants, the application of *Streptomyces sp*. PR69 resulted in a significant difference in the fresh weight of pepper plants compared to the non-inoculated negative control group. Similar results were observed in tests with *A. thaliana*. It was found that *Streptomyces sp*. PR69 produces volatile compounds that increase root length and the number of lateral roots, which may explain the increase in fresh weight of bell pepper plants. This suggests that PR69 has the potential to serve as a growth promoter, but further evaluations are required to assess its effectiveness and the mechanisms through which it promotes growth. The application of *Streptomyces* sp. PR69 in bell pepper plants led to an increase in the fresh weight of the plant and also increased the survival rate of plants inoculated with *P. capcisi* by 40%. It has been reported that bell pepper plants (*C. annuum L. cv elmas*) treated with *Streptomyces rochei* IT20 and *Streptomyces vinaceus drappus* SS14 reduced disease symptoms caused by *P. capsici* by 40% and 60%. When these two strains were co-cultured, the inhibition of the disease increased to 75%. [24]. Interestingly, it has been reported that soil treated with the supernatant of microorganism can show greater antifungal activity than soil treatment with the microorganism itself. As demonstrated by Chen *et al*. [51], in tests on pepper plants, the culture medium of *Streptomyces plicatus* B4-7, added three hours after inoculation with *P. capsici*, resulted in a 75% reduction in root rot. Trinidad-Cruz et al. [25] conducted an *in vivo* evaluation, demonstrating that the culture supernatants of *Streptomyces* strains ABV38 and ABV45 exhibited a wilting-suppressing effect in pepper plants, achieving survival rates of 77% and 94%, respectively. Although our results are lower than those reported in the literature, further trials are necessary to identify the specific compound responsible for the observed antifungal activity.

Additionally, a biosynthetic gene cluster (BGC) with 100% similarity to naringenin was identified in the genome of *Streptomyces* sp. PR69. Naringenin is a well-known flavonoid metabolite commonly found in plants; however, it has also been recently detected in *Streptomyces clavuligerus* [52]. Plant-derived naringenin is recognized for its numerous beneficial properties, including antioxidant, anti-inflammatory, antiviral, and anticancer effects [53]. Moreover, it exhibits antifungal activity against *Candida albicans* [54]. The isolation and characterization of this compound from *Streptomyces* could potentially lead to more effective antifungal treatments.

While extensive research has been conducted on microorganisms that suppress the growth of *P. capsici*, including *Streptomyces* spp., *Bacillus* spp., *Paenibacillus* spp., *Trichoderma* sp., *Clitocybe nuda, and Aspergillus* sp. [52], field application is limited due to the unstable and poor antimicrobial activity in the soil. Therefore, it remains important to discover highly efficient, broad-spectrum antagonistic *Streptomyces* strains for the development of better biocontrol agents [55]. The lack of efficacy during field application may be due to various factors that can affect the effectiveness of the microorganism. These factors include variability from plant to plant, crop to crop, year to year, and interactions with other microorganisms. The discovery of microorganisms with potential antifungal activity opens interesting possibilities for advances in agriculture. Researchers are optimistic about the potential application of these microorganisms in controlling pathogenic fungi that affect crops, which could lead to increased agricultural yields and reduced reliance on chemical fungicides. For example, the strain *Streptomyces* sp. PR69 has shown significant antifungal activity against several plant pathogens in both *in vitro* and pot tests. Future research aims to explore the full potential of these bacteria, including their application in various crops and environmental conditions. Furthermore, future field trials are necessary to further evaluate their effectiveness. These tests will involve evaluating the performance of the strain in various environmental settings, understanding its interactions with other microorganisms, and assessing its impact on non-target species. Successful results could contribute to the management of plant diseases, offering an environmentally friendly and efficient solution.

5. Conclusions

In conclusion, out of the 60 isolates obtained from Cuatro Cienegas Coahuila, the PR69 strain stands out with potential as a biocontrol agent for *Phytophthora capsici* in bell pepper plants. Its efficacy as a growth promoter has been demonstrated both *in vitro* with *Arabidopsis thaliana* and *in*

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vivo with pepper plants. Morphological characterization and analysis of its genome revealed the identity of the isolate as belonging to the *Streptomyces* genus. Genome sequencing further suggested the presence of several secondary metabolites with potential plant growth-promoting, antimicrobial, and antifungal properties. Future research should focus on identifying the specific metabolites responsible for its biocontrol activity and on developing different formulations for field trials on a broader range of pathogens and crops to validate its efficacy.

6. Patents

No patented.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. **Figure S1**: Number of actinomycetes that showed inhibition against phytopathogens **Fl**: *Fusarium lateritium*, **Mp**: *Macrophomina phaseolina*, **Cc:** *Colletotrichum coccodes*, **Pc:** *Phytophthora capsici*, **Fo**: *Fusarium oxysporum*, **Fs:** *Fusarium solani* and **Sr:** *Sclerotium rolfsii*. **Figure S2**. Effect of volatile compounds produced by *Streptomyces* sp. PR69 on phytopathogens using the sealed plate method. **A)** *Colletotrichum coccodes,* **B)** *Phytophthora capsica,* **C)** *Fusarium oxysporum,* **D)** *Sclerotium rolfsii* **E)** *Fusarium solani* **F)** *Macrophomina phaseolina.* **Figure S3**. Gram staining of isolate PR69 under 100x magnification. **Figure S4**. Macroscopic morphological characteristics of isolate PR69 cultured in different solid and liquid media. **A)** ISP1, **B)** ISP2, **C)** ISP3, **D)** ISP4, **E)** ISP5, **F)** ISP6, **G)** ISP7, **H)** M1, **I)** M3**, J)** SFM and **K)** Chitin. **Figure S5**. Qualitative screening of enzyme production of *Streptomyces* sp. PR69 **A)** Cellulase, **B)** Chitinase, **C)** Protease, and **D)** Lipase activity. **Figure S6**. Qualitative evaluation of *Streptomyces* sp. PR69 for siderophore production, phosphate solubilization and nitrogen fixation. **A**) Chrome-Azurol S Assay **B**) Pikovskaya agar medium **C**) Nitrogen-free medium. **Table S1**. Evaluation of the antagonistic activity of 60 actinomycete isolates against seven phytopathogens. Symbols indicate inhibition zones as follows: - no inhibition zone; + inhibition zone less than 2 mm; ++ inhibition zone greater than 2 mm. **Table S2.** Culture characteristics of Streptomyces sp. PR69 in 11 different solid media. **Table S3.** Culture characteristics of Streptomyces sp. PR69 in 11 different liquid media. **Table S4.** *Streptomyces* genomes used for phylogenetic analysis. **Table S5**. Genomic relationship of Streptomyces sp. PR69 with other Streptomyces by DNA-DNA hybridization (DDH) and average nucleotide identity (ANI).

Author Contributions: PKLR, STZ, MMCG and HAA contributed with methodology, experimental design and experiments. PKLR and LJGW performed the statistical analyses. PKLR and HAA wrote the original draft manuscript. PKLR, HAA and STZ contributed with writing—review and editing. HAA and LJGW contributed with funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The *Streptomyces* sp. PR69 sequence was deposited at NCBI under GenBank accession number GCA_026420845.

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