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ORIGINAL ARTICLE

Antimicrobial activity of *Streptomyces* spp. isolated from bean rhizospheric soil against *Xanthomonas* sp.

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Abstract Species of the genus *Streptomyces* are a promising strategy for bacterial disease management in agriculture crops. The present study aimed to isolate and identify *Streptomyces*-like actinobacteria from rhizospheric soil using physical pretreatments and to evaluate their antimicrobial activity against *Xanthomonas* sp. A rhizospheric soil from a bean plantation was pretreated using dry heat or microwave radiation for isolating actinobacteria. Antimicrobial activity was evaluated using the double agar layer method; 73 isolates exhibiting *Streptomyces* colony characteristics were obtained from the soil after dry heat pre-treatment series (50 BVBZ) and microwaves (23 BVBZMW); 34 BVBZ (68%) isolates inhibited *Xanthomonas* sp. growth with 11.2–35.8 mm halos in diameter. Fifteen (15) of 23 BVBZMW isolates (65%) recorded inhibition zones ranging from 15.7 to 73.6 mm in diameter. The 16S rRNA gene sequence analysis confirmed three isolates with greater antimicrobial activity belonging to the genus *Streptomyces*. *Streptomyces* sp. BVBZ 35, BVBZ 47, and BVBZMW 18 shared greater 16S rRNA gene sequence identity with *S. monomycini* NRRL B-2409^T (100%), *S. nogalater* JCM 4799^T (99.38%) and *S. leeuwenhoekii* C34^T (99.45%), respectively. *Streptomyces* sp. BVBZMW 18, which exhibited the highest antimicrobial activity, could only be cultured after microwave irradiation of the soil. *Streptomyces* species isolated from rhizospheric soil using physical pretreatments are a potentially novel antimicrobial source for *Xanthomonas* disease control.

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PALABRAS CLAVE
Actividad antibacteriana;
Antibiosis;
Actinobacterias;
Mancha bacteriana;
Streptomyces monomycini

Actividad antimicrobiana de *Streptomyces* spp. aislados de la rizósfera de frijol contra *Xanthomonas* sp.

Resumen Las especies del género *Streptomyces* son una prometedora estrategia para el manejo de enfermedades bacterianas en cultivos agrícolas. Este estudio tuvo como objetivo aislar e identificar actinobacterias tipo *Streptomyces* de suelo rizosférico luego de aplicar pretratamientos físicos y evaluar su actividad antimicrobiana contra *Xanthomonas* sp. El suelo rizosférico de una plantación de frijol, pretratado con calor seco o radiación electromagnética con microondas, se usó para aislar las actinobacterias. La actividad antimicrobiana de los aislados se evaluó por el método de la doble capa de agar. Se obtuvieron 73 aislados con las características coloniales de *Streptomyces* luego de los pretratamientos de calor seco ($n = 50$, serie BVBZ) y de microondas ($n = 23$, serie BVBZMW). Treinta y cuatro aislados de la serie BVBZ (68%) y 23 aislados BVBZMW (65%) inhibieron el crecimiento de *Xanthomonas* sp., con halos de inhibición de 11.2 a 35.8 mm y 15.7 a 73.6 mm de diámetro, respectivamente. El análisis de la secuencia del gen ARNr 16S confirmó que los tres aislados con mayor actividad antimicrobiana correspondían al género *Streptomyces*. Los aislamientos BVBZ 35, BVBZ 47 y BVBZMW 18 compartieron la mayor identidad de secuencia (ARNr 16S) con *Streptomyces monomycini* NRRL B-24309^T (100%), *Streptomyces nogalater* JCM 4799^T (99.38%) y *Streptomyces leeuwenhoekii* C34^T (99.45%), respectivamente. *Streptomyces* sp. BVEZMW 18 fue el aislado que exhibió la mayor actividad antimicrobiana y solo pudo cultivarse después de la irradiación del suelo con microondas. Las especies de *Streptomyces* aisladas de suelo rizosférico luego de pretratamientos físicos son una fuente de antimicrobianos potencialmente novedosos para controlar enfermedades provocadas por *Xanthomonas*.

Introduction

Mexico is the third chili pepper (*Capsicum annuum* L.) producer at world level and the second vegetable producer at national level with 3 million 681 thousand tons in 2023[46]. Exports of this agricultural product generated USD 1231 million in 2023[46] profits. Chili, like other economically important cultivars, is affected by diseases caused by phytopathogenic organisms that decrease their productivity[32]. Bacterial spot is one of the diseases that mostly affect chili cultivars, caused by four *Xanthomonas* biotypes: *X. vesicatoria*, *X. euvesicatoria* pv. *euvesicatoria*, *X. euvesicatoria* pv. *perforans* and *X. hortorum* pv. *gardneri*[52]. In Mexico, bacterial spot in chili has been reported to affect commercial plantations in states such as Jalisco, Zacatecas, Michoacán, and Chihuahua[15,26]. Bacterial spot is estimated to cause losses of about 30–66% in crop yield, including higher numbers if the environmental conditions are favorable for the pathogen[8,42]. Disease control is based on the continuous use of agrochemicals based on copper or antibiotics, whose indiscriminate use has caused the survival of resistant strains, thus requiring new products for disease management[3,15].

Bacterial biocontrol agents or their bioactive compounds have been reported as potential alternatives for managing diseases caused by *Xanthomonas* spp.[27,31]. The bacterial genus *Streptomyces* is known to be a source of natural bioactive compounds with antibacterial, anti-biofilm, enzymatic or *quorum sensing* inhibitors, among others[6,20]. Moreover, this genus is widely distributed in terrestrial and aquatic environments, with terrestrial environments containing the greatest percentage of new species (80%) and natural products (60%) found from 2015 to 2022[6].

Streptomyces spp. has been reported to be a biological control agent against bacterial diseases, such as the halo blight of beans caused by *Pseudomonas syringae* pv. *phaseolicola*[3], the pineapple white rot caused by *Dickeya zeae*[1], the bacterial panicle blight of rice caused by *Burkholderia glumae*[47], the bacterial wilt caused by *Ralstonia solanacearum*[18]; and the bacterial spike blight caused by *Rathayibacter tritici*[13]. Likewise, the use of *Streptomyces* spp. has been reported against diseases caused by *Xanthomonas* phytopathogenic species. *Streptomyces* spp., isolated from rhizospheric soils of *Artemisia tridentata*, exhibited broad-spectrum antimicrobial activity against phytopathogenic fungi, oomycetes, and bacteria such as *Rhizoctonia solani*, *Pythium ultimum*, and *X. campestris* pv. *campestris*[13]. Bioactive metabolites produced by *Streptomyces* sp. J46 showed leaf suppression of the bacterial spot caused by *X. arboricola* pv. *pruni*[25]. Soil isolate *S. hawaiiensis* SE4 reduces the severity of the rice bacterial blight caused by *X. oryzae* pv. *oryzae*[16]. *Streptomyces* spp. with antibacterial activity could be an alternative to control the bacterial spot caused by *Xanthomonas* spp. in chili pepper and tomato[10,38,51]. The application of the Actinovate® AG product, which contains *Streptomyces lydicus* WYEC108 as the active ingredient, was effective in controlling the bacterial spot on squash caused by *X. cucurbitae*[49].

Moreover, physical or chemical pretreatments of rhizospheric soil are among the strategies used for the isolation of actinobacteria to promote the growth of species that are otherwise uncultivable using conventional methods.

Figure 1 Rhizospheric soil sample in bean cultivation production in the locality of Bañuelos, Zacatecas, Mexico. (A and B) General view of the bean plantation; (C) detail of bean plant collection on rhizospheric soil.

Regarding microwave radiation, Wang et al.[53] reported that microwave irradiation of soil samples increased the number of cultivable actinobacteria compared to non-irradiated samples. Furthermore, the authors noted that certain actinobacteria of the genus *Streptomyces* were cultivable only after microwave irradiation[53].

Considering the above background, soil *Streptomyces* are a promising strategy for bacterial disease management in agricultural crops. Therefore, the present study aimed to isolate and identify *Streptomyces*-like actinobacteria from rhizospheric soil using physical pretreatments and to evaluate their antimicrobial activity against *Xanthomonas* sp.

Materials and methods

Soil samples and pretreatments

Actinobacteria were isolated from a soil sample that belongs to the Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco (CIATEJ) Soil Collection of the Phytopathology Laboratory. The soil sample was maintained in storage at room temperature for three years and three months. The rhizospheric soil sample was collected from a bean (*Phaseolus vulgaris* L.) plantation in the locality of Bañuelos, Zacatecas (23°22′50.7″N; 103°00′44.7″W) in 2014 (Fig. 1). A portion of 100 g of soil was sieved using a fine 8-mesh steel strainer (2.38 mm). The sieved soil was prepared using two physical methods: dry heat and microwave radiation. The dry heat pretreatment was performed heating 10 g of soil at 70 °C for 1 h in a drying oven[11]. The pretreatment with microwave radiation was conducted in accordance with that described by Wang et al.[53] with slight modifications. In a sterile 15-ml test tube, 10 g of soil were deposited and moistened with 4 ml of distilled sterile water. The test tube was placed within a 1 l precipitation glass beaker with 900 ml of water and then irradiated with microwaves at a power of 100% (1100 W) for 3 min using a microwave oven (2450 MHz, Microwave Mod. NN-989B, Panasonic Inverter System Inside). The prepared soil samples were set to cool down for 30 min at room temperature and then mixed with 90 ml of sterile distilled water for 5 min.

Isolation of actinobacteria

Actinobacteria were isolated following the sowing by extension technique, using potato-dextrose agar and adding 2 g/l of yeast extract (PDA-Y; pH 7.0), 12.5 mg/l of nalidixic acid and 50 mg/l of cycloheximide, as described by Trinidad-Cruz et al.[51]. Soil suspensions were diluted in sterile distilled water until 1/1000 dilution. A 100 μl aliquot of 1/100 up to 1/1000 dilutions was scattered in Petri (90 mm in diameter) PDA-Y plates and incubated at 28 °C for 7–14 days. The colonies with similar colony characteristics in morphology to *Streptomyces*[19] bacteria were streaked on the PDA-Y plates until pure cultures were obtained. The isolates were preserved by transferring spores and/or mycelia into cryotubes containing 25% (v/v) glycerol at −80 °C[44]. Pure isolates were named using the ''BV'' code, indicating Biotecnogía Vegetal in Spanish (translated as Plant Biotechnology); ''BZ'' indicating the sampling site Bañuelos, Zacatecas; and ''MW'' indicating those isolates obtained in the pretreatment using microwaves.

Phytopathogenic bacteria

Xanthomonas sp. BV801 (laboratory strain)[26,36] was cultured in nutrient-yeast extract glycerol agar [NYGA; 5 g/l of Bacto™ Peptone (ThermoFisher Scientific, USA), 3 g/l of yeast extract, 20 g/l of glycerol and 15 g/l agar][5] at 28 °C for two days. For *Xanthomonas* sp. BV801 antimicrobial activity essays, 20 ml of NYG broth (NYGB), was cultured at 28 °C at 200 RPM for 18 h; a bacterial suspension was prepared at an $OD_{600\,nm}$ of 1 with fresh NYGB using a spectrophotometer.

Antimicrobial activity assay

The antibacterial activity of the isolates against *Xanthomonas* sp. BV801 was determined by the double agar assay, as described by Dopazo et al.[7] with slight modifications[51]. Previously, the isolates were cultured on PDA-Y plates at 28 °C for seven days. Agar-mycelium 7.5-mm disks in diameter were prepared for each isolate and inoculated using the point of application method[45] on PDA plates. The agar-mycelium disks were placed at 1 cm of distance from the plate border in x shape and incubated at 28 °C for five days. After incubation, the overlay agar was poured onto the PDA plates, which was prepared mixing 400 μl of *Xanthomonas* sp. BV801 suspension with 4 ml of soft NYGA (6 g/l agar) tempered at 48 °C. As control, the overlay agar mixture was poured onto the PDA plates without actinobacteria. The plates were incubated at 28 °C for two days. The inhibition halo was measured from the center of the agar

disc of each actinobacterium to the periphery of the surrounding growth of *Xanthomonas* sp. BV801. In cases where the growth of *Xanthomonas* sp. BV801 did not fully surround the agar disc due to inhibitory effects; therefore, the radius of the inhibition halo – from the center of the disc to the edge of *Xanthomonas* sp. growth – was measured. In both cases, results were expressed as the diameter of the inhibition halo. The diameter or radius of the inhibition zone caused by *Xanthomonas* sp. BV801 was measured using a digital Vernier caliper.

Experimental design and statistical analysis

The experiments were performed using a completely randomized experimental design. For each isolate and control, three replicates were used. Data of the inhibition halos were analyzed using ANOVA (analysis of variance) and Tukey's ($p \leq 0.05$) multiple comparisons of means, using StatGraphics Centurion XVI (version 16.2.04, StatPoint Technologies, Inc. USA) statistical package.

DNA extraction

The BVBZ 35, BVBZ 47, and BVBZMW 18 isolates were cultured in 25 ml of potato-dextrose broth adding up 2 g/l of yeast extract (pH 7.0) at 28 °C with orbital agitation at 200 RPM for five days. The bacterial cultures were centrifuged at 13 000 RPM for 10 min. Each isolate biomass was frozen at −80 °C for 6 h, lyophilized and ground in a mixing grinder at 25 Hz for 5 min (MM 400, Retsch®, Haan, Germany). DNA was extracted from 1-mg biomass using the Dynabeads® DNA DIRECT™ Universal (ThermoFisher Scientific, Waltham, USA) kit as recommended by the producer and stored at −20 °C until subsequent use.

Molecular identification of the actinobacterial strains

The identification of the BVBZ 35, BVBZ 47, and BVBZMW 18 isolates was performed by amplifying the 16S rRNA gene for the polymerase chain reaction (PCR) using oligonucleotides fD1 (5′-CCGAATTCGTCGACAACAGAGTTTGATCCTGGCTCAG-3′) and rD1 (5′-CCCGGGATCCAAGCTTAAGGAGGTGATCCAGCC-3′)[54]. The PCR was performed in a total volume of 50 μl that contained buffer PCR 1×, 1.5 mM de $MgCl_2$, 0.16 mM of each dNTP, 12 pmol of each oligonucleotide, 1.25 *Taq* DNA polymerase units (High Fidelity PCR Enzyme Mix, Fermentas, Waltham, MA, USA) and 4 μl of the DNA extraction. PCR was performed in a thermocycler under the following conditions: one initial denaturalization at 94 °C for 3 min; followed by 35 amplification cycles at 94 °C for 30 s, 58 °C for 30 s and 72 °C for 1.5 min, and one final extension at 72 °C for 10 min. The amplification products were subjected to electrophoresis in agarose gel at 1.2% stained with GelRed® (Biotium, Inc., Fremont, CA, USA) at 90 V for 1 h and visualized under ultraviolet fluorescence. The amplified fragments were purified using the Wizard® PCR Preps DNA Purification Resin (Promega, Madison, WI, USA) kit as recommended by the producer and sequenced by Sanger in Macrogen Inc. (Seoul, KR), using the oligonucleotides 800R (5′-TACCAGGGTATCTAATCC-3′), 1100R (5′-GGGTTGCGCTCGTTG-3′) and rD1.

The sequences obtained from each isolate were assembled, analyzed, and cut using SnapGene® (ver. 4.3.7, GSL Biotech, Chicago, IL, USA). The 16S rRNA consensus sequences of each isolate were compared with the material type sequences available in the GenBank database using the BLASTn (Basic Local Alignment Search Tool) program of the NCBI (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/) website. The sequences of the closest species to the isolates were discharged from the GenBank database[41]; the similarity of the 16S rRNA gene sequence values were calculated using the Pairwise Nucleotide Sequence Alignment For Taxonomy tool available in the EzBioCloud (https://www.ezbiocloud.net/tools/pairAlign) website. The isolate sequences and those that recorded similar values greater than 99% were aligned in a multiple form using the MUSCLE (predetermined parameters) algorithm. The phylogenetic tree was constructed using the neighbor-joining method[40] based on the Kimura-2 parameters nucleotide substitution model[22], and the branch trust value was determined by the bootstrap analysis, with 1000 resamplings[12]. All the analyses were performed in MEGA11[48] software. The 16S rRNA gene sequences of *Streptomonospora salina* YIM 90002^T (AF178988) and *Streptosporangium roseum* DSM 43021^T (CP001814) were used as an external group. The 16S rRNA gene of the BVBZ 35, BVBZ 47, and BVBZMW 18 isolates were deposited in the GenBank database under accession numbers MT756003, OQ569350, and OQ569351, respectively.

Results

Physical pretreatment effects on isolated actinobacteria

Of the 73 isolates obtained, 50 (68%) were recovered (BVBZ 01 to BVBZ 50 series) from the pretreated soil sample with dry heat and 23 (32%) were isolated from the microwave-irradiated samples (BVBZMW 01 to BVBZMW 23 series).

Antimicrobial activity of the isolates

Of the BVBZ series, 34 (68%) isolates showed antimicrobial activity against *Xanthomonas* sp. BV801 observed as inhibition halos in the confrontation assays (11.2–35.8 mm in diameter) (Table 1 and Fig. 2). The BVBZ series isolates caused significant ($p \leq 0.05$) differences in *Xanthomonas* sp. BV801 inhibition halos (Table 1), of which those of BVBZ 35 and BVBZ 47 recorded the greatest inhibition halos with 35.8 ± 0.5 and 34.8 ± 0.7 mm in diameter, respectively.

On the other hand, of the BVBZMW series, 15 (65%) of 23 inhibited *Xanthomonas* sp. BV801 growth with halos from 15.7 to 73.6 mm in diameter (Table 2 and Fig. 2). The greatest significant differences in *Xanthomonas* sp. BV801 inhibition ($p \leq 0.05$) were recorded for isolate BVBZMW 18 (73.6 ± 2.6 mm), followed by BVBZMW 17 (47.4 ± 1.9 mm) and BVBZMW 14 (33.2 ± 1.9 mm) isolates.

Table 1 Inhibition halos (HI) of actinobacteria of the BVBZ series recorded during the antimicrobial assay activity against *Xanthomonas* sp. BV801.

Isolate	HI (mm)[a]	Isolate	HI (mm)[b]	Isolate	HI (mm)
BVBZ 01	13.8 ± 1.2^{opq}	BVBZ 18	30.6 ± 1.5^{b}	BVBZ 35	35.8 ± 0.5^{a}
BVBZ 02	$16.0 \pm 1.0^{l-o}$	BVBZ 19	ND	BVBZ 36	23.7 ± 0.5^{de}
BVBZ 03	ND	BVBZ 20	$21.3 \pm 2.8^{e-i}$	BVBZ 37	$22.8 \pm 1.0^{d-g}$
BVBZ 04	28.8 ± 0.6^{bc}	BVBZ 21	ND	BVBZ 38	ND
BVBZ 05	$17.1 \pm 0.6^{j-o}$	BVBZ 22	$16.9 \pm 0.8^{j-o}$	BVBZ 39	$19.9 \pm 1.5^{f-k}$
BVBZ 06	$15.1 \pm 0.2^{m-p}$	BVBZ 23	$14.3 \pm 0.5^{n-q}$	BVBZ 40	$17.6 \pm 0.5^{j-n}$
BVBZ 07	$14.1 \pm 0.4^{\tilde{n}-q}$	BVBZ 24	27.5 ± 0.3^{bc}	BVBZ 41	23.2 ± 0.6^{def}
BVBZ 08	$16.6 \pm 0.2^{k-o}$	BVBZ 25	$14.4 \pm 0.6^{n-q}$	BVBZ 42	13.9 ± 0.5^{opq}
BVBZ 09	$21.7 \pm 1.4^{e-h}$	BVBZ 26	$21.2 \pm 1.1^{e-i}$	BVBZ 43	ND
BVBZ 10	23.7 ± 1.9^{de}	BVBZ 27	ND	BVBZ 44	$19.0 \pm 0.1^{h-l}$
BVBZ 11	ND	BVBZ 28	ND	BVBZ 45	$17.4 \pm 1.3^{j-\tilde{n}}$
BVBZ 12	$19.4 \pm 1.0^{g-l}$	BVBZ 29	12.5 ± 1.1^{pq}	BVBZ 46	11.2 ± 0.3^{q}
BVBZ 13	$15.4 \pm 0.9^{m-p}$	BVBZ 30	ND	BVBZ 47	34.8 ± 0.7^{a}
BVBZ 14	$15.4 \pm 1.6^{m-p}$	BVBZ 31	$18.0 \pm 0.6^{i-m}$	BVBZ 48	ND
BVBZ 15	ND	BVBZ 32	ND	BVBZ 49	ND
BVBZ 16	25.8 ± 0.3^{cd}	BVBZ 33	$20.2 \pm 0.4^{f-j}$	BVBZ 50	ND
BVBZ 17	ND	BVBZ 34	ND	–	–

ND: non-detectable.
[a] The values of the inhibition halos represent the median of three biological replicates ± standard deviation.
[b] Measurements with different letters in superscript are statistically different according to Tukey's test ($p \leq 0.05$).

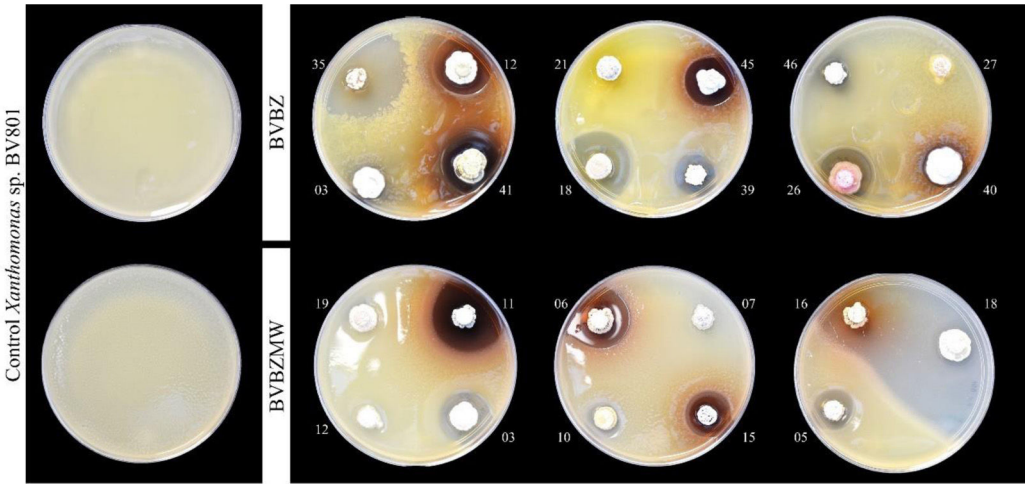

Figure 2 Antimicrobial activity assay *in vitro* of BVBZ and BVBZMW series against *Xanthomonas* sp. BV801 by double layer agar method. The number on the antimicrobial test indicates the isolate code number.

Phylogenetic analysis of *Streptomyces* type isolates

The results of the BLASTn program for the 16S rRNA gene sequences of isolates BVBZ 35 (1125 bp), BVBZ 47 (1289 bp), and BVBZMW 18 (1462 bp) confirmed that they belong to genus *Streptomyces*. Isolate BVBZ 35 shared the greatest rRNA 16S gene sequence identity with *S. monomycini* NRRL B-24309^{T} (100%), followed by *S. olivaceiscleroticus* DSM 40595^{T} (99.73%) and *S. niger* NBRC 13902^{T} (99.73%). For isolate BVBZ 47, the closest type of strain was *S. nogalater* JCM 4799^{T} (99.38%) with rRNA 16S gene sequence similarity with *S. eurythermus* ATCC 14975^{T}, *S. lavenduligriseus* NBRC 13405^{T}, *S. gougerotii* NBRC 13043^{T}, and *S. rutgersensis* NBRC 12819^{T}, sharing a 99.07% similarity value. The comparative analysis of the 16S rRNA gene sequence showed that isolate BVBZMW 18 shared the greatest similarity values with *S. leeuwenhoekii* C34^{T} (99.45%), *S. harenosi* PRKS01-65^{T} (99.38%), and *S. mexicanus* NBRC 100915^{T} (99.25%).

The phylogenetic analysis based on the 16S rRNA gene sequence indicated that isolates BVBZ 35, BVBZ 47 and BVBZMW 18 were grouped with their closest *Streptomyces* genus neighbors (Fig. 3). *Streptomyces* sp. BVBZ 35 formed a well-supported clade with its closest relative, *S. monomycini* NRRL B-24309^{T}, exhibiting 100% sequence identity and a high bootstrap value of 99%. *Streptomyces* sp. BVBZMW 18 formed a distinct branch within the phylogenetic tree

Table 2 Inhibition halos (HI) of actinobacteria of the BVBZMW series recorded during the antimicrobial assay against *Xanthomonas* sp. BV801.

Isolate	HI (mm)[a]	Isolate	HI (mm)[b]
BVBZMW 01	21.9 ± 1.1^{fgh}	BVBZMW 13	ND
BVBZMW 02	ND	BVBZMW 14	33.2 ± 1.9^{c}
BVBZMW 03	18.3 ± 0.4^{hij}	BVBZMW 15	20.8 ± 0.5^{ghi}
BVBZMW 04	15.7 ± 0.9^{j}	BVBZMW 16	30.7 ± 2.2^{cd}
BVBZMW 05	16.6 ± 0.1^{ij}	BVBZMW 17	47.4 ± 1.9^{b}
BVBZMW 06	27.0 ± 0.5^{de}	BVBZMW 18	73.6 ± 2.6^{a}
BVBZMW 07	ND	BVBZMW 19	ND
BVBZMW 08	23.3 ± 1.5^{efg}	BVBZMW 20	ND
BVBZMW 09	24.6 ± 1.1^{efg}	BVBZMW 21	ND
BVBZMW 10	ND	BVBZMW 22	16.4 ± 1.0^{ij}
BVBZMW 11	25.8 ± 1.7^{ef}	BVBZMW 23	21.0 ± 1.1^{gh}
BVBZMW 12	ND	–	–

ND: non-detectable.
[a] The values of the inhibition halos represent the measurements of three biological replicates ± deviation standard.
[b] Measurements with different letters in superscript are statistically different according to Tukey's test ($p \leq 0.05$).

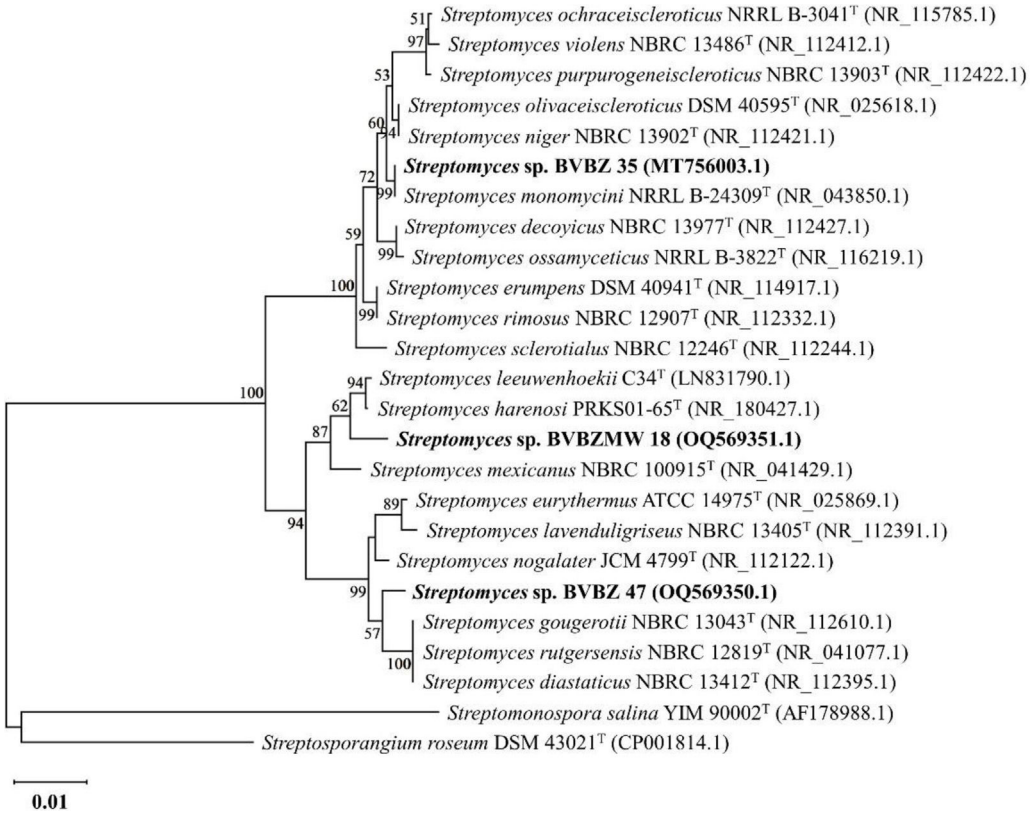

Figure 3 Neighbor joining phylogenetic tree based on the 16S rRNA gene sequence showing the phylogenetic relationships among *Streptomyces* strains isolated from soil (BVBZ 35, BVBZ 47, and BVBZMW 18) and their closest relatives. The bootstrap values (>50%) are shown next to the branches. The 16S rRNA gene sequence of *Streptomonospora salina* YIM 90002^T and *Streptosporangium roseum* DSM 43021^T were used as external group. GeneBank accession numbers are shown in parentheses. Bar = 0.01 substitutions per nucleotide position.

and clustered with *S. leeuwenhoekii* C34^T (99.45% similarity) and *S. harenosi* PRKS01-65^T (99.38% similarity), with a bootstrap support value of 62%. Unlike the two previous isolates, the phylogenetic analysis indicated that the subclade formed by *Streptomyces* sp. BVBZ 47 was closely related to *S. gougerotii* NBRC 13043^T (99.07%), *S. rutgersensis* NBRC 12819^T (99.07%), and *S. diastaticus* NBRC 13412^T (99.06%) (Fig. 3).

Discussion

The soil from a bean plantation showed *Streptomyces* isolates with a weak to strong antibacterial activity against *Xanthomonas* sp. BV801. From *Agave cupreata* agricultural soils, Rincón-Enríquez et al.[37] found that 14 *Streptomyces*-type isolates inhibited the growth of *P. syringae* pv. *phaseolicola* 1448A by 50–100%. Encheva-Malinova et al.[10] reported that *Streptomyces* spp., isolated from Antarctic soils, produced inhibition halos ranging from 11 to 40 mm in diameter against *Xanthomonas* phytopathogenic strains. In a previous study, *Streptomyces* isolates obtained from avocado cv. Hass tree soil recorded similar inhibition halos (15.5–62.7 mm in diameter) against *Xanthomonas* sp. BV801[51]. Other studies mentioned that *S. lydicus* 5US-PDA8 showed inhibition percentages from 9.2 to 70.8% against several *Xanthomonas* strains causing bacterial chili spot[38], whereas *S. lydicus* WYEC 108 recorded an inhibition halo of 10.6 mm in diameter against *X. cucurbitae*[49]. Soil isolate *Streptomyces* sp. J46 showed antibacterial activity against *X. arboricola* pv. *pruni*[25]. In the present study, the result of a significant antimicrobial activity against *Xanthomonas* sp. BV801 suggests that *Streptomyces* (BVBZ 35, BVBZ 47, and BVBZMW 18) are potential candidates for the biological control of these phytopathogens; nevertheless, *in vivo* studies are required to subsequently prove this activity.

On the one hand, actinobacteria, especially those of the genus *Streptomyces*, are a prominent bioactive secondary metabolite source with different biological activities[29]. In the present study, *Streptomyces* sp. BVBZ 35, closely related to *S. monomycini* NRRL B-24309^T, showed antimicrobial activity (inhibition halo of 35.8 mm in diameter) against *Xanthomonas* sp. BV801 phytopathogen bacteria. This activity could be due to the production of secondary metabolites. Similarly, Meidani et al.[28] reported *S. monomycini* ATHUBA 220 antimicrobial activity against non-phytopathogenic bacteria and yeast, which showed the greatest activity against *Lactobacillus fermentum* ATCC 9338 and *Saccharomyces cerevisiae* DSM 70449. As reported by Sadegui et al.[39], *S. monomycini* C 801 showed antimicrobial activity against the phytopathogenic oomycete *Phytophthora drechsleri*. Recently, a *S. monomycini* RVE129 purified bioactive compound, known as setomycin, recorded antibacterial activity against Gram-positive and Gram-negative bacteria[9]. In other studies, antibacterial secondary metabolite production has been reported by *S. monomycini* NRRL B-24309^T as monomycine[19], argolaphos A and B[17]. Subsequent studies are necessary to determine which metabolites of *Streptomyces* sp. BVBZ 35 strain may be implied in *Xanthomonas* sp. BV801 *in vitro* growth inhibition.

On the other hand, *Streptomyces* sp. BVBZ 47 shared the greatest similarity with *S. nogalater* JCM 4799^T (99.38%); however, the phylogenetic analysis indicated that it was closely related to *S. gougerotii* NBRC 13043^T, *S. rutgersensis* NBRC 12819^T, and *S. diastaticus* NBRC 13412^T. Recently, Komaki and Tamura[23] reported that a polyphasic taxonomic study reclassified *S. gougerotii* NBRC 13043^T and *S. rutgersensis* NBRC 12819^T as subsequent heterotypical synonyms of *S. diastaticus* NBRC 13412^T. Based on these findings, the closest *Streptomyces* sp. BVBZ 47 relative was *S. diastaticus* NBRC 13412^T. The production of bioactive compounds for *S. diastaticus* has been reported in several studies, with compounds such as rimocidin, CE-108, oligomycin A and B showing antimicrobial activity against fungi, yeast, and *P. capsici*[33,55]. Rutamycin (also known as oligomycin D), isolated from *S. rutgersensis* NBRC 12819^T, showed antifungal but not antibacterial activity[43]. The production of antimicrobial secondary metabolites of other closer relatives of *Streptomyces* sp. BVBZ 47 have been reported previously. *S. nogalater* JCM 4799^T produces nogalamycin[50], a powerful antibiotic against Gram-positive bacteria[4].

From one *S. lavenduligriseus* strain, Filipin III was purified and identified in three new polyene macrolides, which exhibited from strong to weak antifungal activity against *Candida albicans*[56]. The antimicrobial activity of the closest *Streptomyces* sp. BVBZ 47 relatives, agrees with that observed in the present study in which the isolated strain showed strong antimicrobial activity (inhibition zone of 34.8 mm in diameter) against *Xanthomonas* sp. BV801 Gram-negative bacteria.

The phylogenetic analysis placed *Streptomyces* sp. BVBZMW 18 in a clade with *S. leeuwenhoekii* C34^T and *S. harenosi* PRKS01-65^T, *S. leeuwenhoekii* C34^T, originally isolated from the Atacama Desert, is known to produce the antibiotics chaxamycins A–D, chaxalactins A–C, and hygromycin A[34,35] antibiotics. Among the chaxamycins, chaxamycin D showed a strong antibacterial activity against *Escherichia coli* ATCC 25922 and several strains of *Staphylococcus aureus*[34] whereas chaxalactines A-C recorded a strong antibacterial activity against Gram-positive (*St. aureus* ATCC 25923, *Listeria monocytogenes* ATCC 19115 and *Bacillus subtilis* NCTC 2116) bacteria but weak activity against Gram-negative (*E. coli* ATCC 25922 and *Vibrio parahemolyticus* NCTC 10441) bacteria[35]. Kusuma et al.[24] indicated that the genome of *S. harenosi* PRKS01-65^T, a bacterium isolated from a sand dune, contains several biosynthetic genes with the potential to produce new natural products.

Streptomyces type strains, closely related to the *Streptomyces* strains identified in this study, have been shown to be a source of secondary metabolites with antimicrobial activity. However, Kiepas et al.[21] reported that, due to the complex taxonomy of the *Streptomyces* genus, 16S rRNA gene sequences, as a single molecular marker, are not suitable for species-level assignment. The authors suggest that other approaches, such as multilocus sequence analysis (MLSA) or core genome MLSA (cgMLSA) are needed, as they are more accurate for taxonomic assignment.

In our study, 16S rRNA gene sequencing enabled assignment of the selected isolates to the genus *Streptomyces*, and phylogenetic analysis indicated that their closest relatives are secondary metabolite producers with antimicrobial activity. However, further studies are needed to assign the *Streptomyces* spp. from this study to the species level (either as a previously described species or as a new taxon). Furthermore, it is key to identify the possible secondary metabolites involved in the antimicrobial activity of *Streptomyces* spp.

The results of the present study demonstrated that the pretreatment of the soil samples by the physical methods were effective for obtaining *Streptomyces* isolates with antibacterial activity against *Xanthomonas* sp. BV801. Particularly *Streptomyces* sp. BVBZMW 18 could only be cultured after irradiating the soil sample with

microwaves, which was confirmed by the molecular identification of isolates with significant antimicrobial activity. Likewise, Wang et al.[53] observed that some antagonist antibacterial strains belonging to the genera *Streptomyces*, *Streptosporangium*, and *Lentzea* were only culturable from microwave-irradiated soil samples.

In another study, Niyomvong et al.[30] indicated that the majority of the rare actinobacteria from the genera *Microbispora*, *Micromonospora*, *Nocardia*, and *Nonomuraea* were isolated from microwave-irradiated soil samples compared to non-irradiated ones. Recently, Arango et al.[2] and Hamedi et al.[14] reported that the greatest number of actinobacterial type isolates were obtained from the microwave pretreatment. In the present study, the opposite effect was observed, where the pretreatment with microwaves recorded fewer than half of the *Streptomyces* (23) type isolates, compared with the pretreatment using dry heat (50). Although the pretreatment with microwaves has been used in several studies as a tool for isolating actinobacteria, to our knowledge, it is still unknown how the irradiation of the microwaved samples affects the culturable actinobacteria[53].

The *Streptomyces* type isolates obtained from the bean rhizospheric soil showed antimicrobial activity against *Xanthomonas* sp. BV801, which caused the chili pepper bacterial spot. Among them, *Streptomyces* sp. BVBZ 35, BVBZ 47, and BVBZMW 18 showed a significant antimicrobial activity. The electromagnetic radiation pretreatment was an effective tool for obtaining *Streptomyces* type isolates with antimicrobial activity. This could be observed in the *Streptomyces* sp. BVBZMW 18 strain, which stood out for its inhibitory activity. *Streptomyces* sp. BVBZ 35, BVBZ 47, and BVBZMW 18 are potential candidates for the biological control of the causal agent of bacterial spot in chili pepper crops. Likewise, other unidentified isolates from the microwave-irradiated soil, such as BVBZMW 14, BVBZMW 16 and BVBZMW 17, could be considered in subsequent studies for their antimicrobial activity, similar to that of *Streptomyces* obtained from soil pretreated with dry heat.

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Conflict of interests

The authors declare having no conflict of interest.

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