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Streptomycetes isolated from the rhizosphere of agricultural soils in the Valle del Mezquital, Mexico: *in vitro* production capacity of growth-promoting metabolites and antagonists against phytopathogenic fungi

Streptomicetos aislados de la rizosfera de suelos agrícolas del Valle del Mezquital, México: capacidad de producción *in vitro* de metabolitos promotores de crecimiento y de antagonismo contra hongos fitopatógenos

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ABSTRACT

The Valle del Mezquital, Mexico, is one of the main crop-producing areas in the country. This valley is irrigated by wastewater, primarily from Mexico City. This characteristic creates unique environmental conditions that can impact microorganisms in agricultural soils. In this study, actinomycetes were isolated from the rhizosphere of agricultural soils to characterize them and determine their potential as plant growth promoters and inhibitors of phytopathogenic fungi. Thirteen strains of the *Streptomyces* genus were isolated, and in vitro studies revealed that all could produce indoleacetic acid, siderophores, and organic acids. *Streptomyces thinghirensis* and *Streptomyces lateritius* solubilized phosphates, while *Streptomyces lusitanus* produced HCN. Previously unreported strains with antagonistic activity against plant pathogenic fungi were identified, the main ones being *Streptomyces pseudogriseolus*, *Streptomyces atrovirens*, *Streptomyces lateritius*, *Streptomyces nigra*, and *Streptomyces griseoplanus*. The results obtained provide new knowledge into Streptomycetes that have not been previously studied and may offer tools for the biological control of diseases caused by phytopathogenic fungi, as well as strategies for enhancing productivity under conservation tillage conditions.

Keywords: Actinomycetes; Conservation agriculture; Wastewater irrigation.

RESUMEN

El Valle del Mezquital, México, es una de las principales zonas productoras de diferentes cultivos en el país. Este Valle es irrigado por las aguas residuales provenientes principalmente de la Ciudad de México, esta característica genera condiciones ambientales únicas que pueden incidir sobre los microorganismos de los suelos agrícolas. En el presente trabajo, se aislaron actinomicetos de la rizósfera de suelos agrícolas con el fin de caracterizarlos y determinar su potencial como promotores del crecimiento vegetal y de inhibición de hongos fitopatógenos. Se aislaron 13 cepas del género de *Streptomyces,* a través del estudio *in vitro* se observó que todas fueron capaces de producir ácido indol acético, sideróforos y ácidos orgánicos. *Streptomyces thinghirensis* y *Streptomyces lateritus* fueron capaces de solubilizar fosfatos y *Streptomyces lusitanus* de producir HCN. Se identificaron cepas no reportadas con anterioridad con capacidad antagónica contra hongos fitopatógenos siendo los principales *Streptomyces pseudogriseolus*, *Streptomyces atrovirens*, *Streptomyces lateritius*, *Streptomyces nigra* y *Streptomyces griseoplanus*. Los resultados obtenidos aportan nuevos conocimientos sobre Streptomycetes que no han sido estudiados previamente y pueden ofrecer herramientas para el control biológico de enfermedades causadas por hongos fitopatógenos, así como estrategias para mejorar la productividad en condiciones de labranza de conservación.

Palabras clave: Actinomicetos, Agricultura de conservación, Riego con aguas residuales.

INTRODUCTION

Actinomycetes constitute a heterogeneous group of microorganisms capable of utilizing simple or complex carbon sources and organic molecular compounds such as acids, polysaccharides, lipids, proteins, and aliphatic hydrocarbons. They also utilize ammonium, nitrates, amino acids, peptones, and a large number of proteins as nitrogen sources (Leveau and Bouix, 2000).

Direct mechanisms occur when bacteria synthesize metabolites that facilitate plant growth or increase the availability of nutrients required for plant metabolism, and improve plant nutrition. They include nitrogen fixation, synthesis of phytohormones, vitamins and enzymes, phosphorus solubilization, nitrite production, nitrate accumulation, and siderophore secretion (Gómez *et al*., 2012). In addition to these characteristics, they can also exhibit antagonistic activity, which is an interaction between microorganisms where one interferes with the other, causing the loss or reduction of activity of one of them. This is the basis for true biological control of phytopathogenic microorganisms in plants.

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The Valle del Mezquital (VM) is a unique ecosystem located southeast of Hidalgo, covering 642,653 ha, 60 km northwest of the Mexico City Metropolitan Area. The VM is the region in the world with the second-highest use of wastewater in the agricultural sector. Paradoxically, the economic development of the area is closely linked to the use of this resource in agriculture (Durán-Álvarez *et al*., 2021). Conventional agricultural practices applied in the VM are based on intensive tillage techniques, residue burning, and plowing (Zhang *et al*., 2018), which has generated problems of soil alkalization and desertification. In the VM, there are experimental platforms whose objective is to apply agricultural soil recovery practices. These platforms have been in existence for around 20 years, and strictly adhere to the conservation of agriculture practices. Furthermore, analyzing the biota of agricultural soils provides a better understanding of the biogeochemical cycles that occur. Additionally, it is possible to infer the potential relationships between microorganisms and their impact on crop productivity (Castañeda-Cisneros *et al*., 2024).

Some authors have described how irrigation water has a significant impact on soil microbiota, with prevailing species including Proteobacteria, Firmicutes, and Actinobacteria (Jiang *et al*., 2024), which primarily specialize in degrading toxic contaminants. Therefore, the actinomycetes present in the rhizosphere of crops in the area, as well as the potential impact of wastewater irrigation on these microorganisms, are of interest. This study aimed to study actinomycetes isolated from agricultural soils irrigated with wastewater and subject to conservation agriculture in the VM. The production of molecules involved in plant growth by each microorganism was studied, as well as their possible antagonistic effect against phytopathogenic microorganisms present in the VM.

MATERIAL AND METHODS

Microorganisms, strain propagation, and conservation

The method used for isolating actinomycetes was by serial plate dilutions (Hayakawa, 2008). One g of dry soil from each sample was vigorously shaken in 9 mL of sterile distilled water and heated at 50 °C for 30 min (Oskay *et al*., 2004). This solution (10^{-1}) was diluted five times (10^{-5}) using sterile distilled water. A 50 µL aliquot was taken and distributed onto Petri dishes containing ISP2 (4 g/L yeast extract, 10 g/L malt extract, 4 g/L dextrose, and 20 g/L bacteriological agar at pH 7.3) or ISP3 (20 g/L oats, 1 mL trace salt solution [0.1 g $FeSO_4 \cdot 7 H_2O$, 0.1 g $MnCl_2 \cdot 4 H_2O$, 0.1 g $ZnSO_4 \cdot 7 H_2O$] in 100 mL of distilled water, and 18 g/L bacteriological agar at pH 7.2) media. All strains were propagated both media. The media were supplemented with nystatin (50 µg/mL) and nalidixic acid (25 µg/mL) to inhibit fungal and bacterial contamination. The plates were incubated at 28 °C for 28 d and replated. All plates were reseeded monthly and kept refrigerated until use. The strains *Streptomyces atrovirens*, *Streptomyces lateritius*, *Streptomyces* sp. A-C7, *Streptomyces pseudogriseolus*, *Streptomyces heliomycini*, *Streptomyces anulatus*, *Streptomyces virginiae*, *Streptomyces nigra* CB-8, *Streptomyces griseoplanus* B4-M10, *Streptomyces flavogriseus*, *Streptomyces thinghirensis*, *Streptomyces lusitanus* and *Streptomyces griseoaurantiacus*, were used (Castañeda-Cisneros *et al*., 2020).

In vitro plant growth promotion activities

Phosphate solubilization

The qualitative activity of phosphate solubilization was analyzed on Pikovskaya (PVK) agar (Pikovskaya, 1948). Each strain was inoculated in the center of the plate by puncturing it with a sterile toothpick. A PVK agar plate served as a control. A clear zone around the colony was considered positive after 14 d of incubation at 28 °C. The phosphate solubilization index (SI) was calculated from the ratio of the total diameter (colony plus halo area) to the colony diameter, according to a formula reported by Chouyia *et al*. (2020) expressed in mm.

Organic acid production

Phosphate-solubilizing actinomycetes were transferred to Sandar-Rao and Shina (SRSM-1) agar (Sandar and Shina, 1963). Each strain was inoculated onto the center of the plate using a sterile toothpick and incubated at 28 °C for 14 d. An uninoculated SRSM-1 agar plate served as a control. A color change in the solubilization halo from purple to yellow indicated a positive result. Results were reported using the phosphate solubilization index, expressed in mm. Strains were classified based on their SI as low (SI < 2), intermediate (2 < SI < 4), and high (SI > 4) solubilization (Elias *et al*., 2016).

Siderophore production

Siderophore production was determined on Chromium Azurol S (CAS) agar, according to the method described by Schwyn and Neilands (1987) and modified by Hu and Xu (2011). Strains were inoculated by pinprick in the center of the plate and incubated at 28 °C for 7 d. An uninoculated plate was used as a control. A colony with the formation of an orange-yellow halo was considered positive for siderophore production. Activity diameters were reported using the following scale: 0 = negative; 1 = 1–10 mm halo; 2 = 11–20 mm halo; 3 = 21–30 mm halo; 4 = 31–40 mm halo; and 5 = 41–50 mm halo (Sreevidya *et al*., 2016).

Hydrogen cyanide production

The qualitative determination of hydrogen cyanide (HCN) was evaluated using the method described by Lorck (1948). Strains were streaked onto modified Bennett agar plates (Jones, 1949) (glycine 4.4 g/L, meat extract 1 g/L, enzymatically hydrolyzed casein 2 g/L, yeast extract 1 g/L, and bacteriological agar 15 g at pH 7.3). A Whatman No. 1 filter paper was placed under the lid of the Petri dish, previously soaked in 0.5 % picric acid in 2 % sodium carbonate for 1 minute. All plates were sealed with parafilm and incubated at 28 °C for 7 d. A color change expressed a positive indicator of HCN production in the filter paper; the scale was negative (-) = no color change; weak producer (+) = yellow to light reddish brown; Moderate producer (++) = yellow to medium reddish brown, and heavy producer (+++) = yellow to dark reddish brown (Sreevidya *et al*., 2016).

Indoleacetic acid production

Indoleacetic acid (IAA) production was estimated using the Gordon and Weber (1951) method. Actinomycete strains were grown on ISP2 agar and incubated at 28 °C for 10 d. One mm diameter discs were cut from each plate using a sterile scalpel and transferred to Erlenmeyer flasks containing 100 mL of ISP2 broth supplemented with 0.2 % L-tryptophan. The flasks were maintained at 28 °C with continuous shaking at 125 rpm. After 15 d of incubation, the resulting cultures were centrifuged at 11,000 rpm for 15 min. The reaction consisted of placing 1 mL of supernatant with 2 mL of Salkowski reagent in test tubes, which were incubated for 25 min in the dark at 28 °C. The appearance of a pink-red color indicated the production of IAA (Naik and Gupta, 2020). Absorbance was measured at a wavelength of 530 nm, and the concentration was reported using a calibration curve with IAA as the standard, expressed in micrograms per milliliter (µg/mL). Distilled water was used as the reaction blank.

Ammonia production

To evaluate the capacity of actinomycetes as ammonia producers, the methodology proposed by Cappucino and Sherman (1992) was followed. The strains were inoculated into Erlenmeyer flasks with 10 mL of peptone water (1 g/L peptone and 8.5 g/L NaCl at pH 7) and maintained at 28 ºC with continuous shaking at 120 rpm for 15 d. Then, 0.5 mL of Nessler reagent was added to each culture, and the development of a yellow to brown color was considered a positive result.

Antagonistic activity against plant pathogenic fungi

The *in vitro* antagonistic activity of actinomycetes against 10 plant pathogenic fungi that impact crops was evaluated. These fungi were identified and donated by Dr. Issac Juan Luna Romero of the Plant Pathology Laboratory of the National Polytechnic Institute: *Colletotrichum lindemuthianum*, *Pestalotia* spp., *Helminthosporium maydis*, *Curvularia* spp., *Colletotrichum* spp., *Fusarium* spp., *Alternaria dauci*, *Phytophthora* spp., *Sclerotinia sclerotiorum*, and *Fusarium oxysporum*. The strains were grown on Potato Dextrose Agar (PDA) at 28 °C for 10 d or until complete sporulation. Each fungus was inoculated by puncturing it with a sterile bacteriological loop in the center of a PDA plate, and seven different actinomycetes were plated around it. Control plates were prepared without inoculating the actinomycetes to evaluate fungal growth. The plates were kept at 28 °C for 10 d. In the antagonism experiments, inhibition was identified by the absence of contact between the actinomycetes and the fungus. This was expressed as a percentage of inhibition based on the difference between the diameter of the fungal mycelium on the control plates and the diameter of the fungal mycelium on the plates containing the different actinomycetes. The results were expressed as described by Kumar *et al*. (2014) as: - = no activity; + = weak activity (< 25 % inhibition); ++ = moderate activity (25-50 % inhibition); and +++ = strong activity (> 50 % inhibition).

Data analysis

The mean and standard deviation were calculated based on the replicates of each experiment. Data were analyzed using one-way analysis of variance (ANOVA). Significant differences between means were determined using Tukey's method with a 95 % confidence interval, as calculated with Minitab 18.1 software (Minitab Inc., State College, PA, USA). All analyses were performed in triplicate.

RESULTS

Production of metabolites involved in plant growth

The first stage of the study focused on analyzing the in vitro properties of Streptomyces strains isolated from agricultural soils in the Valle del Mezquital. Of the 13 isolated strains, only 2 (15.38 %) (Fig. 1A) were able to utilize tricalcium phosphate as an insoluble source (Fig. 1B). *S. thinghirensis* had the highest phosphate SI at 3.4, followed by *S. lateritius* with an SI of 2. The strains were transferred to SRSM-1 medium (PVK plus bromocresol purple) to determine the secretion of organic acids. Eleven Streptomyces strains (84.62%) (Fig. 1A) were observed to acidify the medium, as indicated by the formation of yellow halos around the colonies (Fig. 1C). The maximum acidification zone was obtained with *S. thinghirensis* (SI = 5). At the same time, *S. lateritius* (SI = 4), *S. griseoplanus* B4-M10 (SI = 3.2), *S. griseoaurantiacus* (SI = 2.83), *S. anulatus* (SI = 2.69), and *S. lusitanus* (SI = 2.67) showed intermediate indices (Table 1). The 13 strains (Fig. 1A) studied produced siderophores on CAS agar. Different colors were observed in the halos due to the availability of Fe since most of this ion is chelated to CAS (blue); that is, the strain takes up Fe by synthesizing siderophores (orange or purple), which are molecules with a higher affinity for Fe than CAS itself (Fig. 1D). *S. griseoplanus* B4-M10 showed the highest activity with a siderophore diameter of 35 mm (siderophore rating of 4), followed by *S. flavogriseus* and *S. nigra* CB-8 with diameters of 24 and 22 mm (siderophore rating of 3), respectively (Table 1). The strain with the lowest activity diameter was *S. atrovirens*, with a diameter of 4 mm (siderophore rating of 1).

On the other hand, only one of the strains studied tested positive for HCN (Fig. 1A). *S. lusitanus* was found to be a moderate producer (++) of HCN (Fig. 1E). Regarding IAA production (Fig. 1F), all 13 strains exhibited this activity (Fig. 1A). The strains with the highest IAA production were *S. thinghirensis* and *S. griseoplanus* B4-M10, with 231.73 and 229.54 µg/mL of IAA, respectively. At the same time, *S. griseoaurantiacus* produced the lowest concentration (28.79 µg/mL). Finally, ammonia production was observed in 100 % (Fig. 1A) of the strains tested using Nessler's reagent, where the color turns yellowish-brown, and its intensity depends on the NH_3 concentration (Fig. 1G).

Evaluation of Antagonistic Activity

A preliminary qualitative assay evaluated the inhibitory activity of 13 actinomycete strains on the mycelial growth of 10 phytopathogenic fungi impacting agriculture. The strain that exhibited the greatest antagonistic activity was *Streptomyces*

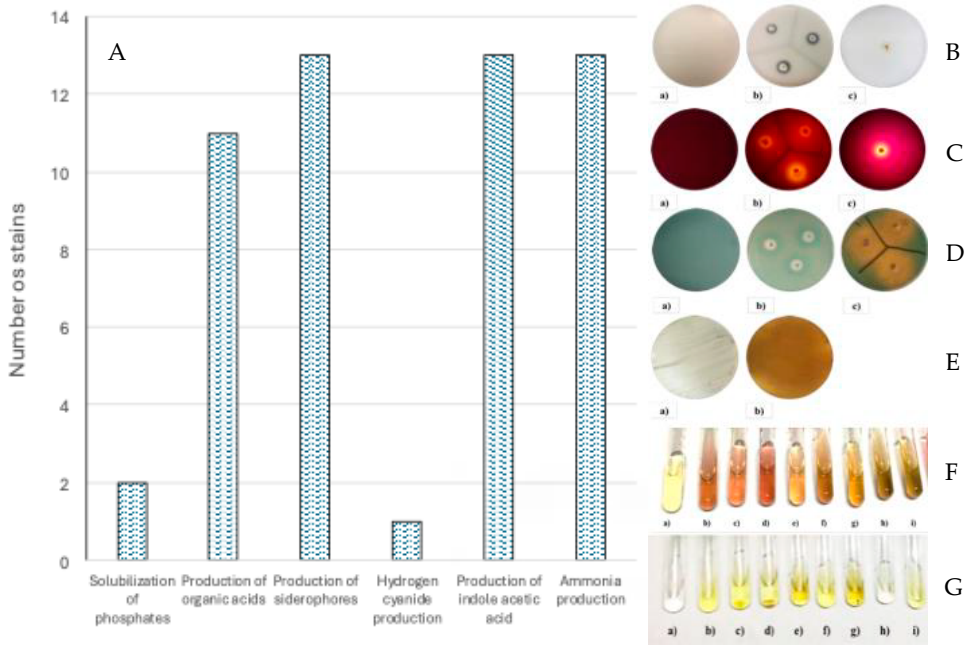

Figura 1. Número total de cepas productoras de moléculas promotoras del crecimiento vegetal y algunos resultados de las pruebas cualitativas en placa de las actividades ensayadas. A = Número de cepas productoras de moleculas promotoras del crecimiento vegetal; B = Solubilización de fosfatos; C = Producción de ácidos orgánicos; D = Producción de sideróforos; E = Producción de cianuro de hidrógeno; F = Producción de AIA y, G = Producción de amoniaco.

Figure 1. Total number of strains producing plant growth-promoting molecules and some results of qualitative plate tests for the activities tested. A = Number of strains producing plant growth-promoting molecules; B = Phosphate solubilization; C = Organic acid production; D = Siderophore production; E = Hydrogen cyanide production; F = IAA production; and G = Ammonia production.

Tabla 1. Producción de metabolitos detectados de las 13 cepas de Streptomicetos aislados de los suelos agrícolas del Valle del Mezquital.

Table 1. Production of metabolites detected from the 13 strains of Streptomycetes isolated from the agricultural soils of the Valle del Mezquital.

	Metabolite production					
Actinomycete	**Phophate solubilization (SI)[1]**	**Organic acids (I.S.)[2]**	**Siderophores classification**	**HCN production**	**IAA (µg/mL)**	**Ammonium production**
S. atrovirens	-	2.2	1	-	46.29 ± 0.02	+
S. lateritius	2	4	1	-	67.71 ± 0.01	+
Streptomyces sp. A-C7	-	2.3	2	-	41.72 ± 0.01	+
S. pseudogriseolus	-	2.42	1	-	32.99 ± 0.11	+
S. heliomycini	-	-	1	-	41.84 ± 0.07	+
S. anulatus	-	2.69	1	-	42.69 ± 0.03	+
S. virginiae	-	2.4	1	-	33.66 ± 0.01	+
S. nigra	-	-	3	-	39.09 ± 0.14	+
S. griseoplanus	-	3.2	4	-	229.54 ± 0.04	+
S. flavogriseus	-	2.23	3	-	52.08 ± 0.02	+
S. thinghirensis	3.4	5	1	-	231.73 ± 0.06	+
S. lusitanus	-	2.67	2	++	30.74 ± 0.11	+
S. griseoaurantiacus	-	2.83	1	-	28.79 ± 0.21	+

IS[1] índice de solubilización en agar PVK; IS[2] índice de solubilización en agar SRSM-1 (PVK+ púrpura de bromocresol). Escala para la clasificación de acuerdo al IS (Marra *et al*., 2015); bajo (IS<2); intermedio (2< IS <4); alto (IS>4). - = Sin actividad; + = con actividad.

SI[1] Solubilization Index on PVK agar; SI[2] Solubilization Index on SRSM-1 agar (PVK + bromocresol purple). Scale for classification according to IS (Marra *et al*., 2015): low (IS<2); intermediate (2< IS<4); high (IS>4). - = no activity, + = with activity.

sp. A-C7 exhibited antagonistic activity against all 10 phytopathogenic fungal strains. *S. griseoaurantiacus* exhibited antagonistic activity against nine phytopathogenic fungi, except for *Fusarium* spp. did not show this activity, while S. *pesudogriseolus* showed antagonistic activity against seven phytopathogenic fungi, except *Fusarium* spp., *Phytophtora* spp., and *F. oxysporum*. The *S. lusitanus* strain only showed antagonistic activity against *C. lindemuthianum*. The results obtained from this step are presented in Table 2.

From the *in vitro* qualitative experiment, actinomycetes with the highest antagonistic potential (+++) were selected and subjected to quantitative tests for 10 d. The results of the dual culture assays between actinomycetes and fungi are shown in Table 3. It was detected that *C. lindemuthianum* was inhibited by 11 actinomycetes (84.62 %), of which *Streptomyces* sp. A-C7 showed the maximum inhibition activity with 95.18 %, followed by *S. griseoaurantiacus* with 91.33 %, *S. griseoplanus* B4-M10 with 88.69 %, and *S. heliomycini* with 81.23 %. *S. atrovirens* and *S. anulatus* strains were unable to affect the radial growth of the other. It was also observed that *A. dauci*, the cause of leaf blight in carrots, was inhibited by six actinomycetes (46.15 %), the most effective of which was *Streptomyces* sp. A-C7 (69.62 %) and *S. pseudogriseolus* (60.76 %). The species *S. atrovirens*, *S. lateritius*, *S. griseoplanus* B4-M10, and *S. flavogriseus* exhibited low inhibition percentages, ranging from 15 % to 30 %.

Streptomyces sp. A-C7 and *S. pseudogriseolus* were the candidates with the highest antifungal spectrum potential. *Streptomyces* sp. A-C7 actively suppressed the growth of all 10 fungi studied, reaching inhibition percentages greater than 65 % for 8 phytopathogenic strains: *C. lindemuthianum*, *Pestalotia* spp., *H. maydis*, *Curvularia* spp., *Colletotrichum* spp., *A. dauci*, *Phytophthora* spp., and *S. sclerotiorum*. Meanwhile, lower inhibition values were observed for *Fusarium* spp. and *F. oxysporum*, with 34.16 % and 32.53 %, respectively (Figure 2A). On the other hand, *S. pseudogriseolus* had inhibitory effects on 6 of the fungi analyzed: *C. lindemuthianum*, *Pestalotia* spp., *H. maydis*, *Curvularia* spp., *A. dauci,* and *S. sclerotiorum* (Figure 2B).

Tabla 2. Actividad antagónica *in vitro* de diferentes actinomicetos aislados de suelos agrícolas del Valle del Mezquital, Hidalgo contra hongos fitopatógenos.
Table 2. *In vitro* antagonistic activity of different actinomycetes isolated from the Valle del Mezquital agricultural soils, Hidalgo, against phytopathogenic fungi.

Actinomycete	Fungi (Inhibition scale)									
	1	2	3	4	5	6	7	8	9	10
S. atrovirens	+++	-	-	-	-	-	++	-	-	-
S. lateritius	+++	++	-	-	-	-	++	-	-	-
Streptomyces sp. A-C7	+++	+++	+++	+++	+++	+++	+++	+++	+++	++
S. pseudogriseolus	+++	++	++	++	+++	-	+++	-	++	-
S. heliomycini	+++	++	-	-	++	-	-	-	+++	++
S. anulatus	+++	-	-	+	-	-	+	-	-	-
S. virginiae	+++	+	-	-	+	-	+	+++	-	-
S. nigra B-C8	+++	-	-	++	+	-	+++	++	+++	-
S. griseoplanus B4-M10	+++	-	+	+	-	-	++	-	-	-
S. flavogriseus	+++	+	+	-	-	-	++	-	-	-
S. thinghirensis	+++	-	+	+	++	-	++	-	-	-
S. lusitanus	+++	-	-	-	-	-	-	-	-	-
S. griseoaurantiacus	+++	+	++	++	++	-	+++	++	+++	+

Hongos: 1 = *C. lindemuthianum*; 2 = *Pestalotia* spp.; 3 = *H. maydis*; 4 = *Curvularia* spp.; 5 = *Colletotrichum* spp.; 6 = *Fusarium* spp.; 7 = *A. dauci*; 8 = *Phytophtora* spp.; 9 = *S. sclerotiorum*; 10 = *F. oxysporum*. - = Sin actividad; + = actividad débil; ++ = actividad moderada, and +++ = actividad fuerte.
Fungus: 1 = *C. lindemuthianum*; 2 = *Pestalotia* spp.; 3 = *H. maydis*; 4 = *Curvularia* spp.; 5 = *Colletotrichum* spp.; 6 = *Fusarium* spp.; 7 = *A. dauci*; 8 = *Phytophtora* spp.; 9 = *S. sclerotiorum*; 10 = *F. oxysporum*. - = no activity; + = weak activity; ++ = moderate activity, and +++ = strong activity.

Tabla 3. Inhibición de los hongos fitopatógeno por los actinomicetos aislados de suelos agrícolas del Valle del Mezquital en estudio.
Table 3. Phytopathogenic fungi inhibition by actinomycetes isolated from agricultural soils of the Valle del Mezquital under study.

Actinomycete	Fungi (% inhibition)									
	1	2	3	4	5	6	7	8	9	10
S. atrovirens	-	-	-	-	-	-	15.19 ± 0.12	-	-	-
S. lateritius	54.22 ± 0.23	-	-	-	-	-	27.85 ± 0.39	-	-	-
Streptomyces sp. A-C7	95.18 ± 0.13	72.22 ± 0.09	68.89 ± 0.27	69.93 ± 0.28	74.44 ± 0.16	34.16 ± 0.39	69.62 ± 0.08	80 ± 0.07	66.67 ± 0.13	32.53 ± 0.13
S. pseudogriseolus	68.23 ± 0.06	73.33 ± 0.12	61.11 ± 0.05	50.33 ± 0.32	-	-	60.76 ± 0.22	-	83.33 ± 0.27	-
S. heliomycini	81.23 ± 0.28	-	-	-	-	-	-	-	75.56 ± 0.09	-
S. anulatus	-	-	-	-	-	-	-	-	-	-
S. virginiae	45.78 ± 0.14	-	-	-	-	-	-	-	-	-
S. nigra B-C8	53.26 ± 0.71	-	-	-	-	-	-	-	76.67 ± 0.02	-
S. griseoplanus B4-M10	88.69 ± 0.23	-	-	-	-	-	20.25 ± 0.02	-	-	-
S. flavogriseus	72.22 ± 0.09	-	-	-	-	-	15.19 ± 0.35	-	-	-
S. thinghirensis	23.14 ± 0.05	-	-	-	-	-	-	-	-	-
S. lusitanus	19.72 ± 0.22	-	-	-	-	-	-	-	-	-
S. griseoaurantiacus	91.33 ± 0.18	-	55.57 ± 0.03	-	-	-	-	-	-	-

Hongos: 1 = *C. lindemuthianum*; 2 = *Pestalotia* spp.; 3 = *H. maydis*; 4 = *Curvularia* spp.; 5 = *Colletotrichum* spp.; 6 = *Fusarium* spp.; 7 = *A. dauci*; 8 = *Phytophtora* spp.; 9 = *S. sclerotiorum*; 10 = *F. oxysporum*; - = sin inhibición.
Fungus: 1 = *C. lindemuthianum*; 2 = *Pestalotia* spp.; 3 = *H. maydis*; 4 = *Curvularia* spp.; 5 = *Colletotrichum* spp.; 6 = *Fusarium* spp.; 7 = *A. dauci*; 8 = *Phytophtora* spp.; 9 = *S. sclerotiorum*; 10 = *F. oxysporum*; - = no inhibition.

C. lindemuthianum, which causes bean anthracnose, was inhibited by 11 of the 13 actinomycetes; only *S. atrovirens* and *S. anulatus* could inhibit its growth. *A. dauci*, the cause of carrot leaf blight, was inhibited by six streptomycetes. *Streptomyces* sp. A.C7 exhibited the greatest inhibition with 69.62 %, followed by *S. pseudogriseolus* with 60.76 %.

DISCUSSION

Evaluation of plant growth promotion capacity

Streptomyces is considered the most abundant actinomycete and possibly the most important due to its ability to produce a wide range of bioactive compounds, antibiotics, and extracellular enzymes. Chouyia *et al*. (2020) reported the phosphate solubilizing activity of *Streptomyces roseocinereus* and *Streptomyces natalensis* with maximum SI of 1.75 and 1.17, respectively. Boubekri *et al*. (2021) found an SI of 3.17 for *S. anulatus* P16 in a medium supplemented with phosphate rock as the sole source of insoluble phosphate. Compared with the present work, the S. *anulatus* A5-M14 strain did not show phosphorus solubilizing capacity; only *S. lateritius* and *S. thinghirensis* showed the ability to solubilize phosphates, with solubilization indices of 2 and 12, respectively. Regarding *S. thinghirensis*, SI is higher than that reported by both authors. The potential of *S. thinghirensis* to efficiently solubilize phosphates has been previously described by Djebaili *et al*. (2020). Rehan *et al*. (2021) analyzed an *S. thinghirensis* HM3 strain isolated from agricultural soils in Saudi Arabia and found it capable of growing on PVK agar. However, the formation of a visible solubilization halo was not evident, they found visible phosphate traces. Among the mechanisms associated with phosphate solubilization by bacteria, acidification, chelation, phosphatase enzymes, and the production of organic acids may be involved (Mohammed, 2020).

Low molecular weight organic acids originate from metabolizing high molecular weight compounds, such as carbohydrates, peptides, and lipids. Among the organic acids produced by *Streptomyces* are succinic, formic, acetic acid, and the most predominant ones, such as oxalic, citric, and gluconic acid (Vargas-Hoyos *et al*., 2021), which are possibly responsible for the decrease in pH and, therefore, the solubilization of minerals.

Siderophores sequester Fe from the rhizosphere, inhibiting pathogen growth or metabolic activity. Jarmusch *et al*. (2021) reported that the *Streptomyces* sp. S29 strain was effective in Fe chelation due to the secretion of siderophores, which inhibited the growth of filamentous fungi. On the other hand, Manigundan *et al*. (2020) reported that strains with higher secretion of siderophores can promote plant growth

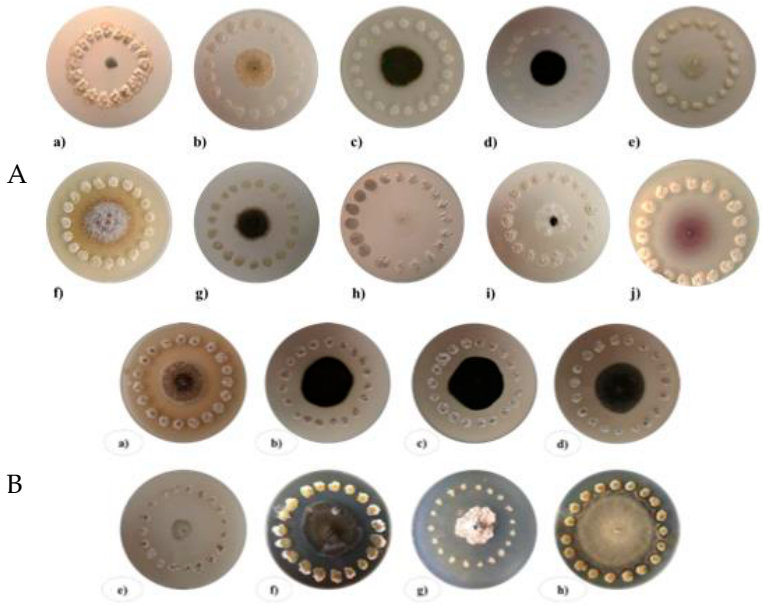

Figura 2. Confrontación dual de la cepa A-C7 de *Streptomyces* sp. contra los hongos fitopatógenos (2A): a) *C. lindemuthianum*; b) *Pestalotia* spp.; c) *H. maydis*; d) *Curvularia* spp.; e) *Colletotrichum* spp.; f) *Fusarium* spp.; g) *A. dauci*; h) *Phytophtora* spp.; i) *S. sclerotiorum* y j) *F. oxysporum*. La cepa *S. pseudogriseolus* A7-M9 (2B) se confronta contra a) *Pestalotia* spp.; b) *H. maydis*; c) *Curvularia* spp.; d) *A. dauci* y e) *S. sclerotiorum*, mientras que la cepa de *S. griseoauranticus* se confronta contra f) *H. maydis*; la cepa *S. heliomycini* se confronta contra g) *S. sclerotiorum* y finalmente *S. flavogriseus* se confronta contra h) *A. dauci*.

Figure 2. Dual confrontation of the *Streptomyces* sp. strain A-C7 against phytopathogenic fungi (2A): a) *C. lindemuthianum*; b) *Pestalotia* spp.; c) *H. maydis*; d) *Curvularia* spp.; e) *Colletotrichum* spp.; f) *Fusarium* spp.; g) *A. dauci*; h) *Phytophtora* spp.; i) *S. sclerotiorum*; j) *F. oxysporum*. The *S. pseudogriseolus* A7-M9 strain (2B) is confronted against a) *Pestalotia* spp.; b) *H. maydis*; c) *Curvularia* spp.; d) *A. dauci* and e) *S. sclerotiorum*, while the *S. griseoauranticus* strain is confronted against f) *H. maydis*; the *S. heliomycini* strain is confronted against g) *S. sclerotiorum* and finally *S. flavogriseus* is confronted against h) *A. dauci*.

and yield better crops. In the present study, the generation of siderophores by *S. lateritius*, *S. flavogriseus*, and *S. lusitanus* was detected; this ability has not been previously reported in these microorganisms, making them an additional option for developing new biotechnological products.

Hydrogen cyanide (HCN) is a volatile secondary metabolite, a byproduct of glycine metabolism, which is highly dependent on glycine availability and environmental Fe levels (Short *et al*., 2018). HCN production by *Streptomyces* sp. has been reported. Anwar *et al*. (2016) evaluated HCN secretion in six actinomycetes obtained from the rhizosphere of wheat and tomato plants grown in Punjab province, Pakistan. Passari *et al*. (2015) found that 68 % of endophytic isolates from medicinal plants produced the metabolite, and most belonged to the genus *Streptomyces*. In the present work, only *S. lusitanus* could produce HCN; no evidence was found in the literature of *S. lusi*tanus as an HCN producer. The fact that *S. lusitanus* produces HCN could indicate its potential role in controlling diseases of fungal or bacterial origin.

Among auxins, indole-3-acetic acid (IAA) is the phytohormone most produced by microorganisms; it is synthesized through the metabolism of L-tryptophan (Widawati, 2020). This metabolite regulates essential biological processes, primarily in plant growth and development, including cell expansion, division, differentiation, fruit development, leaf formation, and trophic responses. Of the microorganisms studied, those that presented the highest IAA production were *S. thinghirensis*, *S. griseoplanus* B4-M10, and *S. lateritius*. The IAA concentrations in the present study are higher than those reported by other authors; Harikrishnan *et al*. (2014) selected IAA-producing microorganisms from the rice rhizosphere, they found that *Streptomyces* sp. VSMGT1014 produced 15.96 µg/mL of IAA, while Detraksa (2018) isolated 95 strains from the sugarcane rhizosphere, and only eleven isolates showed the capacity to produce IAA in a range of 4.76 to 29.02 µg/mL. In the present study, *S. thinghirensis* was the strain with the highest production of IAA with 231.73 µg/mL. This result contrasts with that described by Djebaili *et al*. (2020) who evaluated two strains of *S. thinghirensis*, the first, J4, showed a biosynthesis capacity of 12.8 µg/mL of IAA, and the second, K23, did not produce this auxin. On the other hand, Rehan *et al*. (2021) analyzed *S. thinghirensis* HM3 and detected 86.66 µg/mL of IAA.

Nitrogen (N_2) is essential for all living organisms. It is the most abundant element in the atmosphere, but biochemically, it is not available to plants and most microorganisms, as it is a non-reactive form. The accessible forms are ammonia (NH_3), ammonium (NH_4^+), nitrates (NO_3^-), and nitrites (NO_2) (Shomi e*t al.*, 2021). The release of NH_3 gas by bacteria in vitro is associated with the use of substrates rich in proteins or amino acids, involved in amino acid catabolism and deamination reactions (Vlassi *et al*., 2020). NH_3 production by *Streptomyces* is considered an efficient way to overcome rhizosphere competitors due to its biosynthetic simplicity and low metabolic cost. Avalos *et al*. (2020) demonstrated that *Streptomyces* can produce high levels of NH_3 that affect the growth of gram-positive and negative bacteria over long distances. Borah and Thakur (2020) reported that different *Streptomyces* isolates can produce NH_3, an essential metabolite in suppressing the growth of plant-pathogenic fungi. The 13 strains studied showed the ability to produce ammonia, which suggests that they are involved in N bioavailability processes in plants.

Antagonistic and Inhibitory Activity of Isolated Strains

The antagonism exhibited by actinomycetes is mainly due to the production of lytic enzymes, antibiotics, and parasitism. Different *Streptomyces* species produce antibiotics, and some strains are used in the biological control of plant diseases mainly caused by fungi (Law *et al*., 2017). The results obtained were heterogeneous regarding the antagonistic activity of the 13 *Streptomyces* strains studied. *C. lindemuthianum* was inhibited by 11 of the *Streptomyces* strains; only *S. atrovirens* and *S. anulatus* could not inhibit this fungus. There are no recent reports on the growth inhibition of *C. lindemuthianum* with the 11 strains studied. Regarding the growth inhibition of *Pestolia* spp., only *Streptomyces* spp. A-57 and *S. pseudogriseolus* were able to inhibit it. Regarding the growth inhibition of *H. maydis*, *Curvularia* spp., and *A. dauci*, the antifungal and antibacterial activity of *S. pseudogriseolus* has already been reported; Alekhya and Gopalakrishnan (2014) reported that *Streptomyces* species are capable of inhibiting the growth of *F. oxysporum*, *Macrophomina phaseolina*, and *Rhizoctonia bataticola*, which are pathogens of chickpea and sorghum; Fatmawati *et al*. (2018) found high antagonistic activity against *E. coli*, *Staphylococcus aureus*, and *F. oxysporum*. However, in this study, *S. pseudogriseolus* A7-M9 showed no effect on *F. oxysporum*. Furthermore, Vatsa-Portugal *et al*. (2017) reported that *S. anulatus* S37 induces an early plant response against various diseases and pests. Other *S. anulatus* strains have also shown antagonistic and inhibitory activity against *Phytophtora* sp. (Kunova *et al*., 2016), *F. oxysporium* (Djebaili *et al*., 2021), and *S. sclerotiorum* (Kunova *et al*., 2016). On the other hand, Vijayabharanthi *et al*. (2014) found that *S. griseoplanus* displayed a broad spectrum of activity against entomopathogens. Different strains of this microorganism have also shown activity against bacterial diseases and pests (Kumar *et al*., 2024). For their part, Vurukonda *et al*. (2021) observed that *S. atrovirens* showed antagonistic activity against *Colletotrichum* spp. Regarding *S. lateritius*, it showed inhibitory activity against *Fusarium* spp. (Gromovykh *et al*., 2005), while *S. heliomycini* against *Fusarium graminearum*, and *S. griseoaurantiacus* showed antagonistic activity against *Sclerotium rolfsii* in pepper (Qiu *et al*., 2024).

The results of the present study indicated that strains isolated from the soils of the Mezquital Valley exhibit significant antagonistic and inhibitory activity, likely due to the growth conditions to which they are subjected. It was observed that several of the Streptomyces strains isolated had not been reported for their antagonistic or inhibitory activity, as is the case primarily with *S. pseudogriseolus*, *S. virginiae*, *S. thinghirensis*, and *S. griseoaurantiacus*. Environmental growing conditions likely influence this activity.

CONCLUSIONS

The strains isolated from the agricultural soil under study showed the ability to secrete metabolites related to plant growth, such as siderophores, IAA, and organic acids. Only two strains (*S. thinghirensis* and *S. lateritius*) showed the ability to solubilize phosphates, and one strain (*S. lusitanus*) showed the ability to produce HCN. Previously undescribed strains with antagonistic and inhibitory capacity were identified, such as *S. pseudogriseolus*, *S. atrovirens*, *S. lateritius*, *S. nigra*, S. *griseoplanus*, *S. flavogriseus*, and *S. griseoaurantiacus*, representing new options for the generation of formulations for the biological control of diseases caused by phytopathogenic fungi.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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