ANTAGONIST ACTIVITY OF *Streptomyces* sp. Y20 AGAINST FUNGI CAUSING DISEASES IN PLANTS AND FRUITS †

[ACTIVIDAD ANTAGÓNICA DE *Streptomyces* sp. Y20 CONTRA HONGOS QUE CAUSAN ENFERMEDADES EN PLANTAS Y FRUTOS]

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SUMMARY

Background: Crop microbial pathogens reduce the production and quality of agricultural products. They cause substantial increase costs for producers of fruits, vegetables, and ornamental plants with negative consequences on economy and food security at household, national and global levels. Annually, the losses represent around 40% to 50% for root crops, vegetables, and fruits. Chemical control with fungicides can prevent, kill, mitigate, or inhibit the growth of plant pathogenic fungi. Nevertheless, biological control with microorganisms and natural molecules is an increasingly popular alternative to protect crops. **Objective:** Here, the antagonist activity of soil *Streptomyces* sp. Y20 against the pathogenic fungi causing diseases in plants and fruits was evaluated. **Methodology**: Streptomycetes bacteria was isolated from soils collected at open field cultures of local farms with tomato. The antagonism was evaluated *in vitro* via a dual confrontation experiment against fungal species of *Fusarium*, *Lasiodiplodia*, *Colletotrichum*, *Aspergillus*, *Botrytis*, and *Sclerotium*. *Streptomyces* sp. Y20 was characterized phenotypically and molecularly identified by the 16S rDNA gene. The biosynthetic gene clusters for polyketide synthases (PKS Type I) and non-ribosomal peptide synthase (NSPS) were detected. **Results**: Preliminary, the isolate Y20 was selected by the higher antagonism against *F. oxysporum* f sp. *lycopersici*. Taxonomic characterization of the isolate Y20 by the analysis of the 16S rDNA sequence led to its identification as member of *Streptomyces* genus. Spore surface morphology by Scanning Electronic Microscopy (SEM) showed barrel-like spores. Antagonistic activity of *Streptomyces* sp Y20 was comparable to the commercial strain *S. lydicus* WYEC108 ($P > 0.5$). However, there was a superior antagonism of Y20 strain versus the commercial strain WYEC108 against *F. oxysporum* f sp. *lycopersici*, *Fusarium* sp. CDBB1172, *F. oxysporum*, *Lasiodiplodia* sp., and *Aspergillus* sp. ($P < 0.05$). **Implications:** Soil streptomycetes with *in vitro* antagonistic activity on plant pathogenic fungi could be a natural alternative to the use of chemical fungicides to control plant diseases. **Conclusion**: This study presented a novel soil *Streptomyces* specie which showed *in vitro* antagonism against a diversity of plant pathogenic fungal species. *Streptomyces* strain Y20 could be used as a biocontrol agent.

Key words: Biological control; *Streptomyces*; antagonism; antifungal activity; fungal pathogen.

RESUMEN

Antecedentes: Los patógenos de origen microbiano reducen la producción y calidad de los productos agrícolas. Causan incrementos sustanciales en los costos para los productores de frutas, vegetales y plantas ornamentales con consecuencias negativas sobre la economía y la seguridad alimentaria a nivel local, nacional y global. Anualmente, las pérdidas representan entre el 40% al 50% para los cultivos de tubérculo, vegetales y frutos. El control químico con fungicidas previene, elimina, mitiga o inhibe el crecimiento de los hongos patógenos de plantas. Sin embargo, el control biológico con microorganismos y moléculas naturales es una alternativa para la protección de los cultivos que ha ido creciendo en popularidad. **Objetivo**: En el presente estudio, se evaluó la actividad antagonista de la bacteria del suelo *Streptomyces* sp. Y20 contra hongos patógenos que causan enfermedades en plantas y frutos. **Metodología:** Las bacterias Estreptomicetos se aislaron de suelos obtenidos de campos locales con cultivos de tomate. La actividad

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antagónica se evaluó *in vitro* mediante experimentos de confrontación dual contra hongos patógenos de los géneros *Fusarium*, *Lasiodiplodia*, *Colletotrichum*, *Aspergillus*, *Botrytis*, and *Sclerotium*.asimismo. *Streptomyces* sp. Y20 se caracterizó fenotípicamente e identificó molecularmente mediante el gen ribosomal 16S. Asimismo, se detectaron los genes biosintéticos de sintasas de policétidos (PKS Type I) y las sintasas de péptidos no ribosomales (NRPS). **Resultados**: Inicialmente, el aislado Y20 se seleccionó por su mayor capacidad antagonista contra *Fusarium oxysporum* f. sp. *lycopersici*. La caracterización taxonómica del aislado Y20 por el análisis de la secuencia del gen rDNA 16S lo identificó como una especie perteneciente al género *Streptomyces*. La morfología de la superficie de las esporas observada por SEM las mostró con forma de barril. La actividad antagonista de *Streptomyces* sp. Y20 fue similar a la de la especie comercial *S. lydicus* WYEC108 ($P > 0.5$). Sin embargo, la cepa Y20 mostró una mejor actividad antagonista contra *F. oxysporum* f sp. *lycopersici*, *Fusarium* sp. CDBB1172, *F. oxysporum*, *Lasiodiplodia* sp., y *Aspergillus* sp. ($P < 0.05$) en comparación a la cepa WYEC108. **Implicaciones**: Los Estreptomicetos del suelo con actividad antagonista contra hongos patógenos de plantas pudieran ser una alternativa natural al uso de fungicidas químicos para el control de enfermedades en las plantas. **Conclusión**: Este estudio mostró a una nueva especie del suelo del género *Streptomyces* con actividad antagonista *in vitro* de *Streptomyces* Y20 contra diferentes especies de hongos patógenos de plantas.

Palabras clave: Control biológico; *Streptomyces*; antagonismo; actividad antifúngica; hongo patógeno.

INTRODUCTION

To satisfy the world demand for food in a sustainable way, it is essential to ensure agricultural production without risks to the environment and people. The pests and diseases that affect crops are a factor that puts food security at risk because they damage crops and reduce the availability and access to food (FAO, 2017). Phytopathogenic fungi are microorganisms that every year put agricultural production at risk. They can destroy up to a third of the annual production (Almeida *et al.*, 2019). Approximately, $220 billion per year is the global economic cost of plant diseases, with 20–40% of crop production lost to pests (FAO, 2019). Synthetic fungicides have long been used to control fungi, but these compounds also cause environmental pollution, damage human and animal health, and suffer from the development of resistant strains (Moshi and Matoju, 2017; Akram *et al*., 2018).

Antagonistic bacteria and their metabolites can be an alternative to chemical fungicides in the manage of fungal diseases in the control of postharvest decay (Syed-Ab-Rahman *et al.*, 2018). Several mechanisms are involved in how microorganisms can act against phytopathogens such as parasitism, cross protection, antibiosis, and competition (Shoda, 2000). Bacteria of the genus *Streptomyces* have been widely recognized for their known ability to produce fungicides, antibiotics, extracellular hydrolytic enzymes (lipases, amylases, proteases, chitinases, glucanases, xylanases), and other bioactive compounds that inhibit the growth of phytopathogenic microorganisms in a natural and safe way (Evangelista-Martínez *et al.*, 2017; Chen *et al.*, 2018). The biopesticide market has commercial products based on *Streptomyces* species such as *Streptomyces lydicus* WYEC108 and *Streptomyces griseoviridis* K61 (Lahdenperä, 1987; Yuan and Crawford, 1995). *Streptomyces lydicus* WYEC108 on fungal species of the genera *Fusarium*, *Rhizoctonia*, *Pythium*, *Phytophthora*, *Botrytis*, and *Sclerotinia*. *Streptomyces griseoviridis* K61 inhibits the growth of *Fusarium*, *Phytophthora*, *Alternaria*, *Pythium*, *Rhizoctonia*, and *Botrytis*.

Sustainable agriculture is a useful strategy for biological management of plant diseases and provides fruits and vegetables free of synthetic pesticides (Di Francesco *et al.*, 2016). Therefore, the objective of the study was to evaluate the antagonistic activity of soil Streptomycetes against phytopathogenic fungi that affect plants and fruits in their post-harvest stage.

MATERIALS AND METHODS

Fungi and growth conditions

The fungi used here were obtained from the Fungi Collection preserved at CIATEJ, Southeast Unit. The strains were *Fusarium* sp. CDBB:1172, *Fusarium oxysporum* (from gladiolus corm rot), *Fusarium oxysporum* (from agave tequilana), *Fusarium oxysporum* f. sp. *lycopersici* (from tomato plant), *Lasiodiplodia* sp. M1 (from mango Ataulfo fruit), *Lasiodiplodia* sp. (from coconut palm), *Colletotrichum* sp. M1.1 (from habanero pepper fruit), *Colletotrichum musae* Cm4 (from banana fruit), *Colletotrichum* sp. (from avocado fruit), *Aspergillus* sp. (from sweet orange fruit), *Botrytis cinerea* (from tomato fruit), and *Sclerotium* sp. (from *Aloe vera*). Monosporic cultures were grown on potato dextrose agar (PDA, Difco) plates and incubated at 29 °C for 8-12 days.

Soil sampling and streptomycetes isolation

Soil samples were collected from open field cultures of local farms with tomato var. Pony Express located at Santo Domingo, Oxkutzcab, México (20°11'12.444" N 89°31'28.415" W). All samples were collected with an auger by drilling down to a 10 cm depth. They were

subsequently placed in presterilized plastic bags and were processed after 36 h.

The Streptomycete isolation and colony selection was based on the typical morphological features and was performed as described previously (Evangelista-Martínez, 2014b). Repeated streaking of a spore sample used a toothpick from single colonies onto fresh international *Streptomyces* Project media 2 (ISP 2) agar plates; this step produced pure isolates. A suspension of spores or mycelium cells stored at -20 °C in 20% (w/v) glycerol was used to prepare a working general inoculum (GI) with a turbidity of 0.5 on the McFarland standard.

Molecular identification

The genomic DNA was purified from a spore suspension using the GenElute Bacterial Genomic DNA Kit (Sigma-Aldrich). The 16S rRNA gene amplification and sequencing analysis were performed using the universal oligonucleotides fD1 (5′-CCGAATTCGTCGACAACAGAGTTT GATCCTGGCTCAG-3′) and rD1 (5′-CCCGGGATCCAAGCTTAAGGAGGTGATCCAG CC-3′) (Weisburg *et al.*, 1991). The PCR fragments were amplified as established by Evangelista-Martínez (2014a) using the GoTaq Hot Start Polymerase (Promega). Direct sequencing of both DNA strands was determined at Macrogen (Seoul, Korea). Sequences were assembled and trimmed using CLC Main Workbench 6 (CLC Bio). The sequencing data were BLAST analyzed using the non-redundant GeneBank database (http://www.ncbi.nlm.nih.gov/). Phylogenetic analysis was performed at Phylogeny.fr (http://www.phylogeny.fr). Multiple alignments were generated using the ClustalW (v 2.1); poorly aligned positions and divergent regions were removed with Gblocks (v 0.91b). A phylogenetic tree based on the neighbor-joining method was constructed under Kimura's two-parameter model. Bootstrap confidence analysis was carried out with 1000 replications. Partial sequences of 16S rDNA gene of *Streptomyces* sp. strain Y20 was deposited in the GenBank database under accession MW485004.

Characterization of *Streptomyces* sp. Y20

The phenotypic features of the Y20 isolate were evaluated based on Shirling and Gottlieb (1966) with slight modifications. To evaluate the phenotypic characteristics of Y20 isolate, 2 µl of GI were inoculated in different media: ISP 2 for colony differentiation and color, ISP 7 for melanin production, and IPS 9 for hydrolysis test of complex substrates. The RAL color chart was used for coloring description. To evaluate the growth characteristics of Y20 isolate, 2 µl of GI suspension were inoculated in Petri plates containing different culture agar media: ISP 2, ISP2 added with 0.5% (w/v) pancreatic digest casein, ISP 9, ISP 9 with 0.5% (w/v) pancreatic digest casein, nutrient agar (NA), tryptone yeast extract agar (TYA), Czapek-Dox agar (CDA), King B agar (KB), and PDA. All media were incubated for 14 days at 29 °C; substrate and aerial mycelium growth as well as spore production were determined.

Antibiotic susceptibility by the disk diffusion method was performed as stated in Evangelista-Martínez *et al.* (2020). An antibiotic multidisc for Gram-positive bacteria II (Bio-Rad®, Hercule, CA, USA) was used by triplicate. The inhibitory halo diameter was measured with a caliper.

Scanning electron microscopy

An agar block of 10 × 10 mm with an active mycelium growth of a 14-days culture of Y20 isolate was placed into an empty Petri plate. It was then sealed with 3M Micropore surgical tape and kept at 4 °C. After eight days, the agar pieces were analyzed using a scanning electronic microscope EVO-50 (Carl Zeiss) at the Facultad de Ciencias de la Universidad Autónoma de Querétaro, México.

Antagonistic evaluation

A dual confrontation assay was used to evaluate the antagonistic activity of the Streptomycete isolates on the growth of phytopathogenic fungi (Bredholdt *et al.*, 2007). An initial selection of antagonistic Streptomycetes on the fungal pathogen *F. oxysporum* f. sp. *lycopersici* was implemented. An inoculum of Streptomycetes spores after 15 days of growth was performed with a toothpick and dispersed into a square area of 7 × 14 mm, 1.0 cm from the edge of the ISP 2 agar plates. Spores of the reference strain *S. lydicus* WYEC108 were inoculated at the opposite side of the plate. Thereafter, an agar plug (9 mm diameter) covered with mycelium of a 10–12 days culture of the fungus was placed at the center of the plate and maintained in an incubator at 29 °C. The growth controls consisted of fungus disk in ISP 2 in the absence of Streptomycete isolates. All experiments were performed in triplicate. Measurements were made with a caliper when the radial growth of the fungi colonies grow near the edge in the growth control Petri plates. The percentage of inhibition (PI) was calculated with the formula: PI (%) = (FR − AR)/FR × 100, as described in Evangelista-Martínez *et al.* (2020); FR, represents the radial growth of the fungus (mm) of a control culture, and AR represents the radial growth (mm) in the direction of the Streptomycete.

Subsequent *in vitro* assays to determine the potential biocontrol activity of the selected *Streptomyces* isolates against diverse plant fungal pathogens were

performed. All measurements were conducted in triplicate.

Detection of polyketide synthase Type I and Type II genes (PKS Type I, PKS Type II) and non-ribosomal peptide synthase genes (NRPS)

Detection of biosynthetic genes involved in the production of molecules with antimicrobial activity in *Streptomyces* sp. Y20 was performed by polymerase chain reactions (PCR) with specific oligonucleotides. The PKS Type II gene fragments were amplified with the degenerate oligonucleotides KSα (5′-TSGRCTACRTCAACGGSCACGG-3′) and KSβ (5′-TACSAGTCSWTCGCCTGGTTC-3′) (González *et al*., 2005). The PKS Type I fragments were amplified with the degenerate oligonucleotides K1F (5′-TSAAGTCSAACATCCGBCA-3′) and M6R (5′-CGCAGGTTSCSGTACCAGTA-3′). The NRPS gene fragments were amplified with oligonucleotides A3F (5′-GCSTACSYSATSTACACSTCSGG-3′) and A7R (5′-SASGTCVCCSGTSCGGTAS-3′) (Ayuso-Sacido and Genilloud, 2005). The PCR used GoTaq Hot Start Polymerase (Promega). Samples were visualized in 1.2% agarose gel prepared in 1× Tris-Borate buffer (TBE) and stained with ethidium bromide.

Data analysis

The PI is expressed as means ± standard deviation (SD). The means were compared using an one-way analysis of variance (ANOVA) followed by the Tukey test (P = 0.05). The statistical analyses were performed with the MiniTab v18 program (Minitab, LLC).

RESULTS AND DISCUSSION

Isolation and preliminary selection of antagonistic streptomycetes

A total of 46 Streptomycete-like strains were isolated and preserved at the Actinomycetes Germplasm Bank at CIATEJ. All strains were evaluated for their ability to inhibit the growth of *Fusarium oxysporum* f. sp. *lycopersici*. The results showed that isolates Y20 and Y44 antagonized the fungal pathogen at a PI of 49.2% and 27.1%, respectively. Further antagonistic evaluations over other fungal phytopathogens were conducted for the Y20 isolate.

Molecular characterization

The analysis of the partial 16S rRNA gene sequence (1463 bp) from the isolate Y20 revealed a close relation to other sequences belonged to the *Streptomyces* genus. The phylogenetic tree showed that strain *Streptomyces* sp. Y20 is related to species with antifungal activity and that produce antimicrobial metabolites (Figure 1). The endophytic bacteria *S. cavourensis* produce antifungal metabolites such as flavensomycin, humidin, and bafilomycin; bafilomycin B1 and C1 inhibited the mycelial growth of *Fusarium* spp, *Rhizoctonia solani*, and *Botrytis cinerea* (Skarbek and Brady, 1978; Pan *et al.*, 2015). *Streptomyces californicus* produces borrelidin—an

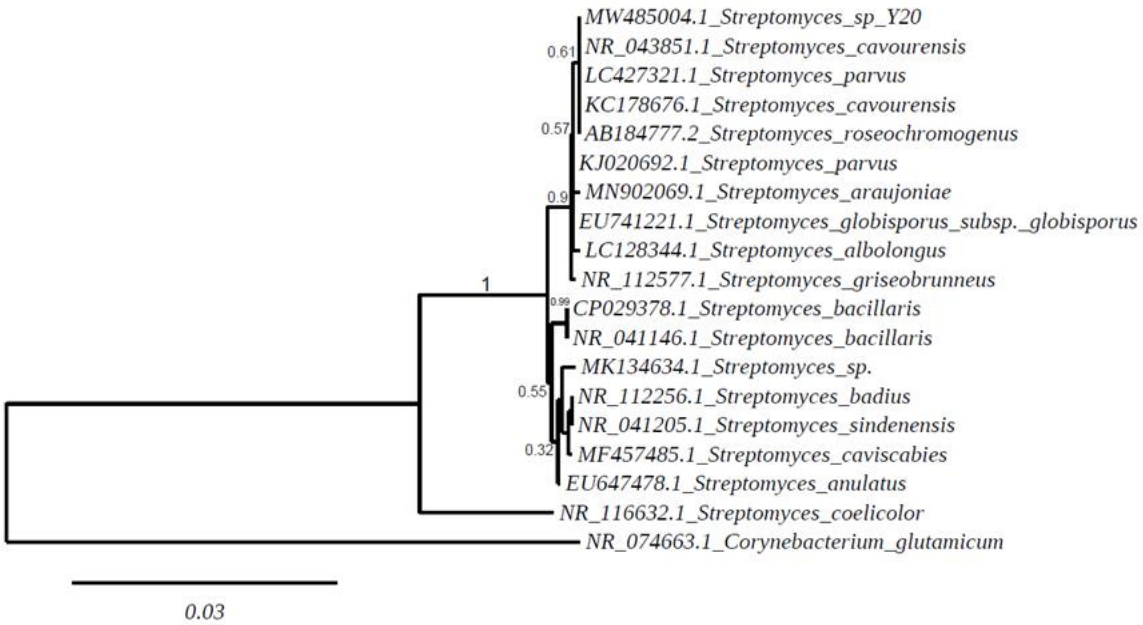

Figure 1. Phylogenetic relationship based on the relationship between the 16S rRNA gene sequences of *Streptomyces* sp. Y20 and other Streptomycetes. The numbers at the nodes indicated bootstrap support levels (n = 1000 re-samplings). The scale bar represents 0.03 nucleotide substitutions per site.

antibacterial and antifungal metabolite that inhibited *F. oxysporum* and *Aspergillus* species (Saisivam *et al.*, 2008). A cell-free ferment filtrate produced by *S. pratensis* inhibited the mycelia growth of *Botrytis cinerea* and diminished the lesion expansion of the mold infection on detached leaves and postharvest fruits (Lian *et al.*, 2017). Moreover, there was antagonism and significant inhibition effects on the wheat scab pathogen *F. graminearum* by *Streptomyces pratensis* S10. *S. pratensis* had control effects on fungal pathogens in the plot experiments (Zhang *et al.*, 2020). Molecular identification was confirmed by its phenotypic features.

Morphological and physiological characterization

Morphologically, the colonies of *Streptomyces* sp. Y20 have a distinctly dusty appearance and a light-ivory substrate mycelium when grown on ISP 2 agar media. There is white to cream-colored aerial mycelia and a cream-colored spore mass. Some physiological features were also observed (Table 1).

Microscopic observation by SEM showed that aerial hyphae morphology was smooth and flexous with a rectiflexible spore chain type with segmented barrel-like spore chains of the hyphae (Li *et al.*, 2016). (Figure 2).

Table 1. Phenotypic characterization of *Streptomyces* sp. Y20.

Test			**Growth on ISP2**	**Color**
Gram staining	+		Substrate mycelium	Cream
Starch hydrolysis	+		Aerial mycelium	White to cream
Casein hydrolysis	+		Spore mass	Cream
Melanin	-		Pigment production	None
Culture media[¥]	**Growth**	**Spore**	**Biosynthetic genes**	**PCR amplification**
ISP 2	+++	+	PKS-I	+
ISP 2 + casein	+++	+	PKS-II	-
ISP 9	++	+++	NRPS	+
ISP 9 + casein	++	+	**Antibiotic[§]**	**Sensitivity**
NA	+++	-	TE	Susceptible
PDA	+++	++	CTX, CF, AM, GE, SXT,	Resistance
CDA	++	+++	LEV, PE, E, DC, DL, FEP	
TYA	+++	-		
King B	+++	+		

[¥] The classification of growth and spore production were (+++), excellent; (++), moderate; (+), poor; (-), not detected.
[§] AM, ampicillin 10 mg; CF, cephalotin 30 mg; CTX, cefotaxime 30 mg; CFM, cefuroxime 30 mg; DC, dicloxacillin 1 mg; E, erythromycine 15 mg; FEP, cefepime 30 mg; PE, penicillin 10 U; TE, tetracycline 30 mg; LVX, levofloxacin 5 mg; GE, gentamicin 10 mg; SXT, trimethoprim-sulfamethoxazole 25 mg.

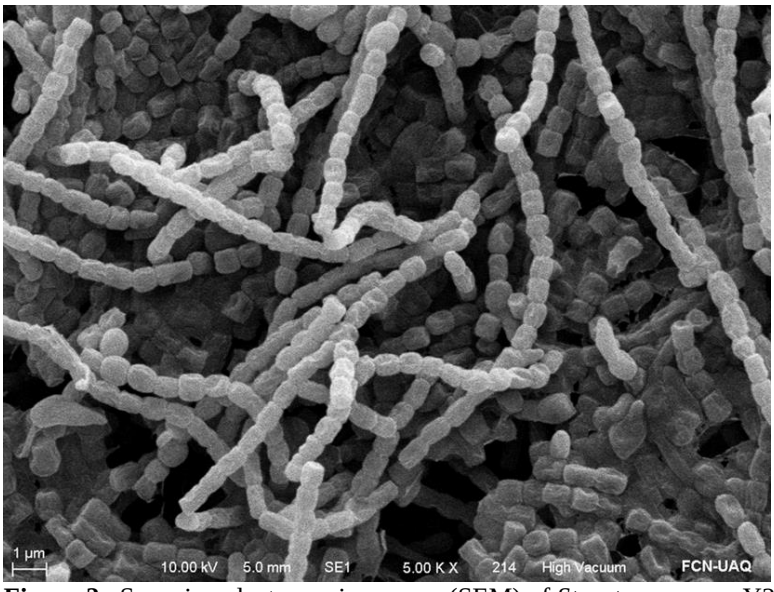

Figure 2. Scanning electron microscopy (SEM) of *Streptomyces* sp. Y20 showing the spore chain morphology.

Antagonistic activity of *Streptomyces* sp. Y20

The antagonistic activities of *Streptomyces* sp Y20 against fungal phytopathogens are shown in Table 2. In general, the bacterial confrontation assays showed non-significant statistical differences for PI between the strain Y20 and *S. lydicus* WYEC108 ($P > 0.05$, capital letters). However, Y20 showed superior antagonism against several *Fusarium* species (e.g., *F. oxysporum* f. sp. *lycopersici*, *Fusarium* sp. CDBB1172, and *F. oxysporum*), *Lasiodiplodia* sp., and *Aspergillus* sp. in contrast to *S. lydicus* WYEC108 ($P < 0.05$). In this sense, we noted that the WYEC108 strain inhibited the mycelial growth of *Colletotrichum* sp. M1.1, *Lasiodiplodia* sp. M1, and *Botrytis cinerea*; significant differences were observed ($P < 0.05$, Figure 3).

The results suggest that *Streptomyces* sp. Y20 could be a potential agent useful to controlling, prevent and/or reduce fungal plant diseases. Previously, control of fungal phytopathogens by several *Streptomyces* species has been studied. *Streptomyces* sp. CACIS-1.16CA and *Streptomyces* sp. CACIS-1.5CA inhibited the growth of *Curvularia*, *Helminthosporium*, *Fusarium*, *Colletotrichum*, *Alternaria*, *Botrytis*, *Rhizopus*, *Aspergillus*,

Table 2. Percentage of inhibition (PI) of *Streptomyces* sp. Y20 against fungal phytopathogens.

ID	Fungi	Host	*Streptomyces* sp. Y20$^{A¨*}$	*S. lydicus* WYEC108^{A}
1	*F. oxysporum* f. sp. *lycopersici* **	Tomato	46.1 ± 1.7 ^{a}	20.4 ± 3.4 ^{b}
2	*F. oxysporum*	Gladiolus	43.4 ± 1.9 ^{a}	46.1 ± 3.9 ^{a}
3	*Fusarium* sp. CDBB1172	CDBB^{+}	44.7 ± 5.8 ^{a}	13.9 ± 3.5 ^{b}
4	*F. oxysporum*	Agave	53.5 ± 4.3 ^{a}	34.7 ± 3.0 ^{b}
5	*Lasiodiplodia* sp. M1	Mango	6.5 ± 4.3 ^{a}	43.3 ± 0.8 ^{b}
6	*Lasiodiplodia* sp.	Coconut	49.8 ± 2.1 ^{a}	33.3 ± 2.8 ^{b}
7	*C. gloeosporioides* M1.1	Habanero pepper	55.6 ± 3.3 ^{a}	66.2 ± 3.2 ^{b}
8	*C. musae* Cm4	Banana	64.2 ± 2.4 ^{a}	68.5 ± 0.6 ^{a}
9	*Colletotrichum* sp.	Avocado	46.1 ± 3.0 ^{a}	43.2 ± 3.2 ^{a}
10	*Aspergillus* sp.	Sweet orange	53.9 ± 1.1 ^{a}	15.6 ± 3.0 ^{b}
11	*Botrytis cinerea*	Tomato	66.5 ± 1.9 ^{a}	73.0 ± 1.8 ^{b}
12	*Sclerotium* sp.	Aloe	32.0 ± 1.1 ^{a}	30.7 ± 5.1 ^{a}

* Different letters indicate significant differences ($P < 0.05$) according to the Tukey test. ** Different letters in each row represent significant differences ($P < 0.05$). $^{+}$ CDBB, Colección Nacional de Cepas Microbianas y Cultivos Celulares del Cinvestav.

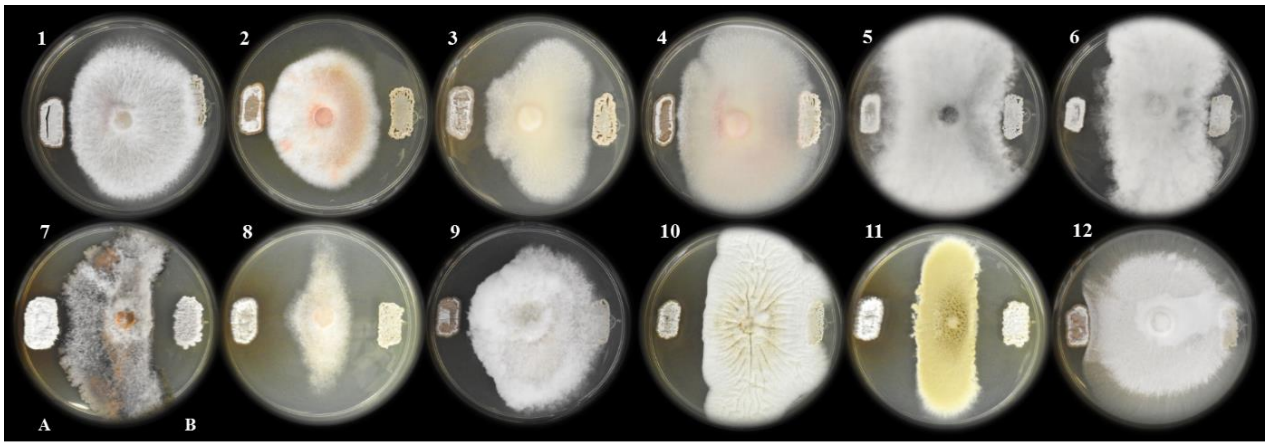

Figure 3. Dual confrontation assay of *Streptomyces* sp. Y20 against fungal pathogens. Both antagonist Streptomycetes were inoculated together in the same assay: A, *Streptomyces* sp. Y20 (left); B, *S. lydicus* WYEC108 (right). Plates were incubated at 29 °C for 8-12 days. Numbers to the left of the Petri plate correspond to the ID number of fungal pathogens as follows: 1. *F. oxysporum* f. sp. *lycopersici*, 2. *F. oxysporum*, 3. *Fusarium* sp. CDBB1172, 4. *F. oxysporum*, 5. *Lasiodiplodia* sp. M1, 6. *Lasiodiplodia* sp., 7. *C. gloeosporioides* M1.1., 8. *C. musae* Cm4, 9. *Colletotrichum* sp., 10. *Aspergillus* sp., 11. *Botrytis cinerea*, 12. *Sclerotium* sp. (further detail in Table 2).

and *Phytophthora capsici* (Evangelista-Martínez, 2014a; Evangelista-Martínez *et al.*, 2020). Rhizospheric *Streptomyces* species exerted antagonist activity against *F. oxysporum* f. sp. *Lycopersici*; additionally, these species promoted vegetative growth and diminished chlorosis and symptoms of wild disease in tomato plants (Abbasi *et al*., 2019). In this sense, *S. samsunensis* showed antagonistic activities against *L. theobromae*. The inhibitory mechanism exerted by streptomycetes may be through the production of diffusible antifungal metabolites or extracellular cell-wall-degrading enzymes that control mango dieback disease caused by *L. theobromae* (Kamil *et al.*, 2018). Several Streptomycete isolates from vermicompost showed *in vitro* antagonism against *C. gloeosporioides*, *C. musae*, *Fusarium* sp. TFPK201, *Fusarium* Foc 1699, and *Pestalotia* sp. (Kawicha *et al.*, 2020). *Streptomyces netropsis* isolated from rhizospheric soil from *Larrea tridentata* exposed antifungal activity over *Macrophomina phaseolina*, *F. oxysporum*, *F. solani*, *F. equiseti*, *Botrytis cinerea*, *Alternaria alternata*, and *C. gloeosporioides* with PI values ranging from 55.02 to 77.27% (Abdelmoteleb and González-Mendoza, 2020).

Detection of biosynthetic gene clusters of secondary metabolites

PCR detection of the biosynthetic clusters of genes involved in the production of specialized secondary metabolites showed DNA fragments corresponding to ~1400 bp for PKS-I and two amplified fragments of ~ 700 bp and ~ 800 bp for NRPS. No biosynthetic genes for PKS type II were detected for Y20. Streptomycetes are a group of bacterium widely recognized as bioactive metabolites producers; some species contain in their genome more than 20 biosynthetic gene clusters for secondary metabolites (Challis and Hopwood, 2003); for instance, 22 secondary metabolite-producing gene clusters in *S. yeochonensis* CN732 have been identified (Malik *et al.*, 2020). Several approaches have suggested that *Streptomyces* genus might produce over 100,000 antimicrobial metabolites. This is a high number of compounds relative to the small percentage that has been identified (Watve *et al.*, 2001). These results suggest that *Streptomyces* sp. Y20 could produce antifungal compounds.

CONCLUSION

Biological control of fungal pathogens with microorganisms is a natural alternative to the use of chemical fungicides in the crop fields. *Streptomyces* sp. Y20 has a wide antagonistic and inhibitory capacity and can inhibit the phytopathogenic fungi that affect horticultural crops. This strain is antagonistic to the growth of *Fusarium* and *Colletotrichum* species that cause wilt and anthracnose disease on a variety of plants and represent a viable option to the control of a wide range of pathogenic fungi.

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Conflict of interest. The authors state no competing interest to declare.

Compliance with ethical standards. Do not apply. The research does not contain any studies involving animals performed by any author.

Data availability. Data are available upon request with the corresponding author at: zevangelista@ciatej.mx

Author contribution statement (CRediT).
G.A. Rejón-Martínez, formal analysis, investigation, and methodology. **D.E. Ríos-Muñiz,** methodology and data curation. **E.A. Contreras-Leal**, investigation, validation, and data curation. **Z. Evangelista-Martínez**, conceptualization, writing –original draft, writing –review & editing, funding acquisition, and supervision.

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