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]

Gabriel A. Rejón-Martínez¹, Diana E. Ríos-Muñiz², Erika A. Contreras-Leal², and Zahaed Evangelista-Martínez*²

¹Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán, Km. 15.5, carretera Mérida-Xmatkuil, C.P. 97100, Mérida, Yucatán, México.
²Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, AC. Subsede Sureste, Tablaje catastral 31264 Km. 5.5, carretera Sierra Papacal-Chuburná Puerto. C.P. 97302, Mérida, Yucatán, México. E-mail: zevangelista@ciatej.mx
* Corresponding author

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: Streptomyces 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 streptomyces 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 species which showed in vitro antagonism against a diversity of plant pathogenic fungal species. Streptomyces strain Y20 could be used as a bioccontrol 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...
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, gluconases, 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 corn 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, Oskutzcab, 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 Streptomyces isolate and colony selection was based on 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’-CCCCGGATCCAGTCTTAAAGAGGTGATCCAG CC-3’) (Weisburg et al., 1991). The PCR fragments were amplified as described 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 spor 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 electron 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 Streptomyces isolates on the growth of phytopathogenic fungi (Bredholdt et al., 2007). An initial selection of antagonistic Streptomyces on the fungal pathogen *F. oxysporum* f. sp. *lycopersici* was implemented. An inoculum of Streptomyces 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 Streptomyces 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 Streptomyces.

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 K5α (5′-TSGRCTACRTCAACGGSCACGG-3′) and K5β (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′-CGCAGGTTSCTGGTACCAGTA-3′). The NRPS gene fragments were amplified with oligonucleotides A3F (5′-GCSTACSYSATSTACACSTCSGG-3′) and A7R (5′-SASGTCVCCCGTGTCGTTAS-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 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 *streptomyces*

A total of 46 Streptomyces-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
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 flexuous with a rectiflexible spore chain type with segmented barrel-like spore chains of the hyphae (Li et al., 2016). (Figure 2).

| 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** |
| ISP 2                 | +++            | +         | PKS-I |
| ISP 2 + casein        | +++            | +         | PKS-II |
| ISP 9                 | ++             | +++        | NRPS |
| ISP 9 + casein        | ++             | ++        | Antibiotic§ |
| NA                    | +++            | -         | TE |
| PDA                   | +++            | ++        | CTX, CF, AM, GE, SXT, |
| CDA                   | ++             | +++       | LEV, PE, E, DC, DL, FEP |
| TYA                   | +++            | -         | |
| King B                | +++            | +         | |

§ 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, levoflaxacin 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.](image-url)
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.

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

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

* 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. gloeosporoides* 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 Streptomyces 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 (Wave 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.

Acknowledgements

G.A. Rejón-Martínez. receives a bachelor grant No. 28651. E.A. Contreras-Leal receives a postdoctoral fellowship from CONACYT 273023. D.E. Ríos-Muñiz receives a postdoctoral fellowship from CONACYT 391737.

Funding. This study was supported by funds granted by Consejo Nacional de Ciencia y Tecnología (No. PN-2016-2900).

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@ciatejm.x

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.

REFERENCES

Abbasi, S., Safaie, N., Sadeghi, A. and Shamsbakhsh, M., 2019. Streptomyces strains induce resistance to Fusarium oxysporum f. sp. lycopersici race 3 in tomato through different molecular mechanisms. Frontiers in Microbiology, 10, pp. 1505. https://doi.org/10.3389/fmicb.2019.01505

Abdelmoteleb, A. and González-Mendoza, D., 2020. A novel Streptomyces rhizobacteria from desert soil with diverse anti-fungal properties. Rhizosphere, 16, pp. 100243. https://doi.org/10.1016/j.rhisph.2020.100243

Akram, S., Khan, S. M., Khan, M. F., Khan, H. U., Tariq, A., Umar, U. U. D. and Gill, A., 2018. Antifungal activity of different systemic fungicides against Fusarium oxysporum f. sp lycopersici associated with tomato wilt and emergence of resistance in the pathogen. Pakistan Journal of Phytopathology, 30, pp. 169–176.
Almeida, F., Rodrigues, M. L. and Coelho, C., 2019. The still underestimated problem of fungal diseases worldwide. *Frontiers in Microbiology*, 10, pp. 214. https://doi.org/10.3389/fmicb.2019.00214

Ayuso-Sacido, A. and Genilloud, O., 2005. New PCR primers for the screening of NRPS and PKS-I systems in actinomycetes: detection and distribution of these biosynthetic gene sequences in major taxonomic groups. *Microbial Ecology*, 49, pp. 10–24. https://doi.org/10.1007/s00248-004-0249-6

Bredholdt, H., Galatenko, O. A., Engelhardt, K., Fjaerik, E., Terekhova, L. P. and Zotevich, S. B., 2007. Rare actinomycete bacteria from the shallow water sediments of the Trondheims fjord, Norway: isolation, diversity, and biological activity. *Environmental Microbiology*, 9, pp. 2756–2764. https://doi.org/10.1111/j.1462-2920.2007.01387.x

Challis, G. L. and Hopwood, D. A., 2003. Synergy and contingency as driving forces for the evolution of multiple secondary metabolite production by *Streptomyces* species. *Proceedings of the National Academy of Sciences*, 100, pp. 14555–14561. https://doi.org/10.1073/pnas.1934677100

Chen, Y., Zhou, D., Gao, Z., Xie, J. and Luo, Y., 2018. Growth promotion and disease suppression ability of a *Streptomyces* sp. CB-75 from banana rhizosphere soil. *Frontiers in Microbiology*, 8, pp. 2704. https://doi.org/10.3389/fmicb.2017.02704

Di Francesco, A., Martini, C. and Mari, M., 2016. Biological control of postharvest diseases by microbial antagonists: how many mechanisms of action? *European Journal of Plant Pathology*, 145, pp. 711–717. https://doi.org/10.1007/s10658-016-0867-0

Evangelista-Martínez, Z., 2014a. Isolation and characterization of soil *Streptomyces* species as a potential biological control agent against fungal plant pathogens. *World Journal of Microbiology and Biotechnology*, 30, pp. 1639–1647. https://doi.org/10.1007/s11274-013-1568-x

Evangelista-Martínez, Z., 2014b. Preliminary study on some actinomycetes and evaluation of their potential antagonism against fungal pathogens. *British Microbiology Research Journal*, 4, pp. 272–281.

Evangelista-Martínez, Z., Quiiones-Aguilar, E. E. and Rincón-Enríquez, G., 2017. Potencial biotecnológico de las actinobacterias aisladas de suelos de México como fuente natural de moléculas bioactivas: compuestos antimicrobianos y enzimas hidrolíticas. *Revista de la UTM: Temas de Ciencia y Tecnología*, 61, pp. 3–12. https://www.utm.mx/edid _anteriores/temas63/T63_E011-2017.pdf

Evangelista-Martínez, Z., Contreras-Leal, E.A., Corona-Pedraza, L.F. and Gastélum-Martínez, E., 2020. Biocontrol potential of *Streptomyces* sp. CACIS-1.5CA against phytopathogenic fungi causing postharvest fruit diseases. *Egyptian Journal of Biological Pest Control*, 30, pp. 117. https://doi.org/10.1186/s41938-020-00319-9

Food and Agriculture Organization of the United Nations, FAO., 2011. Global food losses and food waste: extent, causes and prevention. By Gustavsson, J., Cederberg, C., Sonesson, U., van Otterdijk, R., Meybeck, A. FAO. Rome, Italy. http://www.fao.org/docrep/014/mb060e/mb060e00.pdf [11-01-2022].

Food and Agriculture Organization of the United Nations, FAO., 2019. New standards to curb the global spread of plant pests and diseases. FAO. Rome, Italy. https://www.fao.org/news/story/en/item/1187738/icode/ [01/02/2022].

González, I., Ayuso-Sacido, A., Anderson, A. and Genilloud, O., 2005. Actinomycetes isolated from lichens: evaluation of their diversity and detection of biosynthetic gene sequences. *FEMS Microbiology Ecology*, 54, pp. 401–415. https://doi.org/10.1016/j.femsec.2005.05.004

Kamil, F. H., Saeed, E. E., El-Tarabily, K. A. and AbuQamar, S. F., 2018. Biological control of mango dieback disease caused by Lasiodiplodia theobromae using Streptomyces and Non-streptomyces actinobacteria in the United Arab Emirates. *Frontiers in Microbiology*, 9, pp. 829. https://doi.org/10.3389/fmicb.2018.00829

Kawicha, P., Laopha, A., Chamnansing, W., Sopawed, W., Wongcharone, A. and Sangdee, A., 2020. Biocontrol and plant growth-promoting properties of *Streptomyces* isolated from vermicompost soil. *Indian Phytopathology*, https://doi.org/10.33866/phytopathol.030.02.0458

https://doi.org/10.33866/phytopathol.030.02.0458

https://pdfs.semanticscholar.org/d6db/4c7729acb0481ff236e75629e8f1cf2f47b2.pdf

https://doi.org/10.1016/j.femsec.2005.05.004
Lahdenperä, M. L., Simon, E. and Uoti, J., 1991. Mycostop - A novel biofungicide based on Streptomyces bacteria. *Developments in Agricultural and Managed Forest Ecology*, 23, pp. 258-263. https://doi.org/10.1016/B978-0-444-88728-3.50048-2

Li, Q., Chen, X., Jiang, Y. and Jiang, C., 2016. Morphological identification of actinobacteria. In: Dhanasekaran D. and Jiang Y. (eds). *Actinobacteria -basics and biotechnological applications*: InTech, India. pp. 59–86. https://doi.org/10.5772/61461

Lian, Q., Zhang, J., Gan, L., Ma, Q., Zong, Z. and Wang, Y., 2017. The biocontrol efficacy of *Streptomyces pratensis* LMM15 on *Botrytis cinerea* in tomato. *BioMed Research International*, 2017, pp. 9486794. https://doi.org/10.1155/2017/9486794

Malik, A., Kim, Y. R., Jang, I. H. and Kim, S. B., 2020. Genome-based analysis for the bioactive potential of *Streptomyces yeochonensis* CN732, an acidophilic filamentous soil actinobacterium. *BMC Genomics*, 21, pp. 118. https://doi.org/10.1186/s12864-020-6468-5

Moshi, A. P. and Matouj, I., 2017. The status of research on and application of biopesticides in Tanzania. *Crop Protection*, 92, pp. 16-28. https://doi.org/10.1016/j.cropro.2016.10.008

Pan, H. Q., Yu, S. Y., Song, C. F., Wang, N., Hua, H. M., Hu, J. C. and Wang, S. J., 2015. Identification and characterization of the antifungal substances of a novel *Streptomyces cavourensis* NA4. *Journal of Microbiology and Biotechnology*, 25, pp. 353-357. https://doi.org/10.4014/jmb.1407.07025

Saisivam, S., Bhikshapathi, D. V. R. N., Krishnaveni, J. and Kishan, V., 2008. Isolation of borrelidin from *Streptomyces californicus*—An Indian soil isolate. *Indian Journal of Biotechnology*, 7, pp. 349-355. http://nopr.niscair.res.in/handle/123456789/1849

Skarbek, J. D. and Brady, L. R., 1978. *Streptomyces cavourensis* sp. nov. (nom. rev.) and *Streptomyces cavourensis* subsp. *washingtonensis* subsp. nov., a chromomycin-producing subspecies. *International Journal of Systematic Bacteriology*, 28, pp. 45-53. https://doi.org/10.1099/00207713-28-1-45

Shirling, E. B. and Gottlieb, D., 1966. Methods for characterization of *Streptomyces* species. *International Journal of Systematic Bacteriology*, 16, pp. 313–340. https://doi.org/10.1099/00207713-16-3-313

Shoda, M., 2000. Bacterial control of plant diseases. *Journal of Bioscience and Bioengineering*, 89, pp. 515–521. https://doi.org/10.1016/s1389-1723(00)80049-3

Syed-Ab-Rahman, S. F., Carvalhais, L. C., Chua, E., Xiao, Y., Wass, T. J. and Schenk, P. M., 2018. Identification of soil bacterial isolates suppressing different *Phytophthora* spp. and promoting plant growth. *Frontiers in Plant Science*, 9, pp. 1502. https://doi.org/10.3389/fpls.2018.01502

Watve, M. G., Tickoo, R., Jog, M. M. and Bhole, B. D., 2001. How many antibiotics are produced by the genus *Streptomyces*? *Archives of Microbiology*, 176, pp. 386–390. https://doi.org/10.1007/s002030100345

Weisburg, W. G., Barns, S. M., Pelletier, D. A. and Lane, D. J., 1991. 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology*, 173, pp. 697–703. https://doi.org/10.1128/jb.173.2.697-703.1991

Yuan, W. M. and Crawford, D. L., 1995. Characterization of *Streptomyces lydicus* WYEC108 as a potential biocontrol agent against fungal root and seed rot. *Applied and Environmental Microbiology*, 61, pp. 3119–3128. https://doi.org/10.1128/aem.61.8.3119-3128.1995

Zhang, J., Chen, J., Hu, L., Jia, R., Ma, Q., Tang, J. and Wang, Y., 2020. Antagonistic action of *Streptomyces pratensis* S10 on *Fusarium graminearum* and its complete genome sequence. *Environmental Microbiology*, 23, pp. 1925-1940. https://doi.org/10.1111/1462-2920.15282.