

MOLECULAR IDENTIFICATION AND ANTAGONISTIC POTENTIAL OF THREE STRAINS OF *Streptomyces* AGAINST PHYTOPATHOGENIC FUNGI

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ABSTRACT

Actinobacteria belong to a group of abundant microorganisms in natural ecosystems. Out of these microorganisms, particularly from the species of the genus *Streptomyces*, most of the antibiotics that are used for human health have been characterized and identified. However, they are seldom used as antagonists in agricultural systems; despite it is plausible that species of *Streptomyces* can control important root pathogens. The objective of this study was to obtain the molecular identification and characterization of three *Streptomyces* strains by confronting them against phytopathogenic fungi and oomycetes, in order to determine the *in vitro* antagonistic potential and the effect of volatile compounds produced. The strains of *Streptomyces* were molecularly identified through the sequence of 16S of the RNAr such as: *Streptomyces mauvecolor* (B21), *Streptomyces lasiicapitis* (B22) and *Streptomyces olivochromogenes* (B37) which showed antagonistic potential *in vitro* against *Rhizoctonia solani*, *Fusarium oxysporum* and *Phytophthora capsici*. *S. mauvecolor* showed an average percentage of radial growth inhibition (PICR) for the three pathogens of 62.88 %, *S. lasiicapitis* of 98.72 % and *S. olivochromogenes* of 83.58 %. Similarly, out of the three pathogens, *R. solani* was inhibited in greater proportion by the three strains (93.85 %). A relevant fungistatic action was observed over *P. capsici*, an economically important oomycete, with 100 % inhibition at 24, 48 and 72 h of exposure with *S. lasiicapitis*, first reported as antagonist of root pathogens. The use of these species of *Streptomyces* with high levels of antagonism against pathogens of economically important crops, represents an alternative of low environmental impact.

Keywords: *Fusarium*, *Phytophthora*, *Rhizoctonia*, actinobacteria, antagonism, percentage of radial growth inhibition – PICR.

Citation: Sánchez-García BM, Ramírez-Pimentel JG, Rodríguez-Guerra R, Guevara-Acevedo LP, Raya-Pérez JC, Covarrubias-Prieto J, Mora-Avilés MA. 2022. Molecular identification and antagonistic potential of three strains of *Streptomyces* against phytopathogenic fungi. *Agrociencia*. <https://doi.org/10.47163/agrociencia.v56i5.2793>

Editor in Chief:
Dr. Fernando C. Gómez Merino

Received: May 11, 2022.
Approved: July 11, 2022.
Published in Agrociencia:
August 20, 2022.

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INTRODUCTION

The current demand for a greater quantity of foods is causing the uncontrolled use of synthetic agrochemicals to increase production of crops and to control pests and diseases that appear in each agricultural cycle (Kaur *et al.*, 2019). This results in the degradation of soils, the rise of diseases and pests resistant to the applied products, environmental pollution, and in particular, poses a risk to human health (Jeon *et al.*, 2016).

Among the alternatives for reducing the application of synthetic pesticides, there is the use of microorganisms with distinct biocontrol characteristics, especially antagonism, the induction of resistance and the production of secondary metabolites (Condori-Pacsi *et al.*, 2019). The genus *Streptomyces* is within the microorganisms that show potential as alternatives in the reduction of phytosanitary problems. In terms of number and variety of species identified, *Streptomyces* represents one of the largest taxonomic groups of Actinomycetes and more than 70 % of the natural microbial products reported until now. They are as well, a potential source for controlling biotic and abiotic stress in plants (Passari *et al.*, 2019).

In addition, approximately 60 % of the antibiotics used in agriculture come from *Streptomyces* spp. (Shrivastava *et al.*, 2015). Studies in tomato, wheat, rice, pea and eucalyptus showed that *Streptomyces* spp. have the capacity to produce extracellular proteases, antibiotics, volatile compounds, siderophores, and to promote plant growth through the production of auxins such as the indole acetic acid (Dias *et al.*, 2017). Bacteria *Streptomyces* spp. prevent the development of phytopathogens, due to their capacity to synthesize antibiotics and hydrolytic enzymes that degrade the cell wall of spores of pathogenic fungi (Reyes-Tena *et al.*, 2015). An example of this is kasugamycin (*S. kasugaensis*), which controlled the fungus *Pyricularia oryzae* Cavara which causes rice blight, as well as bacterial diseases caused by *Erwinia* in pear and apple orchards (Kasuga *et al.*, 2017). On the other hand, the streptomycin from *S. griseus* efficiently fought the bacteria *Erwinia amylovora*, the cause of fire blight in pear and apple (Quiñones *et al.*, 2016).

The presence of *Streptomyces* spp. in disturbed and undisturbed soils makes these microorganisms the ideal antagonists to be used in agricultural systems because of their wide adaptability. Thus, the search and characterization of local species of *Streptomyces* shall provide alternatives of biological control for the economically important crops in the region, particularly the consortia of pathogens causing root rot. The objective of this study was to obtain the molecular identification and characterization of three *Streptomyces* strains, by confronting them against phytopathogenic fungi and oomycetes which cause root rot in economically important crops, in order to determine their *in vitro* antagonistic potential and the effect of the volatile compounds produced.

MATERIALS AND METHODS

Strain origin

Three strains of actinobacteria with antagonistic potential, two strains of phytopathogenic fungi and a strain of oomycete from different ecological and

geographic origin within the state of Guanajuato were used in this research. The strains B21 and B22 of actinobacteria were isolated in the rhizosphere of evergreen oak forest in the municipality of Sta. Catarina (21° 17' 97.30" N - 100° 04' 07.30" W and 21° 18' 59.00" N - 100° 07' 22.00" W, respectively), while the strain of actinobacteria B37 was isolated from the maize rhizosphere in the municipality of Pénjamo (20° 20' 33.16" N - 101° 40' 00.68" W). The strains of the phytopathogens *Fusarium oxysporum*, *Rhizoctonia solani* and *Phytophthora capsici* were isolated from the root system of "chilaca" chilli plants that showed symptoms of root rot, in the community of Chirimoya, municipality of San Felipe, Guanajuato, Mexico (21° 36' 16.7" N - 101° 04' 58.3" W).

Morphological characterization of actinobacteria

The actinobacteria were characterized at 14 d of growth of the colonies in Potato Dextrose Agar (PDA) medium (Bioxón®), a culture medium rich in nutrients that made it possible to observe development and sporulation of the actinobacteria (Dávila *et al.*, 2013). The morphological characterization was done by means of macroscopic observations of the colony, such as structures and arrangement of the spore mass, colour, form, size, elevation, texture and diffusible pigment in culture medium. The Gram stain was performed microscopically to verify and classify the three strains as Gram+. Slides were prepared with the actinobacteria, where characteristics were observed such as aerial and vegetative mycelia, grouping of spores, presence of spirals and terminal spores; and they were compared with those described by the manual of Bergey (Bergey *et al.*, 2000).

Molecular identification of the actinobacteria

Extraction of DNA and sequencing of the ribosomal gene 16S

The extraction of genomic DNA was obtained according to the protocol of Nikodinovic *et al.* (2003). For the molecular identification, the ribosomal gene 16S was amplified and sequenced with the universal primers 8F 5'-AGAGTTTGATCCTGGCTCAG-3' and 1492R 5'GGTTACCTTGTTACGACTT-3' (Gunda and Singaracharya, 2012) which generate a band of approximately 1500 pairs of bases (pb). The conditions for the amplification were as follows: denaturalization, 1 cycle 95 °C for 5 min, 35 cycles of annealing at 95 °C for 1 min, extension to 59 °C for 45 s and 72 °C for 1 min, as well as a final extension cycle at 72 °C for 8 min. The genetic sequences obtained were analysed with the program DNASTAR Lasergene v11. The consensus sequences were compared with the sequences deposited in the GenBank through the tool BLAST (Basic Local Alignment Search Tool) for nucleotides of the NCBI (National Center for Biotechnology Information) (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and registered in the data base of NCBI.

Phylogenetic analysis

The phylogenetic tree was constructed by means of paired comparisons seeking similarities of sequences calculated for the gene 16S RNAr (Meier- Kolthoff *et al.*, 2013)

through the web server Genome to Genome distance Calculator (GGDC) (<http://ggdc.dsmz.de/>) (Meier-Kolthoff *et al.*, 2022). The multiple alignment of sequences was made with Clustal W of the MEGA 11 software (Molecular Evolutionary Genetics Analysis) (Tamura *et al.*, 2021).

Confrontations of actinobacteria with antagonistic potential

Three strains of actinobacteria (B21, B22 and B37) were confronted to determine the antagonistic potential against *R. solani*, *P. capsici* and *F. oxysporum*. A fragment of PDA with actinobacteria was dispersed in the centre of a Petri dish with PDA, in a diameter of approximately 3 cm. The inoculated Petri dishes were maintained in darkness at 26 °C for 9 d, time in which the production of spores was observed. After this time had passed, the phytopathogenic fungi *R. solani*, *F. oxysporum* and the oomycete *P. capsici*, of 48 h of growth, were placed in fragments of 0.7 cm diameter at a distance of 2 cm from the margin of the colony of actinobacteria. As controls, fragments of the colony of the same diameter of the phytopathogens were placed in a Petri dish with PDA medium similarly to those of the confrontations but without actinobacteria. Three replications were made per strain of actinobacteria and of the phytopathogens under study. The evaluation was made at 24, 48 and 72 h after the start of the confrontation; the radial growth of the mycelia was measured of the confronted phytopathogens and their controls. The percentage of radial growth inhibition (PICR) was determined using the formula of Ezziyani *et al.* (2004):

$$\text{PICR} = [(R1 - R2) / R1] \times 100$$

where R1 is the radius of the growth of the colony of the control pathogen and R2 is the radius of the colony of the pathogen in confrontation with the strain of actinobacteria.

Production of volatiles by means of the double plate method

Three Petri dishes with PDA medium were inoculated in the centre (3 cm diameter) with the strains of actinobacteria (B21, B22 and B37), with three replications. The inoculated Petri dishes were incubated at 26 °C for 7 days in darkness. After this time, in another Petri dish with PDA medium, 12 fragments were placed of 2 × 2 mm of colony of 48 h growth of fungi and phytopathogenic oomycetes (*R. solani*, *F. oxysporum* and *P. capsici*). Both uncovered Petri dishes were placed in front of each other and were sealed at the point of union with plastic film; the evaluation consisted of measuring the radial growth of the pathogens-control and those confronted with the strains of actinobacteria 48 h after the inoculation of the phytopathogens (Rajani *et al.*, 2021).

Experimental design

The experimental design was completely randomized where each one of the antagonistic strains was confronted with the three pathogenic microorganisms. The experimental unit was a Petri dish with three replications. The statistical analysis

consisted of an analysis of variance and comparison of means with the Tukey test ($p \geq 0.05$), with the statistical utility Minitab® 17 (Minitab Statistical Software).

RESULTS AND DISCUSSION

Morphological characterization of actinobacteria

The morphological characteristics of the actinobacteria colonies cultivated in PDA medium were different for the three isolated strains. The strain B21 showed colonies of firm consistency, with a filamentous appearance, elevated umbilicate form, filamentous and white irregular border (Figure 1A). Strain B22 showed colonies of firm consistency, filamentous appearance, elevated crater form and production of transparent secretions, filamentous irregular border and red-pink colour (Figure 1B); whereas strain B27 presented colonies of soft consistency, shiny appearance, convex elevated form, rough surface and yellow colour with irregular cream-colored border (Figure 1C).

Strain B21 presented aerial mycelia, winding and unfragmented with spiral arrangements of spores (Figure 1A), while strains B22 and B37 displayed long straight chains of spores (Figure 1B and 1C, respectively). The macroscopic morphological characteristics were contrasted with those described by Bergey *et al.* (2000), and it was determined that the selected actinobacteria showed specific characteristics of the genus *Streptomyces* spp. In the Gram stain test, the three strains were found to be Gram positive (Gram+).

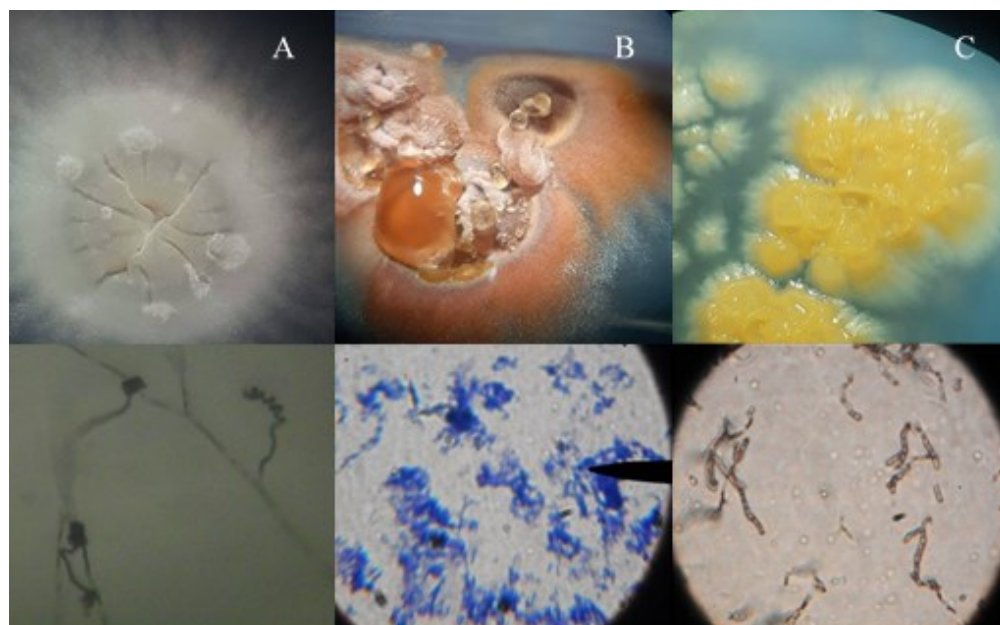


Figure 1. Morphological characteristics of three strains of actinobacteria in PDA medium at 14 d in culture. Upper panel: macroscopic morphology. Lower panel: microscopic morphology (100 ×). A: strain B21; B: strain B22 and C: strain B27.

Molecular characterization of the actinobacteria

The characteristics of the strains and the analysis of sequences of the amplification of gene 16S of rDNA indicated that the strain B21 (1298 pb) showed 100 % similarity with *Streptomyces mauvecolor*; this species is an actinobacterium that has the capacity of producing the polyene macrolide rimocidin, which exhibits a strong inhibitory activity against a wide range of phytopathogenic fungi (Zhao *et al.*, 2019). The interaction between the polyenes and the membranes of the fungi consists in the latter being affected in some of the physiochemical properties of their membranes, such as changes in their ionic permeability, formation of pores and consequently, cellular death. Studies showed that this actinobacterium efficiently controls phytopathogenic fungi such as *Alternaria mali*, *Aspergillus oryzae*, *Botrytis cinerea*, *Colletotrichum coccodes*, *Colletotrichum gloeosporoides*, *Colletotrichum orbiculare*, *Cylindrocarpon destructans*, *Fusarium oxysporum* f. sp. *lycopersici*, *Rhizoctonia solani*, and *Rhizopus stolonifera* var. *stolonifera* at concentrations that range from 2-8 µg L⁻¹ of rimocidin (Jeon *et al.*, 2016).

On the other hand, B22 (1302 pb) showed 100 % similarity with *Streptomyces lasiicapitis*. The information of this species of *Streptomyces* is very scarce. The first report of identification comes from an isolate of the ant head in China and is characterized by producing kanchanamycin (Ye *et al.*, 2017). In 1996, kanchanamycin was originally obtained from *Streptomyces olivaceus*, and an effect of inhibition was observed on the growth of fungi and bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Penicillium notatum*, among others (Solanski *et al.*, 2013). Derived from the scarce information that exists on the effects of *Streptomyces lasiicapitis* (B22) on phytopathogenic fungi, the opportunity is opened to characterize this species as an antagonistic agent along with its profile of synthesis of secondary metabolites.

Finally, the strain B37 (1339 pb) showed a 99.7 % of similarity with *Streptomyces olivochromogenes*. This species has been studied for its production of metabolites, its capacity of antagonism against bacteria and fungi, its ability to degrade pesticides such as the organophosphates in soils contaminated in high concentrations, among other characteristics. Balachandran *et al.* (2016) isolated and characterized the metabolite 2-hydroxy-9,10-anthraquinone from *S. olivochromogenes*. This metabolite showed antibacterial activity against *Pseudomonas aureginosa*, *Proteus vulgaris* and *E. coli*, among others; as well as antifungal activity against *Malassezia pachydermatis* and *C. albicans*, causal agents of diseases that affect human health. Similarly, it showed an effect of biocontrol with an efficiency of 42 % against *Plasmodiophora brassicae*, a disease which affects Chinese cabbage (Zhou *et al.*, 2014).

The three sequences were deposited in the GenBank with the following accession numbers: *Streptomyces mauvecolor* ON361376.1 (B21), *Streptomyces lasiicapitis* ON361556.1 (B22) and *Streptomyces olivochromogenes* ON361562.1 (B37).

The phylogenetic tree grouped the three species of *Streptomyces* into two main clades. In the first clade, *S. lasiicapitis* (B22) (ON361556) was placed among other previously reported accessions of the same species, indicating levels of similitude in the partial

sequence of the gene 16S rRNA of 98.7 to 99.8 %. The location of this accession regarding the other two species of *Streptomyces* indicates an ancestor different from *S. olivochromogenes* or *S. mauvecolor* (Figure 2). The second clade included *S. mauvecolor* (B21) (ON361376) which was also grouped among other accessions of the same species with a similitude of 98.9 to 99.7 %. Finally, and in the same clade, *S. olivochromogenes* (B37) (ON361562) was located, which was consistent sharing the same node with EU589442 and closeness to MH251060, with a similitude of 99.7 % and 98.5 %, respectively. It is important to note that in the phylogenetic tree, another prominent accession (MN620387), reported as *S. olivochromogenes*, but with a lower similitude

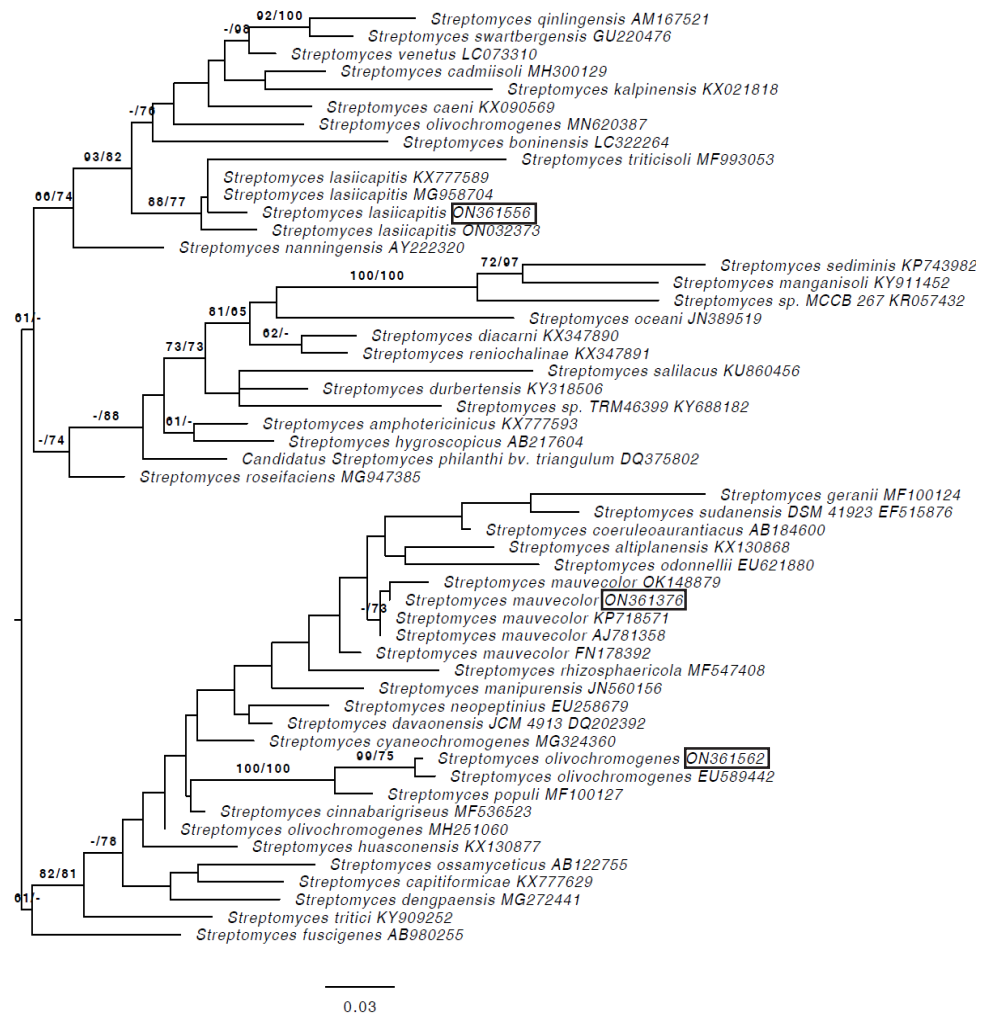


Figure 2. Phylogenetic tree of three species of *Streptomyces* from the sequencing of the gene 16S rRNA by the statistical method of maximum likelihood. The accessions with rectangle are the species reported in this document. The numbers above the branches are support values when they are higher than 60 % of maximum likelihood (left) and maximum parsimony (right).

(95.22 %) and located in a different clade. In this case, there is the possibility that this species was different from *S. olivochromogenes* due to its higher similitude to the other species close to it (Figure 2).

Antagonistic potential of the actinobacteria

The results of the confrontation between actinobacteria and phytopathogens showed a widely variable range of the PICR, which depended on each interaction. *S. mauvecolor* (B21) showed a PICR of 62.88 % for the three pathogens, inhibiting in a higher proportion *R. solani* (89.86 %) (Table 1). Jeon *et al.* (2016) reported that this species did not have the capacity to inhibit the growth of *P. capsici* at concentrations of up to 128 $\mu\text{g L}^{-1}$. This result is consistent with those obtained in this study, as *S. mauvecolor* was able to strongly inhibit the development of *R. solani*; however, it had an inhibition of *P. capsici* (44.66 %) and *F. oxysporum* (54.13 %). Větrovský *et al.* (2014) reported that the hydrolytic enzymes produced by *S. mauvecolor* after 21 d of cultivation in wheat straw are in the order ($\text{nmol min}^{-1} \text{mL}^{-1}$) of activity level of the enzymes 1, 4- β -xilosidase, endoxylanase, 1,4- β -glucosidase and 1,4- β -manosidase. This could signify that the hydrolytic enzymes produced by the strain B21 of *S. mauvecolor* were sufficient (in type and activity) to inhibit *R. solani* and *F. oxysporum*, but not *P. capsici* for its conformation of cell wall.

On the other hand, *S. lasiicapitis* showed inhibition percentages of 96 to 100 % from the first hours of exposure for the three pathogens. This is the first report where the antagonistic capacity of *S. lasiicapitis* is demonstrated, thus it has great potential in the control of pathogens. The type of metabolite synthesized by *S. lasiicapitis* reported as kanchanamycin was isolated from *Lasius fuliginosus*, the jet-black ant (Ye *et al.*, 2017). The genus *Streptomyces* is commonly found in microbiomes of insects such as the Southern pine beetle (*Dendroctonus frontalis*) and exhibits mutualism by producing a series of secondary metabolites that protect them from entomopathogenic fungi through the compounds frontalamide A, frontalamide B and mycangimycin. Similarly, the actinobacterium as antagonistic agent.

Table 1. Percentage of Radial Growth Inhibition (PICR) by actinobacteria over phytopathogenic fungi and oomycetes.

Actinobacteria strains	<i>R. solani</i>			<i>P. capsici</i>			<i>F. oxysporum</i>			Average (%)
	24 [†]	48	72	24	48	72	24	48	72	
<i>S. mauvecolor</i> B21	64.07b [‡]	81.61a	89.86b	0.00b	33.13b	44.66b	16.67b	25.76b	54.13b	62.88
<i>S. lasiicapitis</i> B22	96.67a	94.07a	96.18a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	98.72
<i>S. olivochromogenes</i> B37	89.26a	91.40a	95.51ab	0.00b	32.86b	57.69b	93.75a	95.23a	97.54a	83.58
Promedio (%)	83.33	89.02	93.85	33.33	55.33	67.45	70.14	73.66	83.89	

[†]Hours after inoculation; [‡]Means with different letter within column are different (Tukey $p \leq 0.05$).

Streptomyces formicae isolated from the head of the Japanese carpenter ant (*Camponotus japonicas*), exhibited specific antifungal activity in the phytopathogens *Phytophthora infestans* and *Corynespora cassiicola* (Kett *et al.*, 2021). These reports indicate that the characterization of *S. lasiicapitis* is still incipient in terms of hydrolytic enzymes and synthesized metabolites. However, the results presented here indicate the important capacity of this actinobacterium as antagonistic against *Streptomyces olivochromogenes* exhibited more variable levels of inhibition, notably the inhibition of growth of *R. solani* (95.5%) and *F. oxysporum* (97.5 %); however, the PICR values indicated that it took longer time, or the efficiency of *S. olivochromogenes* was lower, for inhibiting the growth of *P. capsici* with reduced percentages of inhibition (57.7%) in comparison to its activity over the other two pathogens (Table 1). This species has clusters of 40 potential biosynthetic genes, including 7 polyketide synthases (PKSs), 4 non-ribosomal peptide synthases (NRPSs), 4 bacteriocins, 4 terpenes, 3 siderophores, 2 lanthipeptides and 1 lasso peptide (Dohra *et al.*, 2017). In addition, according to Balachandran *et al.* (2016), the 2-hydroxy-9,10-anthraquinone is the active principle of the antimicrobial and antiproliferative activity of *S. olivochromogenes* against bacteria and fungi. The above marks the basis for determining the structure and function of the bioactive compounds and their biosynthetic pathways and transport systems, of this important antagonist. Independently of the values of PICR at 72 h during the interaction between the actinobacteria and the pathogens *in vitro*, it was observed that the species *S. lasiicapitis* completely inhibited the growth of the three pathogens, suggesting an antagonistic machinery that is broader than the other two species of actinomycetes. *S. mauvecolor* and *S. olivochromogenes* required more time to reach relevant PICR values, and showed a less robust antagonistic system, in particular for the control of *P. capsici* (Figure 3). In all of the cases the inhibition of the phytopathogens by the three species of *Streptomyces* occurred without presenting contact with each other. Thus, it suggests the production of metabolites secreted and diffusible in the culture medium (Rodríguez-Villareal *et al.*, 2014).

No differences were found in the PICR among the three species of *Streptomyces* spp. over *R. solani*, given that the three were efficient in the inhibition of the growth of this pathogen. However, the differences shown in the PICR of *P. capsici* and *F. oxysporum* suggest differential antagonistic mechanisms for more complex pathogenic systems. Although *S. mauvecolor* (B21) and *S. lasiicapitis* (B22) both come from the evergreen oak forest rhizosphere, it was observed that they have different mechanisms of performance in the inhibition of phytopathogenic agents. Regarding this, it was reported that the differential in the action mechanisms among species of *Streptomyces* may be due to the versatile production of secondary metabolites that act as antibiotic, fungicidal and siderophore substances used as active principles in competitive strategy for colonizing the area in which they are found, even when the strains belong to the same species and ecological niche (Anwar *et al.*, 2016).

The antagonism of *Streptomyces* spp. is linked to the activity used (antibiosis, competition, parasitism, induction of resistance, etc.) and the differential production

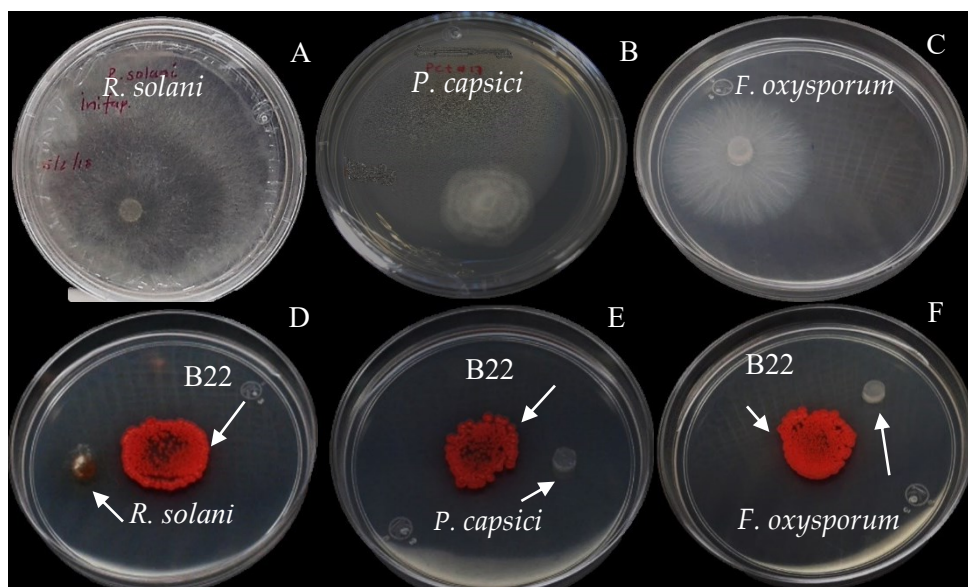


Figure 3. Growth development of pathogens in the presence or absence of actinomycetes after 72 h of confrontation. A: *Rhizoctonia solani* control; B: *Phytophthora capsici* control; C: *Fusarium oxysporum* control; D: *Rhizoctonia solani* vs. *Streptomyces lasiicapitis* confrontation; E: *Phytophthora capsici* vs. *Streptomyces lasiicapitis* confrontation; F: *Fusarium oxysporum* vs. *Streptomyces lasiicapitis* confrontation.

of antibiotic and fungicidal substances is given by the sporulation of *Streptomyces* spp. where the production of secondary metabolites is initiated, due to the exhaustion of available food, as well as the growth rate of the pathogen to antagonize, and to the virulence of the antagonistic species being tested (Kunova *et al.*, 2016). Therefore, these results indicate that the species of *Streptomyces* deploy different antagonistic mechanisms and in turn produce different bioactive molecules involved in the antagonism against different pathogens, all of which forms part of monitoring the characterization of these species.

Production of volatiles by the double plate method

The radial growth of the three pathogens responding to the possible presence of volatiles of the three species of *Streptomyces* was not inhibited and a growth similar to the controls was observed in absence of *Streptomyces* spp. Thus, no evidence was found of the presence of volatile compounds or effect in case of their synthesis, in the inhibition of the growth of the three phytopathogens. Boukaew *et al.* (2013) reported that the volatile compounds generated by *Streptomyces philanthi* (RM-1-138) inhibited the pathogenic fungi *Rhizoctonia solani* (PTRRC-9), *Pyricularia grisea* (PTRRC-18), *Bipolaris oryzae* (PTRRC-36) and *Fusarium fujikuroi* (PTRRC-16), with more pronounced effects with *S. philanthi* at 14 d of growth (52.85 – 100 %) than at 7 days of growth (17.03-89.40 %). In addition, they identified by means of gas chromatography-

mass spectrometry (CG/EM) 17 and 36 compounds at 7 and 14 days of cultivation, respectively. Similarly, Wang *et al.* (2013) demonstrated that volatile compounds of *Streptomyces alboflavus* inhibited *Fusarium moniliforme*, *Aspergillus flavus*, *A. ochraceus*, *A. niger* and *Penicillium citrinum* *in vitro* by 24.8 % with mycelia of 5 d of cultivation, identifying by means of CG/EM analysis 27 types of volatile organic compounds. Therefore, a possible reason of not observing an effect of volatile compounds in the inhibition of pathogenic fungi in this study could be due to the time of induction and exposure to them.

The production of volatile compounds by microorganisms such as *Streptomyces* spp. is dynamic and complex, given that they are produced in small quantities, and according to other authors, the antifungal activity varies with the age of the colony of the fungus, conditions and time of cultivation, temperature, among others. Thus, other assays will be required to determine the presence and analysis of the possible volatile compounds synthesized by the species of *Streptomyces* studied here.

CONCLUSIONS

This study demonstrated the antagonistic activity of three species of *Streptomyces*, which showed more than 80 % inhibition *in vitro* against phytopathogenic fungi. The differential percentages of inhibition of radial growth in each interaction, established that the antagonistic mechanisms of *S. mauvecolor*, *S. lasiicapitis* and *S. olivochromogenes* are also ruled by the hydrolytic enzymes and secondary metabolites synthesized in each confrontation.

S. lasiicapitis was the actinobacterium with superior capacity of inhibition of radial growth, including *P. capsici*, one of the most recalcitrant pathogens in terms of control. No evidence was found of activity of volatile metabolites. Therefore, a more specific characterization is needed to determine the synthesis and activity level under experimental conditions with variables reported by other authors.

ACKNOWLEDGEMENTS

To the Mexico's National Council for Science and Technology (CONACYT) for the doctorate scholarship (Num. 206005), awarded to Bertha María Sánchez García; and to the National Institute of Technology, Roque (TecNM-Roque) for facilitating installations to implement this research.

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