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INHIBITORY ACTIVITY OF THERMO-RESISTANT METABOLITES PRODUCED BY *Streptomyces* SP. CACIS-1.16CA AGAINST HUMAN AND PLANT PATHOGENIC BACTERIA

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ABSTRACT

For many decades, members in the genus *Streptomyces* have been considered the principal microbial producers of secondary metabolites with antibiotic activity. In this study, we evaluated the inhibitory activity of a cell-free supernatant produced by *Streptomyces* sp. CACIS-1.16CA against human and plant bacterial pathogens. We also assessed the thermal stability of the compounds. An initial screening to evaluate the antibacterial activity of compounds diffused from a CACIS-1.16CA culture into agar discs found inhibitory effects against *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus pumilus*, and *Pectobacterium carotovorum.* A cell-free supernatant from cultured CACIS-1.16CA confirmed the inhibition effects on the susceptible pathogens. Thermally treating the supernatant did not significantly affect the inhibitory activity, indicating *Streptomyces* sp. CACIS-1.16CA produces some thermally stable antibacterial metabolites. This antibacterial activity is consistent with the detection of biosynthetic gene clusters for polyketide synthase Types I and II and non-ribosomal peptide synthase.

Keywords: antibiotic; pathogenic bacteria, *Pectobacterium carotovorum,* secondary metabolites, streptomycetes

INTRODUCTION

Streptomycetes (Streptomycetaceae) are the group of microorganisms considered to have the highest production of bioactive compounds, and for many years they have been considered the best source of new antibiotics (**Genilloud, 2017**). The genus *Streptomyces* belongs to this bacterial family, and with more than 600 diverse species described, they produce more than half of the known antibiotics (**Nithya *et al*. 2018**). Other biologically active compounds produced by members of the genus include metabolites that function as plant growth factors, siderophores, anti-protozoans, antifungals, antivirals, insecticides, pesticides, herbicides, immunomodulators, antitumor agents, and enzyme inhibitors (**Harir *et al*. 2018**).

Despite this diversity of compounds, the discovery of new antibiotics has significantly declined, which is why new strategies have been developed for finding novel molecules. One of these strategies relies on the isolation of microorganisms in unexplored environments, with the goal of identifying new bioactive compounds. Microorganisms inhabiting oceans, soil halophiles, plant endophytes, and less studied species have been preferred. For example, a genomic study of *Streptomyces yeochonensis* CN732, an acidophilic neutrotolerant bacterium isolated from soil, identified 22 biosynthetic clusters of secondary metabolites (**Malik *et al*. 2020**).

Food poisoning and foodborne illnesses are a primary public health problem worldwide. According to the World Health Organization (WHO), foods contaminated with bacteria, viruses, parasites, and chemicals cause more than 200 different diseases, from diarrhoea to cancer. Each year, approximately 600 million people around the world acquire an infection from consuming contaminated food (**WHO, 2020**). Hence, the search for new compounds against human pathogenic bacteria will be directed toward the bacteria priority list established by **WHO (2017**). Carbapenem-resistant bacteria from the *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and several Enterobacteriaceae species comprise the critical priority category (Level 1); high-priority bacteria (Level 2) include methicillin-, vancomycin-, clarithromycin-, cephalosporin-, and fluoroquinolone-resistant species (*Enterococcus faecium*, *Staphylococcus aureus*, *Helicobacter pylori*, *Campylobacter* spp., Salmonellae, and *Neisseria gonorrhoeae*). Another area of interest is the search for new compounds effective in reducing the deleterious effects of pathogenic bacteria in agricultural crops. The current catalog naming the ten most important pathogenic bacteria for plant pathologists includes *Pseudomonas syringae*, *Ralstonia solanacearum*, *Agrobacterium tumefaciens*, *Xanthomonas oryzae* pv. *oryzae*, *X. campestris*, *X. axonopodis*, *Erwinia amylovora*, *Xylella fastidiosa*, *Dickeya* (*D. dadantii* and *D. solani*), and *Pectobacterium carotovorum* (**Mansfield *et al.* 2012**).

Streptomyces sp. CACIS-1.16CA is a soil bacterium isolated from a Wetland of International Importance (RAMSAR site) located at Los Petenes Biosphere Reserve in Campeche State, Mexico. This bacterium has shown antagonistic activity against the phytopathogenic fungi *Curvularia* sp., *Aspergillus niger*, *Helminthosporium* sp., *Fusarium* sp., *Alternaria* sp., *Phytophthora capsici*, *Colletotrichum* sp., and *Rhizoctonia* sp., all isolated from diseased plants. The CACIS-1.16CA strain produces a yellow water-soluble metabolite that accumulates in culture medium and correlates with an inhibitory effect on fungal growth (**Evangelista-Martínez, 2014**). Streptomycetes are also important for producing secondary metabolites with anti-candida activity (**Córdova-Dávalos *et al*. 2018**). In view of the above, the objectives of this study were to evaluate the antibacterial activity of a cell-free supernatant produced by *Streptomyces* sp. CACIS-1.16CA against human and plant pathogens and to determine the thermal stability of the compounds in vitro.

MATERIAL AND METHODS

Microorganisms and cultures

Human pathogens used in this study were *Escherichia coli* ATCC 17775, *Salmonella enterica* subsp. *enterica* serovar Typhimurium ATCC 14028 (*Salmonella* Typhimurium), *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Salmonella enterica* serovar Enteriditis ATCC 33090 (*Salmonella* Enteriditis), and *Pseudomonas aeruginosa* ATCC 33090. The plant pathogen bacteria were *Pseudomonas syringae* pv. *phaseolicola*, *Bacillus pumilus*, and *Pectobacterium carotovorum*. All strains were maintained on nutrient agar (NA), excepting the *Pseudomonas* species, which were grown on King B agar. The bacterial inoculums were prepared in Mueller-Hinton (MH) broth and King B broth, respectively. The *Streptomyces* sp. CACIS-1.16CA strain was maintained on International Streptomyces Project No. 2 Agar (ISP2).

Preparation of bacterial cultures

Bacteria inoculums were prepared according to the Clinical and Laboratory Standards Institute method (**CLSI, 2012**). The broth media was inoculated with a loopful of a bacterial colony, mixed, and maintained in an orbital shaker (150 rpm, 18 h at 37 °C). A 0.1 mL volume of a bacterial suspension was adjusted to a 0.5 optical density (OD_{600}) on the McFarland scale and dispersed on MH or King B agar media in a Petri dish. The Petri plates were ready to test for antibacterial activity after absorption of the cell suspension.

Evaluation of antibacterial activity

A preliminary screening to evaluate the inhibitory effect of diffusible metabolites was performed using the agar disc-diffusion method. Initially, a suspension of CACIS-1.16CA spores was distributed on NA medium at the edge of a Petri dish, leaving a square space without bacteria (3 cm^2) at the center. The inoculated plates were incubated for 14 days at 29 °C. The growth of the strains was then inactivated by placing the Petri plates in a sealed chamber containing chloroform for 30 min. Subsequently, a 6 mm diameter mycelial-free agar disc was removed from the center of the Petri dish, deposited on seeded plates inoculated with pathogenic bacteria (as described above), and incubated at 37 °C for 24 h. The diameters of the inhibition halos were measured with a caliper, with all measurements conducted in triplicate.

Production of antibacterial metabolites in submerged cultures

ISP2 medium supplemented with 50 ppm of antifoam A (Sigma-Aldrich) was used. Cultures (30 mL) were grown at 30 °C in 250-mL siliconized flasks with stainless steel springs at 150 rpm in an orbital shaker (**Kieser *et al.* 2000**). Cultures were inoculated with a spore suspension that had been washed twice in sterile distilled water and adjusted to an initial optical density of 0.05. Growth was monitored by optical density measurements at 450 nm. Samples to be assayed for antibacterial activity were drawn at appropriate time intervals and centrifuged at 16,000 × g in a microcentrifuge (Hettich); the supernatants (SN) were then withdrawn, and filter sterilized (0.22 µm, Millipore) into fresh tubes before storing at −20 °C until the assays. The SNs were analyzed using a Jenway 6715 UV/VIS spectrophotometer (Cole-Palmer), with the absorbance spectra ranging from 220 to 740 nm. All measurements were conducted in triplicate.

Evaluation of SN antibacterial activity by the paper disk-diffusion assay

The production of inhibitory compounds accumulated during the growth of the CACIS-1.16CA strain was analyzed by the paper disk-diffusion method (**Carović-Stanko *et al*. 2010**). These experiments were performed using the pathogenic bacteria that were inhibited in the previous tests. A 10 µL SN fraction collected after 96 h of incubation was deposited on 6 mm diameter filter paper discs (Whatman) and placed on the pathogen-inoculated Petri plates. After 24 h of incubation at 37 °C, the diameters of the inhibition's zones were measured with a caliper. Kanamycin (30 mg/mL) was used as the positive control. All experiments were conducted in triplicate.

Effect of temperature on antibacterial activity

At this stage of the study, the effect of temperature on the antibacterial activity of the compounds produced by *Streptomyces* sp. CACIS-1.16CA was evaluated. Initially, the total SN fractions collected from the submerged cultures were heated to 100 °C for 3 min and assayed on *E. coli* ATCC 17775. Subsequently, one 96-h SN fraction was heated at 100 °C for 60 min, and another SN fraction was treated at 121 °C for 15 min in an autoclave. These two treatments were evaluated on the five selected pathogenic strains. The determination of antibacterial activity of the SNs was performed as previously described, with 10 µL of the SN deposited on 6 mm diameter filter-paper discs before placement on the surfaces of MH Petri dishes inoculated with the pathogenic bacteria. In all cases, the Petri plates were incubated at 37 ° C for 24 h, and all experiments were performed in triplicate. The diameter of the inhibition zone was measured with a caliper. For the last experiment, the residual activity (RA) of the SNs was determined based on **Carvalho and Van Der Sand** (**2016**), where: RA (%) = (MSNT − 6) / (MSNC − 6) × 100. MSNT is the mean size of the inhibition halos obtained with the heat-treated SN (mm); MSNC is the mean size of the inhibition halo (mm) for the unheated control sample; and 6 is the diameter of the paper disc (mm).

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

Detecting the biosynthetic genes involved in the production of molecules with antimicrobial activity in *Streptomyces* sp. CACIS-1.16CA was performed by polymerase chain reactions (PCR) with specific oligonucleotides. 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 the oligonucleotides A3F (5′-GCSTACSYSATSTACACSTCSGG-3′) and A7R (5′-SASGTCVCCSGTSCGGTAS-3′; **Ayuso-Sacido and Genilloud, 2005**), and the PKS Type II gene fragments were amplified with the degenerate oligonucleotides KSα (5'-TSGCSTGCTTCGAYGCSATC-3') and KSβ (5' TGGAANCCGCCGAABCCGCT 3') (**Metsä-Ketelä *et al*. 1999**). The PCR conditions were implemented using Expand High Fidelity PCR system (Roche) (**Ayuso-Sacido and Genilloud, 2005; Metsä-Ketelä *et al*. 1999**). The PCR products were visualized in 1.5 % agarose gel prepared in 1× Tris-Borate buffer (TBE; Invitrogen) and stained with ethidium bromide.

Data analysis

The inhibition halo sizes from the diffusion experiments are expressed as means ± standard deviation (SD). The means were compared using an analysis of variance (ANOVA) followed by the Fisher test ($p = 0.05$). A statistical analysis to evaluate the effect of temperature on the antibacterial activity of the SNs was performed with an ANOVA followed by the Dunnet test ($p = 0.05$). The statistical analyses were performed with the MiniTab v18 program (Minitab, LLC).

RESULTS

Antibacterial activity of *Streptomyces* sp. CACIS-1.16CA

The initial screening to evaluate the antibacterial activity of *Streptomyces* sp. CACIS-1.16CA had the purpose of selecting those human and plant pathogenic bacteria susceptible to the compounds produced by the strain. The microorganisms inhibited by the compounds that spread to the agar discs were *E. coli*, *S. aureus*, *E. faecalis*, *B. pumilus*, and *P. carotovorum* (Table 1). Significant differences were found among the susceptible bacteria, with *E. coli* and *B. pumilus* having the largest inhibition halos and *S. aureus* having the smallest. These results indicated that the extracellular metabolites produced by the CACIS-1.16CA strain diffused into the growth medium and exerted an inhibitory activity against the bacteria.

Table 1 Primary screening to detect bacteria inhibition by *Streptomyces* sp CACIS-1.16CA

Microorganism	Inhibition halo (Ø mm)
Staphylococcus aureus	12.7 ± 0.8^{b}
Salmonella Enteriditis	-
Salmonella Tiphymurium	-
Escherichia coli	13.8 ± 0.4^{a}
Enterococcus faecalis	13.4 ± 0.5^{ab}
Pseudomonas aeruginosa	-
Pseudomonas syringae pv *syringae*	-
Bacillus pumilis	13.7 ± 0.2^{a}
Pectobacterium carotovorum	13.0 ± 0.4^{ab}

[*] Media non-shared letter are significantly different ($p < 0.05$). (-) No inhibition detected.

Growth phase-dependent production of antibacterial compounds

The production of antibacterial metabolites was examined to determine the growth phase in which the production of the inhibitory metabolites begins. A growth curve for *Streptomyces* sp CACIS-1.16CA was determined for 0 to 156 h and antibacterial activity of supernatants on *E. coli* was evaluated. Assays using the filter paper disc-diffusion method detect inhibitory halos with SN's starting at 48 h, which coincides with the exponential growth phase. The extracellular compounds accumulated during the stationary growth phase reaching the maximum inhibitory activity at 96 h (■) (**Figure 1**). Bacteria from the *Streptomyces* genus are characterized by their production of extracellular metabolites with antibiotic activity and a battery of hydrolytic extracellular enzymes, which can act synergistically to inhibit the growth of microorganisms (Chater et al., 2010). Based on this, a heat treatment of 100 °C for 3 min (■) was applied to the SNs from the different culture times to confirm whether the antibacterial activity was due solely to secondary metabolites, or if hydrolytic proteins contributed to the inhibition. The heat treatments did not affect the SNs ability to inhibit *E. coli*, resulting in an inhibitory halo similar to that of the unheated sample (**Figure 1**). Therefore, there were no statistically significant differences ($p > 0.05$, Dunnet test) between the heated and unheated SNs. In general, a direct correlation between the diameters of the inhibitory halos and the growth phases of the CACIS-1.16CA strain was observed.

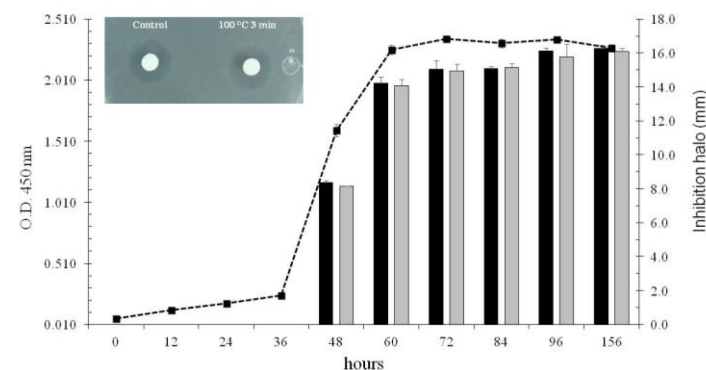

Figure 1 Growth of *Streptomyces* sp. CACIS-1.16CA and the antibacterial activity of the supernatant against *Escherichia coli*. An aliquot of SN was heated to 100 °C for 3 min (■); unheated control SN (■). O.D., optical density. No significant differences ($p > 0.05$, Dunnet test). The inner box showed the antibacterial activity for 96 h SN

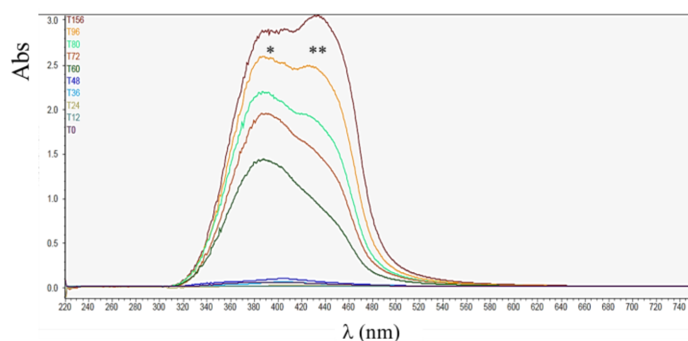

Figure 2 UV/Vis absorption spectral analysis of the cell-free supernatant (SN) from a submerged culture of *Streptomyces* sp. CACIS-1.16CA. The SN was collected from time zero to 156 h. *Peak 1; **Peak 2

The ultraviolet/visible (UV/Vis) absorption spectrum of the collected SNs confirm the accumulation of extracellular metabolites that could exerted the inhibitory activity (**Figure 2**). The spectral scan detected two maximum absorption peaks. Peak 1 is between 380 and 400 nm (at the UV spectrum limit), appears at 60 h of growth, and continues to accumulate until 156 h. Peak 2 is between 420 and 440 nm (visible spectrum) and begins at 60 h of growth, though in a smaller proportion than Peak 1, except at 156 h, when maximum production is reached.
An evaluation using the filter paper disc-diffusion method using 96-h SN confirmed that the extracellular compounds accumulated during the growth of the CACIS-1.16CA strain have antibacterial activity against other pathogenic bacteria (**Figure 3**). There were significant differences between the means, indicating that *E. coli* and *E. faecalis* were the most susceptible to inhibition, while *S. aureus*, with the smallest inhibitory halo, was the least susceptible.

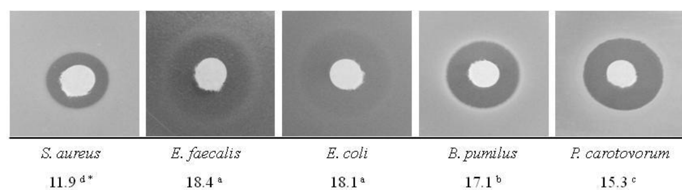

Figure 3 Antibacterial activity of the extracellular compounds accumulated during the growth of *Streptomyces* sp. CACIS-1.16CA as assayed by the filter paper disc-diffusion method. A 96-h supernatant was used for the assays. *Media not sharing a letter are significantly different ($p < 0.05$)

Thermal stability of the compounds produced by *Streptomyces* sp. CACIS-1.16CA

Considering the thermal stability of compounds contained into the supernatants, the 96-h SN was exposed to the more drastic conditions of 100 °C for 60 min and 121 °C for 15 min (**Figure 4**). These two treatments were evaluated with the five pathogenic bacteria using the paper disc-diffusion method. The RA of the SN determines the percentage of activity retained by the heat-treated SN in comparison to the unheated SN. The ANOVA results and Dunnet test ($p > 0.05$) indicated there were no significant differences between the SNs heated to 100 °C for 60 min and the control. Under these conditions, the treated SN retained its antibacterial activity above 94 %. Statistically significant differences were observed between the SN treated at 121 °C for 15 minutes respect to the unheated control SN and the SN heated for 60 min at 100 °C (ANOVA and Dunnet test, $p < 0.05$). Nevertheless, despite being exposed to drastic conditions (autoclaved), the SN retained more than 80 % of its antibacterial activity against the pathogenic bacteria (**Figure 4a**). This can be seen in **Figure 4b**, which shows the inhibitory halos diminished by between 10 and 20 %. The decreased activity may be due to the destruction of other antibacterial compounds present in the mixture.

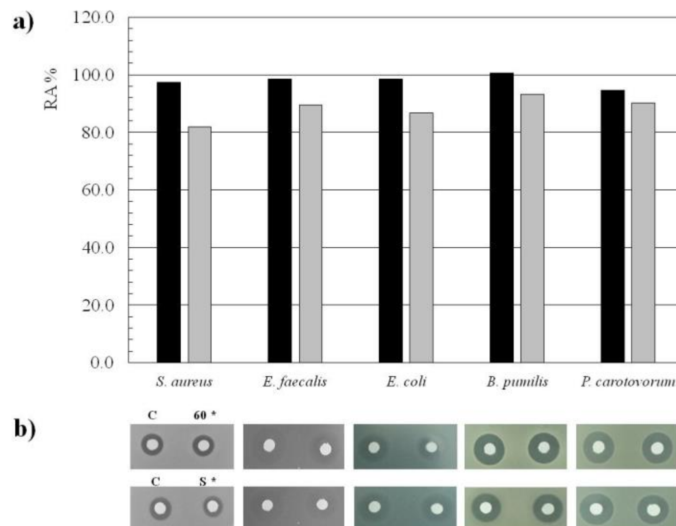

Figure 4 Effect of heat on the activity of the 96-h supernatant (SN) from *Streptomyces* sp. CACIS-1.16CA against human and plant pathogens. a) Percentage of retained activity (RA); aliquots of SN were heated to 100 °C for 60 min (■) or 121 °C for 15 min (■), and activity was compared to an unheated control sample. b) Inhibition halo detection in the Petri plate assay; * 60, 100 °C for 60 minutes, and S, 121 °C for 15 min. $^{+}$, no significant difference with respect to the control (p >0.05, Dunnet test); $^{\&}$, significantly different with respect to the control (p <0.05, Dunnet test)

Detection of biosynthetic gene clusters of secondary metabolites

The biosynthetic genes in the PKS-I, PKS-II, and NRPS clusters in the *Streptomyces* sp. CACIS-1.16CA genome were detected by PCR (**Figure 5**). The amplified fragments corresponded to ~700 bp for NRPS, to ~1200-1300 bp for PKS-I, and to ~ 900 bp for PKS-II. These results indicate a potential for the strain as a producer of compounds with antimicrobial activity and a different chemical nature.

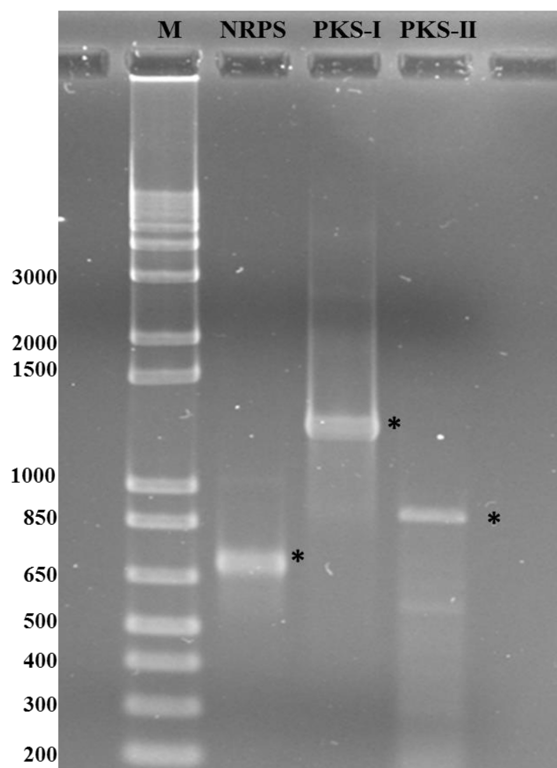

Figure 5 PCR amplification of biosynthetic gene clusters from genomic DNA *Streptomyces* sp. CACIS-1.16CA. M: DNA molecular weight marker (1 Kb Plus Ladder, Invitrogen). * Size of amplified DNA fragment

DISCUSSION

Bacterial resistance to antibiotics is a wide-spread process, with the most important consequence being a growing number of compounds that are no longer effective in treating disease (**Lin *et al.* 2015**). This fact has led to a search for alternatives in the form of new compounds by using strategies that allow for the quick evaluation of a large number of molecules (**Murray *et al.* 2019**). However, a great number of studies are still based on traditional microbiological methods and focus on screening extreme and little-explored environments to find novel microorganisms producing new compounds (**Genilloud, 2017**; **Hug *et al.* 2018**).

Streptomyces sp. CACIS-1.16CA is a bacterium that was isolated from the Los Petenes Biosphere Reserve on the northwest coast of the Yucatan peninsula, México, and it includes land and a marine portion. The area is characterized by a naturally fragmented environment on karstic soils, where the presence of spring water holes and hammocks cause important variations in salinity and pH (**Hernández-Montilla *et al.* 2016**). The antibacterial compounds, including the yellow pigmented metabolite, have been observed to begin accumulating and diffusing on solid media simultaneously with the initiation of the sporulation process (**Barka *et al.* 2016**). The inhibition halo that formed in the bacterial pathogens after exposure to the agar discs from CACIS-1.16CA confirmed the presence of synthesized metabolites with antibacterial activity. The SN obtained from the CACIS-1.16CA strain inhibited the growth of both human and plant pathogenic gram-negative and gram-positive bacteria but did not inhibit *Salmonella* and *Pseudomonas* species. Antibacterial activity has been observed with other species; *S. flavogriseus* ACTK2 has exhibited antimicrobial activity against *S. aureus*, *B. subtilis*, *E. coli*, and *Enterobacter aerogenes* (**Dezfully and Ramanayaka, 2015**), and *Streptomyces* sp. ES2 (isolated from estuaries) produced antibacterial compounds against *E. coli*, *P. aeruginosa*, *B. subtilis*, *E. aerogenes*, and *Proteus mirabilis* (**Al-Ansari *et al.* 2019**). The production of inhibitory compounds began after 48 h of growth and reached maximum inhibitory activity at 96 h. Similarly, studies have shown that marine *Streptomyces* sp. BT-408 produces its highest concentrations of the polyketide antibiotic SBR-22, which is effective against methicillin-resistant *S. aureus*, 96 hours after growth initiation (**Sujatha *et al.* 2005**).

For many pathogenic microorganisms, particularly *S. aureus*, the formation of biofilm is an important feature, not only because it contributes to the adherence process to various surfaces, but also because it confers resistance to the inhibitory action of antibiotics and antiseptic agents. Therefore, it is important to search for the biofilm formation-inhibiting compounds produced by streptomycete species. Some studies have found strains with antibiofilm activity such as *Streptomyces* sp. MC11024, which inhibits biofilm formation in *S. aureus* (**Suzuki *et al.* 2015**). Likewise, *Streptomyces* sp. GCAL-25 inhibits cell growth and biofilm formation in *Candida albicans* (**Córdova-Dávalos *et al.* 2018**). Therefore, it will be important to assess whether the CACIS-1.16CA SN can inhibit biofilm formation in any of the species tested in our work here.

In relation to plant pathogens, the antibacterial activity of *Streptomyces* species against phytopathogenic microorganisms has also been studied. For example, a novel *Streptomyces* strain promotes the vegetative growth of rice plants and exhibits antibacterial activity against *Burkholderia glumae*, a pathogen of rice (**Suárez-Moreno *et al.* 2019**). *Pectobacterium carotovorum* is a common soil pathogen that causes soft rot in a wide range of plants, including tomato, potato, pumpkin, and other vegetables (**Cui *et al.* 2019**). This pathogen causes soft rot in *Agave tequilana*, the raw material to produce the alcoholic beverage tequila. In our study, the compounds produced by *Streptomyces* sp. CACIS-1.16CA had antibacterial activity against *P. carotovorum* that was isolated from agaves affected with this disease.

The cell-free SN of *Streptomyces* sp. CACIS-1.16CA showed thermal stability after exposure to elevated temperatures, retaining 80 % of its residual activity against gram-positive and gram-negative bacteria after heating to 121 °C. Similarly, a heat treated (100 °C for 45 min) filtered supernatant from the stationary phase of a *S. hygroscopicus* culture retained compounds of a non-protein nature that maintained antifungal activity against *Colletotrichum gloeosporioides* and *Sclerotium rolfsii* (**Prapagdee *et al*. 2008**). Likewise, an extract obtained from a *Streptomyces* sp. TQR12-4 culture heated to 100 °C for 2 h retained its antifungal activity against *Fusarium udum* (**Hong-Thao *et al.* 2016**). In contrast, an extract produced by the endophytic actinomycete R18, with antibiotic activity against diverse gram-negative bacteria, lost its activity when exposed to peptidase enzymes or temperatures above 80 °C for a few minutes, suggesting the activity was related to a peptide-like molecule (**Carvalho and Van Der Sand, 2016**).

The growth of CACIS-1.16CA on liquid media and the accumulation of compounds evidenced by the spectral scanning peaks (between 380 and 440 nm) could be related to the antibacterial activity. The streptomycete secondary metabolites identified belonging to the natural substances of the following chemical structure: polyketides, pyrones, peptides, siderophores, g-butyrolactones, butenolides, furans, terpenoids, fatty acids, oligopyrroles, deoxysugars, and many of them included into the group of compounds called cryptic compound (**van Keulen and Dyson, 2014**). Spectroscopic characteristic of 16-membered ring macrolide antibiotics leucomycin and maridomycin indicated a UV absorption at 230-280 nm (**Omura and Nakagawa, 1975**). Sceliphrolactam, a polyene macrocyclic lactam had a signature at 333 and 420 nm (**Oh *et al.* 2010**). Tunicamycin a nucleoside antibiotic (870 Da) identified from culture broth of *Streptomyces lysosuperificus* showed an absorption spectrum with a maximum absorption peak at 260 (**Atta, 2011**). Generally, polyene quantification is based on their characteristic UV-visible absorption spectrum, in aqueous or organic solvents have three or four peaks of maximum absorption, which depends on the number of double bonds in the chromophore (**Barreales and Aparicio, 2021**).

Biosynthetic gene clusters are organized groups of genes involved in the production of specialized metabolites, some of which display antimicrobial activity. Polyketide synthase genes (PKS Types I and II) as well as genes from compounds synthesized by NRPSs were detected in *Streptomyces* sp. CACIS-1.16CA, which indicates a high possibility to macrocyclic polyketides (PKS-I), polycyclic aromatic polyketides (PKS-II), and non-ribosomal peptide compounds (NRPS). Streptomycetes are a widely recognized group of antibiotic producers, with genomes containing more than 20 biosynthetic gene clusters for secondary metabolites, representing 5 % of their genome (**Challis and Hopwood, 2003**). Whole genome sequencing and analysis of *Streptomyces fildesensis* showed a 9.47 Mb genome size with 42 predicted biosynthetic gene clusters and 56 putative clusters representing a 22% of total genome content (**Nuñez-Montero *et al.* 2019**). It is important to point out that, in addition to the fact that while many *Streptomyces* species have PKS Types I and II and NRPS biosynthetic clusters, some species also produce a third type of synthase. PKS Type III has the peculiarity that it does not use an acyl-carrying protein to produce metabolites (**Risdian *et al*. 2019**).

CONCLUSION

With the emergence and rapid spread of resistant bacteria in a diversity of fields, the world needs new antibiotics to be developed, and not only for treating human diseases. We need new antimicrobial molecules to control the emergence of food-borne pathogens, plant bacterial pathogens, and nosocomial infections. We also need drugs that can stop bacteria by preventing their ability to form biofilms or by affecting their chemical communications with each other. The isolation of microorganisms from unexplored environments with a goal of identifying new bioactive compounds is an important strategy for finding novel antibiotics. Our experimental findings describe the production of heat-resistant compounds produced by a *Streptomyces* species isolated from a poorly studied ecosystem. Future work on these compounds should focus on separating and purifying the compounds to probe their activities against drug-resistant bacteria or superbugs. The isolation of these compounds from the crude extracts and chemical analyses would be necessary for structure elucidation.

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