ORIGINAL ARTICLE

Potential of *Streptomyces* sp. strain AGS-58 in controlling anthracnose-causing *Colletotrichum siamense* from post-harvest mango fruits

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Abstract

Anthracnose is a cosmopolitan fungal disease affecting many tropical and subtropical plants and was first associated with the members of the genus *Colletotrichum*. In this study, we evaluated the potential antifungal activity of *Streptomyces* sp. AGS-58 to inhibit anthracnose-causing *Colletotrichum* spp. on mango fruits. Additionally, a goal of this study was to investigate if the extracellular metabolites produced by the AGS-58 strain could inhibit fungal conidial germination in vitro and control post-harvest anthracnose disease on mango fruits. Antagonistic streptomycetes were initially isolated from Jalapeño chili pepper soil fields and were preliminary selected for their antagonism against *C. siamense*. The isolate identified as *Streptomyces* sp. AGS-58 exhibited the highest inhibitory activity against *C. siamense* CT1. The metabolites obtained from the excreted droplets that accumulated on AGS-58 colonies were studied for their inhibitory effect on conidial germination. According to morphological evidence, extracellular exudates inhibited conidial germ tube formation and hyphal extension. These results suggest that the exudate contains antifungal metabolites that can inhibit the growth of *C. siamense*. The inhibitory activity was similar to that of Benlate. Spraying mango fruits with the extracellular exudate affected the germination of *C. siamense* conidia and thus prevented anthracnose progression. To the best of our knowledge, this study suggests the potential of *Streptomyces* sp. AGS-58 and its extracellular secondary metabolites in controlling anthracnose-causing fungi that infect post-harvest fruits.

Keywords Biocontrol · Post-harvest disease · Streptomycetes · Antifungal · Anthracnose

Introduction

Anthracnose is a cosmopolitan fungal disease that affects many tropical and subtropical plants (Cannon et al. 2012). The term anthracnose is used to describe a group of fungal diseases with a particular set of symptoms, including the development of dark, sunken spots, or lesions, often with a raised rim, on affected foliage, stems, and fruits. The symptoms vary according to the plant part affected and can manifest as seedling tumbling, dark circular spots on leaves, stem necrosis, and fruit lesions (Saxena et al. 2016). However, the most commonly observed damage is to the fruits in the form of dark, depressed, and necrotic lesions with concentric acervuline rings, leading to pre- and post-harvest rot (Dean et al. 2012; Saxena et al. 2016).

While *Colletotrichum* spp. cause a considerable portion of the damage, anthracnose is not limited to one fungal species or genus. The best-known *Colletotrichum* species is *C. gloeosporioides*, a species that causes damage to a variety of crops, fruits, vegetables, legumes, and trees. This pathogen is responsible for one of the major pre- and post-harvest diseases affecting many fruits globally (Chowdhury and Rahim 2009; Siddiqui and Ali 2014). In addition, *C. gloeosporioides* infects unripe fruits and remains latent until the fruits are stored and allowed to ripen. At this stage, the lesions caused by the disease appear in a progressive manner. This critically deteriorates fruit quality, consequently incurring economic losses as the affected fruits do not meet the

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requirements for consumption and exportation. The losses caused by this disease after harvest range from 5 to 60% of the overall production, whereas in humid conditions, the damage may reach 100% of the export volume (Kamle and Kumar 2016).

Anthracnose affects fruit production from high-value crops in different markets, especially chili pepper, mango, papaya, and banana (Cannon et al. 2012; Ali et al. 2016). Globally, the most important and widespread form of decay in mangoes is anthracnose caused by *C. gloeosporioides*. However, anthracnose symptoms have also been associated with other *Colletotrichum* species, e.g., *C. asianum*, *C. fructicola*, *C. tropicale*, *C. alienum*, and *C. siamense* (Tovar-Pedraza et al. 2019). *C. siamense* has been commonly isolated from avocados and mangoes with disease symptoms (Giblin et al. 2018). Nowadays, several *Colletotrichum* spp. are being isolated from diseased mangoes in southern China, with the dominant species being *C. asianum*, *C. siamense*, and *C. fructicola*, accounting for 30%, 30%, and 25% of isolates, respectively (Li et al. 2019). A further study of diseased mango fruits showed that the *Colletotrichum* spp. *C. asianum*, *C. fructicola*, *C. siamense*, *C. tropicale*, and *C. scovillei* caused lesions, with larger and more evident being caused by *C. asianum* and *C. siamense* (Wu et al. 2020).

Currently, the main strategy to extend the shelf life of fresh fruits and to reduce the post-harvest decay of fruits by fungal pathogens is with the use of synthetic fungicides such as benomyl, carbendazim, thiabendazole, and mancozeb. However, there have been increasing concerns regarding the use of these substances owing to both health hazards and the environmental accumulation of toxic residues, which have led to the development of alternative strategies for crop protection (Akem 2006; Edirisinghe et al. 2014). Moreover, a lack of effective control of these pathogens has been observed and is attributable to strains displaying resistance toward these synthetic products (Torres-Calzada et al. 2015), thus highlighting the necessity to search for alternative approaches to manage these diseases (Rojo-Báez et al. 2017). A strategy that fits with the sustainable agriculture concept relies on the post-harvest biological control of fruit disease. This strategy is based on the growing interest in food that is free from chemical pesticide residues. Several groups have been actively studying microbial antagonists to control the post-harvest decay of fresh fruits and vegetables, and it appears to be a promising strategy to manage fruit diseases in a wide range of crops (Di Francesco et al. 2016). Some species of the *Streptomyces* genus effectively control both pre- and post-harvest anthracnose in several crops by acting against *Colletotrichum* spp. This significantly impairs the pathogen's growth (Shimizu et al. 2009; Sadeghian et al. 2016). Streptomycetes are known to secrete a diverse repertoire of secondary metabolites (antibiotics, antifungals, and cell-growth inhibitors) and extracellular enzymes (Chater et al. 2010). The former is associated with the mycelium and/

or is secreted into the culture medium. In many *Streptomyces* spp., liquid droplets accumulate on top of the sporulated colonies. These may be colorless or not and contain vesicles that serve as protein and polyketide antibiotic reservoirs (Schrempf et al. 2011).

In this study, we evaluated the antifungal potential of the *Streptomyces* sp. AGS-58 against *C. siamense* that causes post-harvest anthracnose in fruits. Additionally, we examined the inhibition of conidial germination by the extracellular exudates that are accumulated as droplets on the top of colonies, and we explored their ability to control anthracnose in mango fruits.

Materials and methods

Fungal strains and culture conditions

Colletotrichum spp. were isolated from fruits displaying anthracnose symptoms. *Colletotrichum* strain CT1 was isolated from mango fruits procured form the local market. Monosporic cultures were cultured on potato dextrose agar (PDA; Difco, USA) plates and incubated at 29 °C for 8–10 days. Stock cultures was maintained on PDA slants and stored at 4 °C. Molecular identification and phylogenetic analysis were conducted as described in Online Resource 1 and 2. DNA was amplified with primers for partial actin (ACT), partial chitin synthase (CHS-1), partial glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and rDNA-ITS (ITS) genomic regions.

Isolation of streptomycetes

Soil samples were collected from open cultivation fields of Jalapeño chili pepper in Aguascalientes, Mexico. All samples were collected using an auger by drilling down to a 10 cm depth. They were subsequently placed in presterilized plastic bags and were processed after 36 h. Streptomycete isolation was performed as described previously (Evangelista-Martínez 2014a). Emerging colonies were selected based on the typical morphological features of streptomycete colonies. Pure isolates were obtained by repeated streaking onto fresh International Streptomyces Project media 2 (ISP2) plates. For long-term preservation, a suspension of spores was stored at −20 °C in 20% (w/v) glycerol. A working general inoculum (GI) of spores was prepared to obtain a turbid suspension of 10^8 CFU/ml.

Antagonistic activity of *Streptomyces* species against *Colletotrichum* spp.

The antagonistic assays of streptomycetes against anthracnosis-producing *Colletotrichum* strain CT1 were conducted by placing 2 µl of the GI onto a Petri dish containing ISP2,

at 1 cm distance from the edge. After 30 min of incubation at room temperature, a PDA agar disc of 0.6 cm diameter covered with an actively growing fungal mycelium was punched from sporulating areas near the periphery and was placed at the center of the plates. Cultures were stored at 29 °C ± 2 °C for 7 days. Media plates inoculated with only fungal pathogen were used as controls. The percentage of inhibition (PI) was calculated using the following formula: PI (%) = (FR − AR)/FR × 100 (Evangelista-Martínez 2014a). All measurements were conducted in triplicate.

An initial screening assay to select antagonistic streptomycetes was conducted against *C. siamense* CT1. Subsequent in vitro assays to evaluate the potential biocontrol activity of the selected *Streptomyces* strain were performed as dual confrontation tests with the cultures of *C. siamense* and were contrasted with that of *Streptomyces lydicus* WYEC 108 (Actinovate®), a biocontrol agent used against fungal root and seed rots (Yuan and Crawford 1995). PI was calculated as previously indicated. All measurements were conducted in triplicate.

Inhibitory effects of extracellular exudates on *Colletotrichum* spore germination

The inhibitory activity of the extracellular droplet-like exudates from *Streptomyces* sp. AGS-58 colonies on fungal spore germination was evaluated using a two-step procedure: the production of extracellular droplets and inhibitory assays. Initially, 2 μl of GI was placed at four equidistant points on the edge of NA Petri plates and was allowed to dry completely. After 10 min, 3 μl of the *C. siamense* CT1 spore suspension (10^6 spores/ml) was inoculated in the center of the plate. The plates were stored at 29 °C ± 2 °C for 12 days, and extracellular droplets that accumulated on the sporulated colonies were collected each day. For inhibitory tests, the exudates were diluted in sterilized distilled water (SDW) to obtain working solutions (WSs) at the following concentrations: 100, 10, 1, and 0.1 mg/ml. The spore germination inhibition assay was performed using the cavity slide technique using a 30 μl sample containing 15 μl of 2X potato dextrose broth mixed with 5 μl of a freshly harvested fungal spore suspension (10^6 spores/ml). The slides were immediately seeded with 10 μl of the WS and subsequently mixed and covered with a coverslip. The final concentrations of the exudates added to the mix were 50, 5, 0.5 and 0.05 mg/ml. The prepared slides were maintained in a humid environment in Petri dishes at 29 ± 2 °C under dark conditions. Cavity slides containing only SDW were used as a positive control. After 6–24 h of incubation, spore germination was observed under a contrast phase microscope (Olympus model CX31). The experiment was performed in duplicate, and spore germination was evaluated based on the presence of germ tubes. A conidium was considered germinated if its germ tube was

longer than the conidia itself. Results were expressed as the percentage of spores germinated compared to the control using the mean values from duplicate experiments. The percentage of spore germination inhibition was calculated using the following formula: % inhibition of spore germination = (SGC − SGT)/SGC × 100, where SGC is the mean number of germinated spores in the control set, and SGT is the mean number of germinated spores in the test set.

In vivo evaluation of droplet-like exudate from *Streptomyces* sp. AGS-58 to control anthracnose disease of mango fruits

To evaluate the effect of droplet-like exudates on anthracnose progression, an experiment with detached mango fruits was conducted. A completely randomized experimental design was used along with replicates. Mango fruits (*Mangifera indica* var. Ataulfo) were obtained from local markets. Quality indices used were uniformity of shape and size, freedom from decay and defects, and flesh color and firmness. Mango fruits were sanitized by soaking in 0.5% sodium hypochlorite solution for 3 min. Subsequently, fruits were rinsed twice in SDW and air-dried inside a laminar flow cabinet. The fruits were then moistened with a stock solution of the concentrated exudate and 50 mg/ml WS; they were then allowed to dry on Whatman paper. Subsequently, a 5 mm diameter *C. siamense* CT1 mycelial plug was seeded on the fruit surface. After inoculation, the fruits were incubated at 28 °C ± 2 °C in humidity chambers containing wet paper towels. All measurements were conducted in duplicate with three mangoes for chamber.

After 10 days, the diameter of the resulting lesion was measured, and the results were presented as mean. Control fruits were treated with SDW and the commercially available fungicide Benlate® (Benomyl, 50 mg/ml).

Characterization of *Streptomyces* sp. AGS-58

The morphological, biochemical, and physiological features of the AGS-58 isolate were evaluated as described previously (Shirling and Gottlieb 1966). Bacterial growth and spore production were recorded after a 15-day incubation at 29 °C ± 2 °C. The morphological features were observed on ISP2 medium, while the carbohydrate utilization test was performed in 24-well plates containing International Streptomyces Project agar media 9 supplemented with different carbon sources at 1% (w/v). Growth response was evaluated on diverse culture media. Melanin production was evaluated on tyrosine agar media (ISP 7 medium). A phosphate solubilizing test was conducted using the National Botanical Research Institute's phosphate growth medium (Nautiyal 1999). In addition, bacterial growth of the isolate in a nitrogen-low medium (Ashby) was determined (Franco-Correa

et al. 2010). The enzymatic index was determined to identify chitinase, xylanase, lipase, gelatinase, and cellulase activities on the base ISP 9 medium (Evangelista-Martínez 2014b). The tolerance toward sodium chloride and pH was evaluated using ISP 2 media supplemented with 2.5, 5, 7.5, 10, and 15% (w/v) salt and by adjusting the pH to 4–9, respectively. All evaluations were conducted in duplicate. Finally, either the relative susceptibility or the resistance to antimicrobials was studied by the disc diffusion method. Briefly, 0.1 ml of an AGS-58 GI solution was evenly distributed onto Petri plates containing ISP2 media in duplicate. Once the solution was absorbed, an antibiotic multidisc for gram-positive bacteria II (Bio-Rad®, Hercule, CA, USA) was placed on the surface of the media and the plate was incubated at 29 °C ± 2 °C for 15 days.

Molecular identification

The AGS-58 isolate was identified by nucleotide sequence analysis of the ribosomal 16S DNA gene. Genomic DNA was extracted from freshly harvested spores using the Pure Yeast/bact Kit B (Qiagen) (Evangelista-Martínez 2014a). The 16S rDNA gene was amplified using the universal 63F (5`-CAGGCCTAACACATGCAAGTC-3`) and 1389R (5`-ACGGGCGGTGTGTACAAG-3`) primers (Integrated DNA Technologies Inc) (Osborn et al. 2000). Polymerase chain reaction (PCR) was performed using a 50 μl solution containing 50 ng DNA, 1X PCR HiFi buffer (Invitrogen), 2 mM MgSO$_4$, 0.2 mM of each dNTP (Invitrogen), 0.4 μM of each primer, and 1.25 U Platinum *Taq* High Fidelity DNA polymerase (Invitrogen). Amplification was conducted in a Veriti thermal cycler (Applied Biosystem®) and consisted of an initial denaturation step at 94 °C for 3 min, followed by 35 cycles at 94 °C for 1 min, 55 °C for 45 s, and 68 °C for 1 min; the final extension was performed at 68 °C for 10 min. The amplified fragment was purified using the PureLink PCR Purification Kit (Invitrogen) and was verified by nucleotide sequencing of both the strands at LANGEBIO (National Laboratory of Genomics for Biodiversity, CINVESTAV Irapuato, Mexico). Using the BLASTN v 2.7.1. software, a 1288-bp fragment of the sequence was analyzed for homology. The 16S rDNA gene sequences from type strains of several genera were retrieved from the nonredundant GenBank database (Altschul et al. 1990). These sequences, along with additional streptomycete strains, were used as a reference and any unidentified or unpublished sequences were not included. A phylogenetic analysis was performed using the Molecular Evolutionary Genetic Analysis software version 7.0 (Kumar et al. 2016). Analysis was conducted using the Jukes-Cantor substitution model, and the tree was constructed using the neighbor-joining method. The partial sequence of the 16S rDNA gene was deposited

in the GenBank database under the accession number KY694530.

Screening for antibiotic biosynthetic gene clusters

PCR screening for the biosynthetic genes involved in the production of antimicrobial molecules by *Streptomyces* sp. AGS-58 was based on amplifications with specific primers. Polyketide synthase (PKS) type I gene fragments were amplified using the following degenerate primers: K1F 5`-TSAAGTCSAACATCCGBCA-3` and M6R 5`-CGC AGGTTSCSG TACCAGTA-3`. Non-ribosomal peptide synthetase (NRPS) gene fragments were amplified using the following degenerate primers: A3F 5`-GCSTAC-SYSATSTACACSTCSGG-3` and A7R 5`-SASGTCVC-CSGTSCGGTAS-3` (Ayuso-Sacido and Genilloud 2005). Gene fragments of the PKS type II gene were amplified using the degenerate primers KSα 5`-TSGRCTACRTC AACGGSCACGG-3` and KSβ 5`-TACSAGTCSWTCGCC TGGTTC-3` (González et al. 2005). Each reaction mixture (25 μl final volume) comprised 50 ng of DNA template, 1X Colorless GoTaq Flexi buffer, 2 mM MgCl$_2$, 0.2 mM of each dNTP (Invitrogen), 0.4 μM of each primer, 1.25 U GoTaq Hot Start polymerase (Promega), and 10% dimethyl sulfoxide. The PCR amplification program was as previously reported (Ayuso-Sacido and Genilloud 2005; González et al. 2005). All amplification products were visualized on 1.5% (w/v) agarose gels prepared in 1X Tris–borate-EDTA buffer (Invitrogen) and were stained with ethidium bromide. Bands in the ranges of 1200–1400, 800–900, and 700 bp were classified as products of the PKS-I, PKS-II, and NRPS genes, respectively.

Statistical analysis

Data are expressed as the means ± standard deviation (SD), and comparisons by one-way ANOVA tests were performed at P = 0.05 using R v2.14.0 statistical software (R Development Core Team®). Mean values were assessed using a Tukey multiple range test.

Results

Isolation and preliminary selection of antagonistic streptomycetes

A total of 68 streptomycete-like strains were isolated and preserved at the Actinomycetes Germplasm Bank at CIATEJ. All strains were evaluated for their ability to inhibit the growth of *C. siamense* CT1. The results demonstrated that 11 isolates antagonized the fungal pathogen and showed

that isolate AGS-58 was one of the most efficacious in inhibiting the growth of the pathogen by > 40% (Table 1).

In vitro antagonist activity of *Streptomyces* sp AGS-58

To validate the antagonistic potential of *Streptomyces* sp. AGS-58, an in vitro set of independent experiments was conducted to compare this isolate with *S. lydicus* WYEC108. The first confrontation experiment was conducted to estimate the inhibitory effect on *C. siamense* CT1. As observed on Fig. 1, the bacterial-fungal confrontation assay revealed a higher inhibitory activity of *Streptomyces* sp. AGS-58 on the fungal growth than that of *S. lydicus* strain WYEC108. Clearly, AGS-58 inhibited mycelial growth up to 50%, while WYEC108 only efficiently decreased the growth of *C. siamense* until 50% ($P < 0.05$). For the second antagonist assay, *Streptomyces* sp. strain AGS-58 showed higher percentages of mycelial growth inhibition than *S. lydicus* WYEC108 (PI: 66.7% [± 2.8 SD] and 40% [± 1.8 SD], respectively. *Streptomyces lydicus* could not display greater inhibitory effects than AGS-58, in either case.

Inhibition of *Colletotrichum* spore germination in vitro is mediated by the extracellular exudate

After collecting the translucent exudate droplets accumulated on top of the AGS-58 strain colonies, a homogenous WS was prepared (100 mg/ml). Mixtures of the *C. siamense* conidial suspension and the WS were added to the cavity of concave microscope slides and were stored in a humid chamber for 24 h. As observed in Fig. 2A, B, WS concentration was sufficient to completely inhibit the conidial germination of *C. siamense* CT1, suggesting that the exudates contain antifungal metabolites that have an inhibitory effect on the conidial germination process.
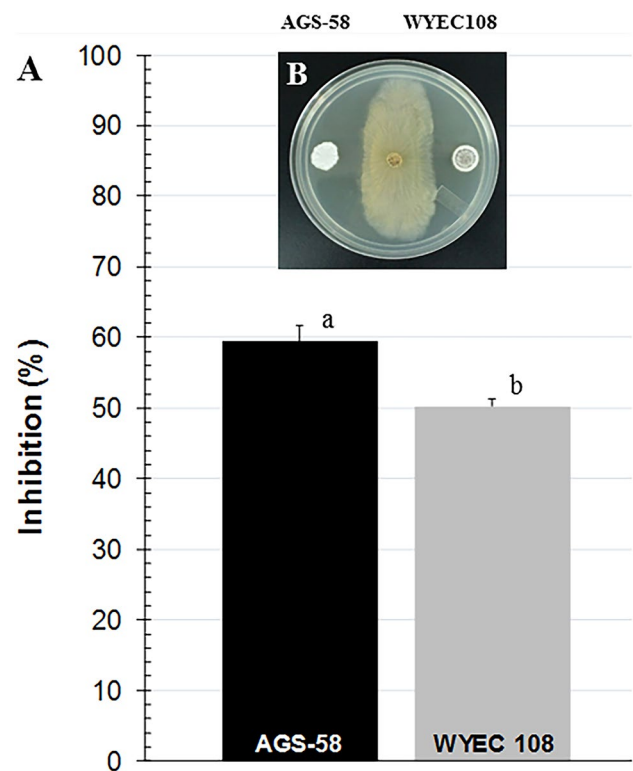

Fig. 1 Inhibitory activity of *Streptomyces* sp. AGS-58 and *Streptomyces lydicus* WYEC108. **A**) Mean percentage of inhibition of *C. siamense* CT1 by *Streptomyces* sp. AGS-58 (black columns) and *S. lydicus* WYEC108 (gray columns). **B**) Comparison of inhibition of mycelial growth on *C. siamense*. *Means with different letter(s) are significantly different ($P < 0.05$)

Considering that *Colletotrichum* is the most important pathogen causing anthracnose disease among all mango production areas worldwide, we evaluated the effects of droplet-like exudates on the vitality of *C. siamense* CT1 spores. We mixed the WSs with a conidial suspension and incubated them for 6 h (Fig. 3). These mixtures were then assayed, as described above. Quantitatively, the number of germinated spores decreased proportionally to the increase in exudate concentration. Conidial germination was inhibited by < 5% in the presence of 0.05 mg/ml of WSs, whereas the inhibitory activity increased by up to 30% when the WS concentration was higher (> 0.5 mg/ml). Concentrations above 5 mg/ml completely inhibited the spore germination process. Significant differences were observed when the treatments were compared ($P < 0.05$). Treatment of spores with 50 mg/ml of WS had a lethal effect on conidial germination. Exposure to MS resulted in morphological changes in conidial development, such as reduced conidial germ tubes, limited hyphal extension, and disrupted appressoria formation.
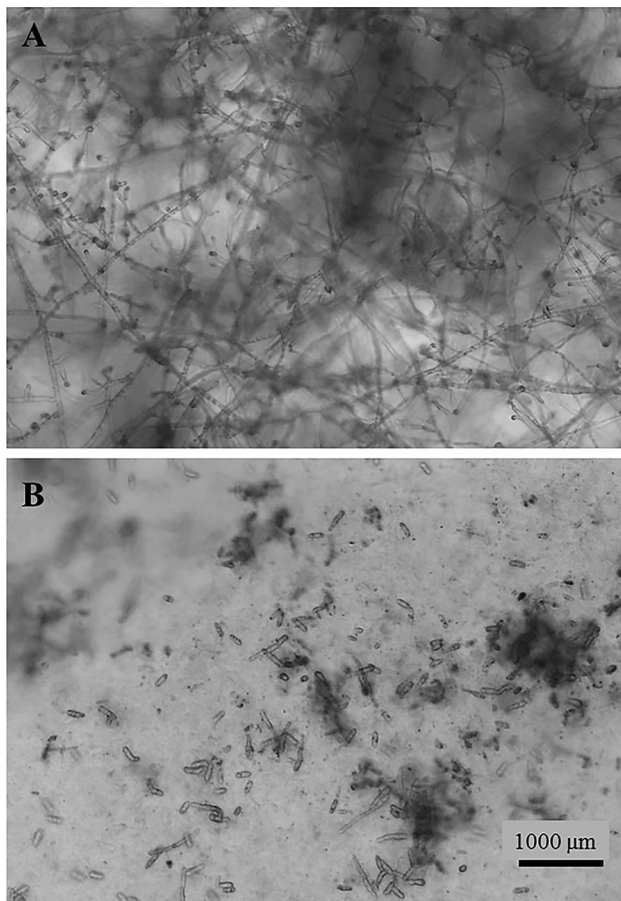
Table 1 Primary screening to detect streptomycetes displaying antagonist activity against *Colletotrichum* species

AGS Id	Inhibition (%) *Colletotrichum siamense* CT1
4	37 ± 4.6
6	27 ± 3.9
7	26 ± 8.5
9	22 ± 4.9
10	23 ± 0.6
12	26 ± 2.1
13	23 ± 2.4
17	21 ± 5.9
20	11 ± 1.0
25	19 ± 3.1
58	49 ± 1.1

Fig. 2 Inhibition of conidial germination by extracellular exudates from *Streptomyces* sp. AGS-58. Assays were conducted using the cavity slide technique and were observed at 24 h. Phase contrast microscopy at 40×. **A**) Control conidial germination; **B**) Conidial suspension exposed to WS

Fig. 3 Percent inhibition of *Colletotrichum siamense* CT1 conidial germination mediated by extracellular exudates. Effects on hyphal growth extension and morphology were quantified at 12 h after treatment. *Means with different letter(s) are significantly different (P < 0.05)

Assay of fruit protection against anthracnose using the extracellular exudate from AGS-58

Applying the exudate onto the mango fruit surface was effective at controlling anthracnose. Mangoes treated with a superficial cover containing the exudate solution before being inoculated with *C. siamense* CT1 had lesions of reduced size, similar to that observed after treatment with the commercial fungicide (P < 0.05). In contrast, fruits inoculated with the pathogen without previous treatment with a protective agent developed anthracnose lesions. Thus, treatment with the exudate produced by *Streptomyces* sp. AGS-58 controls *C. siamense* growth and disease development (Fig. 4).

Morphological, biochemical, and physiological characterization of the AGS-58 isolate

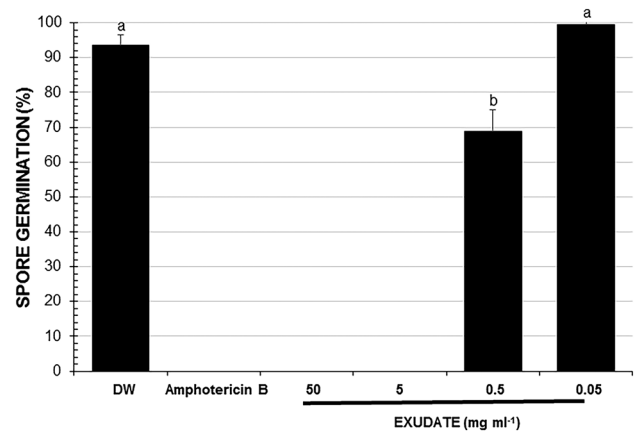Morphologically, AGS-58 colonies displayed yellow-colored substrate mycelia, white to cream aerial mycelia, and a massive spore mass with a gray-to-black appearance. The sporulation morphology of the aerial hyphae was of recti-flexibiles (Li et al. 2016). In addition, some biochemical and physiological features were observed (Online Resource 3).

Molecular identification and detection of biosynthetic genes

The analysis of the partial 16S rRNA gene sequence (1298 bp) from the AGS-58 isolate revealed a high similarity (98%) with the streptomycete species sequences previously deposited in the GenBank database. This close relation to other *Streptomyces* species, along with its phenotypic features, confirmed that the AGS-58 isolate belongs to the *Streptomyces* genus. The phylogenetic tree based on the neighbor-joining method showed that the AGS-58 strain was related to a particular *Streptomyces* sp. that can produce antimicrobial metabolites, such as *S. avermitilis* that produces avermectine, a pesticide with activity against insects, mites and ticks, and helminths (Ikeda et al. 2003). Other closely related members included species that can produce antibacterial, antifungal, and herbicide metabolites (Schwartz et al. 2004; Singh et al. 2009; Zhang et al. 2013; Wu et al. 2017). Additionally, the 16S rRNA gene sequences from *S. coelicolor* 14,426, as well as those from two streptomycete species used as commercial biofungicides (*S. lydicus* WYEC 108 and *S. violaceusniger*) were included in the analysis. Both bioagents were clustered within a separate group (Fig. 5). Screening for the antibiotic gene clusters in *Streptomyces* sp. AGS-58 revealed the presence of PKS type I, PKS type II, and NRPS genes. Specific amplification of chromosomal DNA revealed amplicons of approximately 1,200 bp, 900 bp, and 700 bp for PKS type I, PKS type II, and NRPS
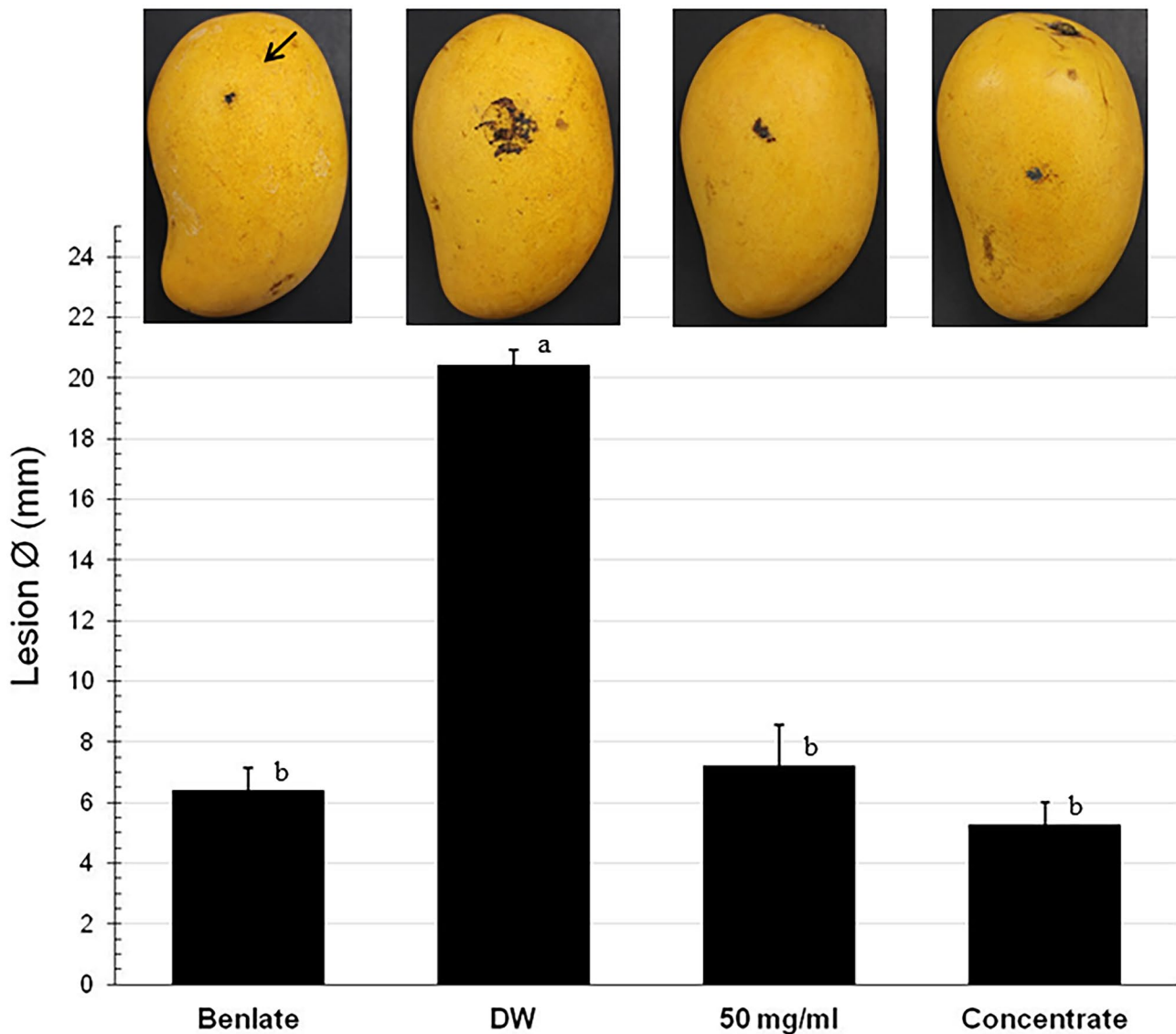
Fig. 4 In vivo anthracnose control mediated by extracellular exudates. The diameters of the lesions caused by *Colletotrichum siamense* CT1 on mango fruits were measured after 10 days. Damage to mango fruits is indicated by black arrows. *Means with different letter(s) are significantly different (P < 0.05)

genes, respectively (Online Resource 3). For *Streptomyces* species, the most frequently amplified biosynthetic antibiotic cluster corresponds to PKS-II sequences (71.2%), whereas PKS-I and NRPS are only detected in 48% and 37% of species (González et al. 2005).

Discussion

Reportedly, suboptimal handling and storage conditions of post-harvest fruits and vegetables led to global losses of around 40%–50% for root crops, fruits, and vegetables (Sawicka 2019). The post-harvest decay of fruits is caused by several fungal and bacterial microorganisms. Primarily, the control of fungal diseases involves the use of synthetic chemical fungicides. However, there is a growing interest in the post-harvest control of fruit decay using alternative biological methods instead of hazardous fungicides, which pose a concern regarding food safety and environmental issues.

Actinobacteria are an important group of plant-associated, spore-forming bacteria that have been studied for their natural ability to use mechanisms for biocontrol, to promote plant growth, and to interact with plants (Palaniyandi et al. 2013). Nowadays, commercially available products based on *Streptomyces* species have been used to control soil-borne and foliar fungal phytopathogens. For example, Mycostop®
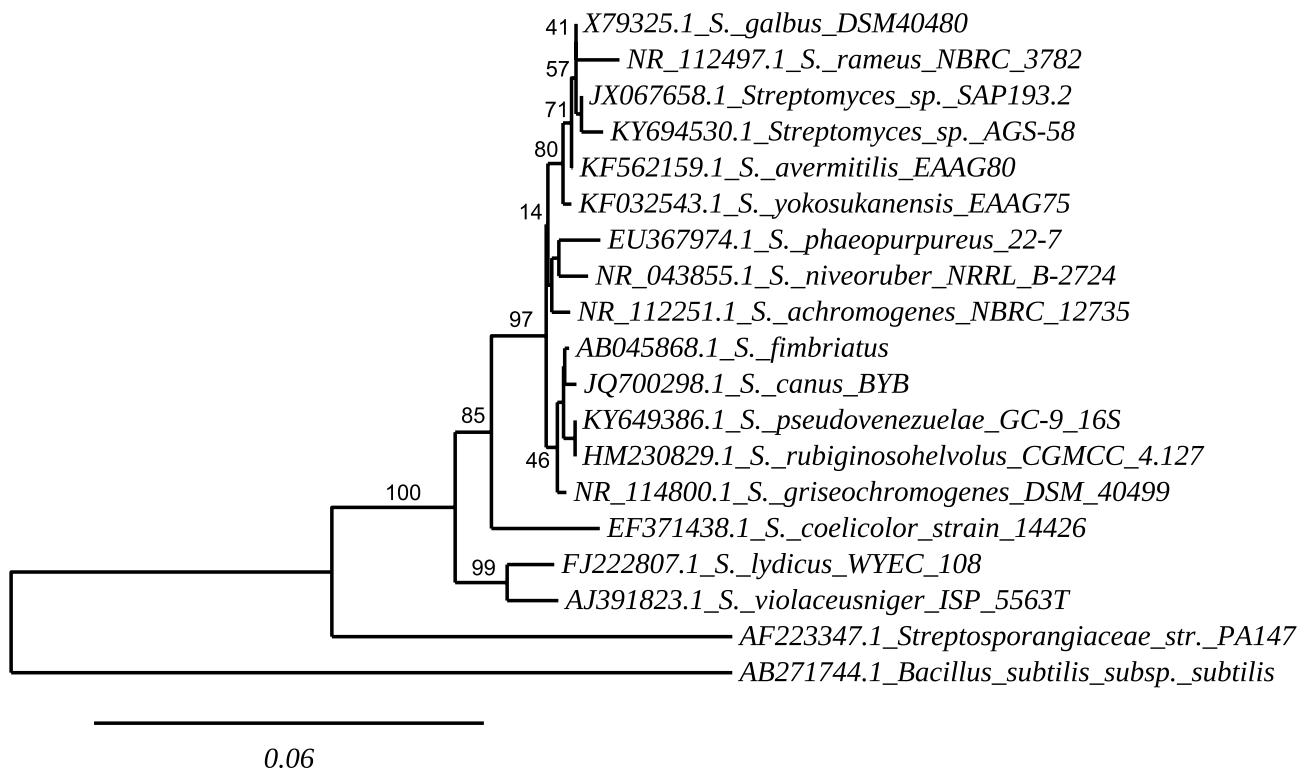
```
        41 ┌ X79325.1_S._galbus_DSM40480
      57 ┌─┤
         │  └── NR_112497.1_S._rameus_NBRC_3782
      71 ┤
         │ ┌─ JX067658.1_Streptomyces_sp._SAP193.2
      80 ┤ └── KY694530.1_Streptomyces_sp._AGS-58
         │ ┌ KF562159.1_S._avermitilis_EAAG80
      14 ┤ └ KF032543.1_S._yokosukanensis_EAAG75
         │ ┌── EU367974.1_S._phaeopurpureus_22-7
         │ ├── NR_043855.1_S._niveoruber_NRRL_B-2724
      97 ┤ └── NR_112251.1_S._achromogenes_NBRC_12735
         │ ┌ AB045868.1_S._fimbriatus
         │ ├ JQ700298.1_S._canus_BYB
      85 ┤ ├ KY649386.1_S._pseudovenezuelae_GC-9_16S
         │ 46 ┬ HM230829.1_S._rubiginosohelvolus_CGMCC_4.127
     100 ┤    └ NR_114800.1_S._griseochromogenes_DSM_40499
         │ └── EF371438.1_S._coelicolor_strain_14426
         │ 99 ┌ FJ222807.1_S._lydicus_WYEC_108
         │    └── AJ391823.1_S._violaceusniger_ISP_5563T
         │──────── AF223347.1_Streptosporangiaceae_str._PA147
         └──────── AB271744.1_Bacillus_subtilis_subsp._subtilis
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0.06

Fig. 5 Phylogenetic relationship between *Streptomyces* sp. AGS-58 and closely related members of the genus based on the 16S rDNA gene. The numbers at the nodes represent percentage values that indicate bootstrap support levels (n = 1,000 resamplings). The scale bar represents 0.06 nucleotide substitutions per site

contains the *S. griseoviridis* K61 strain and Actinovate® contains *S. lydicus* WYEC108. Although several antagonistic streptomycete strains have been studied as potential biocontrol agents, *Streptomyces* sp. CACIS-1.16CA significantly impairs the mycelial growth of several fungal pathogens, including *Colletotrichum* sp., a strain that causes anthracnose on *Jatropha curcas* trees (Evangelista-Martínez 2014a). Furthermore, a preliminary study focused on the novel CACIA-1.46HGO *Streptomyces* sp. strain demonstrated that the strain produces secondary metabolites and extracellular enzymes that possibly act in a combined manner to inhibit the mycelial growth of some fungal pathogens (Evangelista-Martínez 2014b). Similarly, the abundant production of an extracellular droplet-like exudate by *Streptomyces* sp. AGS-58 was observed. This exudate may either accumulate on colonies or be released into the media inhibit the mycelial growth of *C. siamense* (Bonev et al. 2008). An inhibitory effect was also observed against fungi from different genera, including *Fusarium, Aspergillus, Corynespora, Lasiodiplodia, Phomopsis, Bipolaris*, and *Rhizoctonia* (Online Resource 4). Moreover, the antagonistic activity displayed by *Streptomyces* sp. AGS-58 was similar to that of *S. lydicus* WYEC 108. These antagonistic assays confirm the excellent inhibitory activity of the *Streptomyces* sp. AGS-58.

The droplets accumulated on *Streptomyces* sp. AGS-58 colonies were confirmed to have an inhibitory effect on

Colletotrichum spore germination. Spore germination in the absence of the inhibitory compound begins with the formation of a germ tube that ultimately elongates and then differentiates into a melanized appressoria (Nesher et al. 2008). In contrast, the exudate from AGS-58 impaired conidial germination by affecting germ tube formation. Many *Streptomyces* spp. produce these exuded droplets, and the accumulated polyketide-like compounds and proteins participate in defense and survival. The blue droplets that appear on *S. coelicolor* sporulated lawns contain the colored polyketide actinorhodin and several different proteins arranged into densely packed vesicles (Schrempf et al. 2011). The droplets produced by *S. lividans* contain vesicles that store enzymes and other specific proteins, as well as the antibiotic undecylprodigiosin. These lipid vesicles inflict lesions on *Aspergillus proliferans* and *Verticillium dahlia* conidia and induce damage to *Escherichia coli* (Schrempf and Merling 2015). The biosynthesis of linearmycin (an antifungal and antibacterial agent) has been linked with the production of extracellular vesicles by *Streptomyces* sp. Mg1 (Hoefler et al. 2017).

The antibiotics produced by streptomycetes have been used as alternative fungicides in soils to replace synthetic chemical compounds. Reportedly, *Streptomyces violaceusniger* YCED9 produces nigericin, a guanidylfungin A-like compound, and geldanamycin that inhibit *Pythium, Fusarium*, and

Phytophthora spp. (Trejo-Estrada et al. 1998). Soil samples previously treated with a culture filtrate enriched with azalomycin produced by *S. malaysiensis* (strain MJM1968) had decreased fungi, including *Fusarium oxysporum*, *Rhizoctonia solani*, *Cladosporium cladosporioides*, *Fusarium chlamydosporum*, *C. gloeosporioides*, *Alternaria mali*, and *Pestalotia* spp. (Cheng et al. 2010). The extracellular metabolites from *Streptomyces violascens* MT7 achieved biological control of the toxigenic fungi that cause citrus and papaya rotting, whereas the incidence of the diseases caused by *Geotrichum candidatum* and *Rhizopus stolonifer* on oranges and papaya fruits decreased (Choudhary et al. 2015). In addition, a recent study conducted by Boukaew et al. (2021) found that the volatile organic compounds produced by *Streptomyces salmonis* PSRDC-09 exhibited high antifungal activity against several *Colletotrichum* spp., thus demonstrating the potential of this genus for controlling fungal diseases.

The typical symptoms of anthracnose in mango fruits are sunken, necrotic, dark brown-to-black lesions that are visible before or after the harvest. The fruits may prematurely drop from their trees. Fruit spots usually coalesce and can eventually penetrate deep into the fruit, resulting in extensive fruit decay (Nelson 2008). The current strategies for anthracnose control being used by farmers include hot water, fungicides, calcium carbonate, natural antioxidants, chitosan, plant extracts and essential oils, and antagonistic microorganisms, which have demonstrated promising results (Korsten 2006; Osorio et al. 2012; Luo et al. 2015; Khaliq et al. 2016; Gonçalves et al. 2021; Khaliq et al 2021; Limon et al. 2021; Aguirre-Güitrón et al. 2022).

This is the first study that uses extracellular exudate droplets containing vesicles for post-harvest biocontrol purposes. An aqueous solution sprayed onto mango fruits that contained *Streptomyces* sp. AGS-58 droplets decreased anthracnose to a similar extent as the commercial fungicide benomyl. A significant reduction was observed in the lesion size. Mango crops, in addition to *Colletotrichum* spp., are particularly susceptible to infection by *Lasiodiplodia theobromae*, the causal agent of stem-end rot of fruits. Extracellular exudates from *Streptomyces* sp AGS-58 exerted a protective effect on mango Ataulfo fruits, controlling the damage caused by *L. theobromae* (Online Resource 5).

These results are promising, as the current control practices rely on synthetic fungicides or on expensive physical treatments (Kumar et al. 2007). Future studies on anthracnose control in detached mango fruits may include correlations with variables that indicate fruit quality, as well as with post-harvest physiologies, such as skin color, firmness, total soluble solids, titratable acidity, ascorbic acid, and cell membrane permeability (Luo et al. 2015; Lawson et al. 2019).

Currently, there is a global trend to develop ecofriendly agrochemicals using microbial metabolites. These metabolites have relatively shorter environmental life spans, are biodegradable, and therefore, represent a lower risk of residual toxicity than conventional halogenated chemical structures (Saxena 2014). In this regard, for the post-harvest control of fungal diseases, more reliable effects may be obtained using technologies that are based on a formulation containing *Streptomyces* metabolites intended to be sprayed onto fruits. Some benefits may be obtained from the stability of the metabolite formulation rather than using living microorganisms. The use of metabolites instead of strains may trigger streamlining of the processes to replace chemically synthesized pesticides (Rey and Dumas 2017).

Declarations

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest All authors declare that they have no conflict of interest.

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