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Isolation and Molecular Identification of *Serratia* Strains Producing Chitinases, Glucanases, Cellulases, and Prodigiosin and Determination of Their Antifungal Effect against *Colletotrichum siamense* and *Alternaria alternata* In Vitro and on Mango Fruit

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Abstract: Microorganisms represent a viable option for the control of phytopathogens. From the surface of healthy mangoes, different bacteria were isolated. For all isolated bacterial strains, we determined their antimicrobial activity against a fungal strain that caused anthracnose in mangoes and against *Alternaria alternata*, both in the culture medium and directly on mangoes. The bacterial strains with the highest antifungal activity were identified by sequencing the 16s rRNA gene. Two species of *Serratia* were identified: *marcescens* and *nematodiphila*. Finally, the chitinolytic, glucanolytic, and cellulolytic activity and prodigiosin production of bacteria with antifungal activity was determined. Five fungal strains were isolated from mangoes with anthracnose. Only one strain was responsible for anthracnose in mangoes. This fungal strain was identified as *Colletotrichum siamense*. Against *C. siamense* and *A. alternata* in vitro and in mango selected strains of *Serratia* showed antifungal activity. Finally, the *Serratia* strains produced chitinases, glucanases, cellulases and prodigiosin, and the two *S. marcescens* strains did not produce hemolysins. The three *Serratia* strains isolated in this study can potentially be used in the biological control of anthracnose caused by *C. siamense* and *A. alternata* on mango.

Keywords: *Colletotrichum siamense*; *Alternaria alternata*; *Serratia marcescens*; *Serratia nematodiphila*; mangoes

1. Introduction

The genus *Colletotrichum* comprises several important species that are among the most common fungal pathogens that damage various tropical and subtropical fruit and vegetables. Most crops around the world are susceptible to one or more *Colletotrichum* species. *Alternaria alternata* is another fungus that causes damage to fruit and vegetables around the world. *Colletotrichum* spp. and *A. alternata* are associated with economically important diseases in cereals, legumes, vegetables, and perennial crops, including fruit trees [1]. The importance of *Colletotrichum*, *A. alternata*, and the anthracnose disease they cause in horticultural products is a growing threat to global agriculture, and more than 50% of fruit and vegetable losses are caused by *Colletotrichum* spp. and *A. alternata* [2]. The late onset of fungus disease symptoms in a crop is often devastating and leads to significant losses after harvest. Tropical fruit crops in which *Colletotrichum* and *A. alternata* cause severe losses include papaya, avocado, and mango. The high susceptibility of fruit, such as mango, to *Colletotrichum* and *A. alternata* exposes the need for alternative *Colletotrichum* and *A. alternata* prevention and control methods [3].

Anthracnose is considered the most important disease affecting mango (*Magnifera indica* L.) crops. It is characterised by the presence of dark spots on leaves, flowers, and peduncles; on fruit, dark sunken irregular lesions appear. The disease manifests a high degree of severity under a humid climate, reaching the pulp of the fruit, which generates a bitter taste [4]. Mango anthracnose is generally attributed to species of the genus *Colletotrichum* and, to a lesser degree, to the genus *Alternaria*. In the case of *Colletotrichum*, most cases of anthracnose are attributed to only two species: *gloeosporioides* and *asianum*. However, there are reports that other *Colletotrichum* species also cause anthracnose in mangoes, including *C. alienum*, *C. fructicola*, *C. tropicale*, and *C. siamense* [5]. Recently, Tovar-Pedraza et al. [5] reported that *C. siamense* is a highly virulent species on mango in Mexico.

The most common method of combating these phytopathogenic fungi is the use of chemical pesticides. However, it is widely documented that pesticides are toxic to humans, animals, and plants [6]. In Mexico, the most commonly used pesticides to combat anthracnose in mangoes are azoxystrobin, trifloxystrobin (extremely toxic to aquatic plants and animals, as well as humans if inhaled), pyraclostrobin (toxic to aquatic plants and animals), cyprodinil (toxic to fish, bees, and aquatic invertebrates), fludioxonil (highly toxic to aquatic organisms, with long-lasting effects), thiabenzadol (toxic to humans), mancozeb (acute toxicity), chlorothalonil (harmful for human health, toxic to fish), and copper derivatives [7].

Therefore, it is necessary to search for non-toxic control alternatives for phytopathogenic fungi. One approach is the use of microorganisms that inhibit the growth of phytopathogenic fungi. In general, biological control is based on the action of living organisms (animals, plants, or microorganisms) that can reduce the populations of a pest. There are reports of different microorganisms that exert an inhibitory or antagonistic effect on different phytopathogenic fungi, representing a viable option for the control of phytopathogens. The use of different bacteria, such as *Bacillus subtilis* [8], and *Brevundimonas diminuta* [9] can control anthracnose in mango, caused mainly by *C. gloeosporioides*. However, the antagonistic effect of other microorganisms, such as strains of the genus *Serratia*, on *Colletotrichum* spp. or *A. alternata* has not been reported. There are some reports of the inhibitory effect of *Serratia* on other fungal genera [10,11]. It is possible that strains of the genus *Serratia* could be used for biological control of *Colletotrichum* spp. and *A. alternata*.

In addition, it should be noted that most studies on the antagonistic effects of bacteria to control anthracnose have been carried out with *C. gloeosporioides*. Currently, there are only two studies on the in vitro antagonistic effects of bacteria on *C. siamense* [12,13]. However, there are limited reports on the use of *Serratia* species as a biological control agent or on its antifungal activity, and no studies have investigated the antagonistic effects of species of the genus *Serratia* against *C. siamense* or *A. alternata*. In this context, the objective of this study was to isolate bacteria with in vitro antifungal activity against *C. siamense* and *A. alternata*, molecularly identify them, determine their chitinolytic, glucanolytic, and cellulolytic activity and prodigiosin, and determine their antagonistic effects against *C. siamense* and *A. alternata* in mangoes.

2. Materials and Methods

2.1. Isolation and Identification of Mango Phytopathogenic Fungi

2.1.1. Isolation of Fungi from Mangoes with Anthracnose

Ataulfo mangoes with evident anthracnose damage were obtained from a public market in the city of Pachuca, Hidalgo. The mangoes were transported to the laboratory under aseptic conditions and in sterile plastic bags. In the laboratory, the mangoes were disinfected by immersion in a sodium hypochlorite solution at 200 mg/mL for 10 min. Subsequently, the mangoes were washed with sterile tap water and dried in a laminar flow hood for 10 min. Using sterile dissecting forceps, the cuticle was removed from the area damaged by anthracnose; the damaged mango pulp was then minced with a sterile platinum loop, and the sample was streaked onto sterile potato dextrose agar (PDA) in Petri dishes. The plates were incubated at 28 °C for 7 days, and conidia production was

determined microscopically with cotton blue staining with lactophenol [14]. The fungal strains that showed conidia formation were planted on potato dextrose agar plates and incubated at 28 °C for 7 days. The fungal cultures were stored and refrigerated until use.

2.1.2. Verification of the Pathogenicity of the Isolated Fungal Strains

Preparation of the Mangoes

Physiologically ripe mangoes were bought at a market in the city of Pachuca, Hidalgo. The Ataulfo mangoes showed no bumps or signs of anthracnose and were transported to the laboratory under aseptic conditions in sterile plastic bags. In the laboratory, the mangoes were wiped with a clean cloth to remove dirt and subsequently disinfected and dried, as described above.

Preparation of the Fungal Inoculum

Each fungal strain that formed conidia was used to inoculate a 250 mL rectangular dilution bottle with 40 mL of PDA agar in an inclined position. The bottles were incubated at 28 °C for 14 days, and subsequently, 50 mL of a sterile solution of Tween 20 (0.1%) in distilled water was added to each bottle; the mixture was stirred for 1 min to release the conidia. The obtained suspension was filtered through sterile cotton gauze, and the number of conidia in the filtered suspension was estimated using a Neubauer chamber. For each fungus, a conidia suspension in sterile distilled water of approximately 1×10^5 conidia per mL was prepared.

Inoculation of Mangoes and Detection of Anthracnose Signs

Disinfected mangoes were wounded with a bodkin to a depth of 1 mm, and each wound was inoculated with 20 µL of the conidia suspension (1×10^5 conidia/mL) of each fungal strain. As a negative control, wounds were inoculated only with sterile distilled water. Inoculated mangoes were stored at 28 °C for 14 days, and five mangoes were used for each suspension of conidia of each fungus and five for the control. Inoculated mangoes were checked daily for visible signs of anthracnose. Only one fungal strain, called MID, caused anthracnose in mangoes.

2.1.3. Molecular Identification of the Mid Fungal Strain

DNA was extracted from approximately 500 mg biomass of strain MID using a ZR Fungal/Bacterial DNA MiniPrep kit. The amount of DNA obtained was measured using an Epoch spectrophotometer system (Agilent Technologies Mexico, Mexico City, Mexico). From the total DNA obtained, the 18S-ITS1-5.8S-ITS2-26S gene fragment was amplified with oligonucleotides ITS5 and ITS4, according to the conditions previously described by White [15]. A 1% agarose gel was used to check the size of the expected fragment (500 nt) at 100 V for 1 h. Sequencing of the 18S-ITS1-5.8S-ITS2-26S gene fragment was carried out by the CINVESTAV National Laboratory of Experimental Services, and two sequences were obtained for each oligonucleotide. The sequences were manually edited using Chromas 2.4, (2012), forming a consensus sequence for each isolate. The consensus sequences of each of the samples were compared with the BLAST program of the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA) to determine similarities among biological sequences. The consensus sequence was aligned with the BLAST search results using Clustal W built-in MEGA 6.0 [16]. A dendrogram was generated using the neighbor joining method to observe phylogenetic relationships with different holotype sequences of the defined species.

2.2. Isolation and Identification of Bacteria with Antifungal Activity

2.2.1. Bacterial Isolation

Physiologically ripe Ataulfo mangoes were bought at a market in Pachuca, Hidalgo. The mangoes showed no bumps or anthracnose lesions and were transported to the laboratory under septic conditions. In the laboratory, each mango was placed in a plastic bag,

and 100 mL of sterile peptone diluent (0.1%) was added and rubbed onto the bag for 1 min. Subsequently, via surface extension, Petri dishes containing agar for standard methods (SMA) were streaked and incubated for 24–48 h at 35 °C. Plates with 20 to 100 bacterial colonies were selected for isolation. Colonies with different morphologies were selected and Gram-stained. The selected strains were streaked in tubes with slanted SMA and incubated at 35 °C for 24 h. The tubes with the cultures were stored in a refrigerator until use.

2.2.2. In Vitro Antifungal Effect of Isolated Bacteria

Alternaria alternata ATCC 42012 and *C. siamense*, which were isolated from mangoes with anthracnose, were used. The studies were carried out using the dual growth method in culture plates, applying PDA according to the methodology described by Saha et al. [17]. For this, each of the bacterial strains was cultured in a tube with 3 mL of trypticase soy broth (CST) at 35 °C for 48 h. Subsequently, the cultures were centrifuged at 1507× *g* for 30 min, and the bacterial supernatant was collected in sterile tubes. In the central parts of PDA plates, *C. siamense* or *A. alternata* were streaked; then, in these plates, 50 µL aliquots of the supernatant of the same bacterium were placed on the PDA agar at four equidistant points to form the corners of an imaginary square surrounding the *C. siamense* or *A. alternata* inoculum. Each spot where the supernatant was placed was 2 cm from the *C. siamense* or *A. alternata* streaking point. Once the supernatant was absorbed by the PDA agar, the plates were incubated at 28 °C for 14 days. Daily, the radial growth of *C. siamense* or *A. alternata* against the supernatant of each bacterial type was measured. PDA plates streaked with *C. siamense* or *A. alternata* were included as a control, but sterile distilled water was used instead of the bacterial supernatant. The studies were conducted in triplicate.

2.2.3. Identification of Bacterial Strains with Antifungal Activity

Seven bacterial strains (B1, B2, B3, B4, B5, B6, B7) were identified, four of which (B4, B5, B6, B7) were biochemically identified using the automated VITEK 2 kit (bioMérieux, Marcy-l'Étoile, France) following the manufacturer's instructions. The other three strains (B1, B2, B3) were molecularly identified. For this, DNA was extracted from approximately 500 mg biomass of each strain using a ZR Fungal/Bacterial DNA MiniPrep kit. The amount of DNA obtained was measured using an Epoch spectrophotometer system (Agilent Technologies Mexico, Mexico City, Mexico). From the total DNA obtained, the 16s rRNA gene was amplified with the universal oligonucleotides 27F (5′AGAGTTTGATCMTGGCTCAG3′) and 1492R (5′TACGGYTACCTTGTTACGACTT3′), according to the conditions previously described by White [15]. A 1% agarose gel was used to check the size of the expected fragment (1500 nt) at 100 V for 1 h. Two sequences were obtained for each oligonucleotide and manually edited using Chromas v2.4, (Technelysium Pty. Ltd., South Brisbane, Australia, 2012), forming a consensus sequence for each isolation. The consensus sequences of each of the samples were compared with the BLAST programme of the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA) to determine similarities among biological sequences. The consensus sequence was aligned with the BLAST search results with Clustal W built-in MEGA 6.0 [16]. A dendrogram was generated using the neighbor joining method to observe the phylogenetic relationships with different holotype sequences of the defined species. The biochemical identification in VITEK 2 and the sequencing of the 16s rRNA gene were both carried out by the CINVESTAV National Laboratory of Experimental Services, Mexico. Molecular study identified two strains of *Serratia marcescens* and one of *S. nematodiphila*.

2.3. Antifungal Effect of S. marcescens and S. nematodiphila Strains against the Conidia of Colletotrichum and Alternaria

The determination of the antifungal effect of the *Serratia* strains against *C. siamense* and *A. alternata* ATCC 42012 conidia was determined following Gutiérrez-Román et al. [18]. Briefly, equal volumes of a conidial suspension of *C. siamense* or *A. alternata* in sterile tap

water (1×10^6 conidia/mL) and cells of *Serratia* strains (two *S. marcescens* or *S. nematodiphila*) grown in Luria Bertani medium (LB) to a density of 1×10^9 cfu/mL were mixed. The control contained the conidial suspension of *C. siamense* or *A. alternata* mixed with sterile LB medium. Twenty microliters of each mixed was applied to a glass slide and incubated in a humid chamber at 30 °C in the dark. Slides were observed by microscopy and photographed every 6 h for 24 h. The percentage of conidia germination was estimated by counting a total of 100 conidia per slide under a microscope at 100× magnification. Percent germination of conidia were evaluated, and the images were captured with a Zeiss AxioCam ERc 5s. The percent conidial germination (±standard error) was calculated from mean values as: $CG\ \% = (C - T)/C \times 100$; Where, CG = conidial germination, C = percentage of germinated conidia in control, and T = percentage of germinated conidia in treated samples. The studies were conducted in triplicate.

2.4. Chitinolytic Activity Assay

2.4.1. Preparation of Colloidal Chitin

Colloidal chitin was prepared as reported by Zarei et al. [11]. Briefly, 24 g chitin powder obtained from shrimp shell (C7170, Sigma-Aldrich Co., St. Louis, MO, USA) was added to 790 mL concentrated HCl and left at 4 °C overnight with vigorous stirring. The mixture was added to 8 L of ice-cold ethanol 95% (v/v) with rapid stirring and maintained at 4 °C overnight. The precipitate was collected by centrifugation at 5000× g for 20 min at 4 °C and was then washed with sterile distilled water until the colloidal chitin had a pH of 7.0.

2.4.2. Chitinolytic Activity

The assay was carried out using the agar diffusion technique. Tubes containing 3 mL of trypticase soy broth (TSB; Bioxon, Becton Dickinson, Ciudad de México, Mexico) were inoculated with individual *Serratia* strains (two *S. marcescens* and *S. nematodiphila*) and incubated at 35 °C for 24 h. The cultures were washed twice in sterile isotonic saline solution (ISS; 0.85% of NaCl) by centrifuging at 3500× g for 20 min and resuspending the pellets in ISS at about 10^9 CFU/mL. However, according to Zarei et al. [11], a minimum culture (MC) medium containing colloidal chitin was prepared with the following ingredients and proportions: 0.5% colloidal chitin, 0.03% peptone, 0.03% yeast extract, 0.07% K_2HPO_4, 0.03% KH_2PO_4, 0.05% $MgSO_4.7H_2O$, 1.5% agar, 0.2% NH_4NO_3, 0.1% NaCl (w/v), and 0.1% (v/v) trace elements (pH 7). The culture medium was sterilised by autoclaving and poured into Petri dishes. Separately, 20-µL aliquots of washed cultures of *Serratia* strains were placed on Petri dishes containing the culture media with colloidal chitin. Each washed culture was inoculated in triplicate. Once the washed cultures were absorbed by the agar, inoculated plates were incubated at 35 °C for 2 days. After incubation, the appearance of a translucent zone around the inoculum in the culture medium was considered chitinolytic activity [11]. The diameter (mm) of the resulting translucent zone for each *Serratia* strain were measured, and the average was obtained for each strain.

2.5. Glucanolytic Activity

Glucanase enzyme production of *Serratia* strains was determined following the procedure described by Renwick et al. [19]. The MC medium described above was now prepared containing a substrate of β-1,3-glucanase: laminarin (0.01%, Sigma-Aldrich, St. Louis, MO, USA). The *Serratia* strains (two *S. marcescens* and one *S. nematodiphila*) were cultured on the MC medium containing laminarin and incubated at 35 °C for 2 days. After incubation, 0.1% w/v Congo red solution (Sigma-Aldrich, St. Louis, MO, USA) was applied to cultivated plates, and the formation of clear zones around colonies was considered positive to β-1,3-glucanase activity by depolymerisation of laminarin [19].

2.6. Cellulolytic Activity

The cellulolytic enzyme production assay of *Serratia* strains was performed according to Ashwini and Srividya [20]. The MC medium described above was now prepared

containing a substrate of β-1,4 cellulase: carboxy methyl cellulose (1%, Sigma-Aldrich, St. Louis, MO, USA). The *Serratia* strains (two *S. marcescens* and *S. nematodiphila*) were cultured on the MC medium containing carboxy methyl cellulose and incubated at 35 °C for 2 days. After incubation, 0.1% (*w*/*v*) Congo red solution (Sigma-Aldrich, St. Louis, MO, USA) was applied to cultivated plates, and the formation of clear zones around colonies was considered positive for β-1,4 cellulase activity [20].

2.7. Prodigiosin Production

Prodigiosin production was determined following Slater et al. [21]. The *Serratia* strains (two *S. marcescens* and one *S. nematodiphila*) were inoculated in tubes containing Luria Bertani medium (LB) and incubated at 20 °C for 18 h to reach a density of approximately 1×10^9 cfu/mL. Separately, 1 mL of each culture was placed in a clean tube and centrifuged at 10,000× *g* for 10 min. The supernatant was discarded, and the pellet was resuspended in acidified ethanol (4% 1 M HCl in ethanol) to extract prodigiosin from the cells. Cell debris was removed by centrifugation at 10,000× *g* for 10 min, and the supernatant was transferred to a cuvette for measurement of absorbance at 534 nm. The relative prodigiosin concentration was expressed per cell ($A_{534\ mL}{}^{-1}$ OD_{600} unit).

2.8. Antagonistic Effects of S. marcescens and S. nematodiphila against C. siamense and A. alternata on Mango

Physiologically ripe "Ataulfo" mangoes were used; they were cleaned and sanitised, as described above. A 1×10^4 conidia/mL-suspension of *C. siamense* or *A. alternata* was prepared as described above. Simultaneously, supernatants collected from 48 h cultures at 37 °C in CST of the two strains of *S. marcescens* (B1 and B3) and of the strain of *S. nematodiphila* (B2) were obtained as described above. Two inoculation methods were tested: (a) inoculation in the mango wound and (b) inoculation of the mango on the integral or undamaged surface of the mangoes. For the first case, (a) the mangoes were wounded with a bodkin to a depth of 1 mm, and the wounds were inoculated with 20 µL of the supernatant of *S. marcescens* B1, *S. marcescens* B3, or *S nematodiphila* B2. The mangoes were kept in a laminar flow hood for 30 min so that the supernatant could be absorbed by the pulp of the mangoes or dried. Subsequently, 20 µL of the conidia suspension (final concentration of 10^2 conidia per mango) was placed onto the same spot and left for another 30 min in a laminar flow hood at room temperature. For *A. alternata*, only this procedure was tested. For the second case, (b) the entire process mentioned above in section a was carried out, but instead of causing a wound, the three supernatants of *Serratia* strains and *C. siamense* were inoculated on the intact surface. In all cases, as a negative control, mangoes were inoculated only with sterile distilled water, and other mangoes inoculated only with the supernatant of each of the 3 *Serratia* strains were used.

Finally, for both cases, the inoculated mangoes were stored for 14 days in a bioclimatic chamber at 30 °C and 90% relative humidity. All treatments were performed in triplicate. The mangoes were checked daily for visible signs of anthracnose. Damage to mangoes related to anthracnose was recorded according to the eight-level hedonic scale reported by Corkidi et al. [22]. This hedonic scale considers 5 levels of damage: 0–1% (1), 1–5% (2), 6–9% (3), 10–49% (4), 50–100% (5). The experiment was repeated three times.

2.9. Hemolytic Activity of Serratia Marcescens Strains

Hemolytic activity of the *S. marcescens* strain was assessed according to Gerhardt et al. [23]. Briefly, *S. marcescens* strains were inoculated separately in 3 mL of trypticase soy broth (TSB; Bioxon, Becton Dickinson, Ciudad de México, Mexico) and incubated at 35 ± 2 °C for 18 h. Then, *S. marcescens* strains were streaked on tryptone soya agar (TSA) plates supplemented with 5% goat blood and incubated at 30 °C for 48 h. After incubation, the plates were observed against light for transparent zones formation around the bacterial growth. A clear zone indicates β-hemolysin production, dark colored zone indicates α hemolysin production and green colored zone indicate γ hemolysin production [23].

2.10. Statistical Analyses

All experiments on the antagonistic effect, both in vitro and in mangoes, and antifungal effect on conidia, chitinolytic, glucanolytic, cellulolytic, and prodigiosin assays were repeated three times separately. Differences between the means were determined using ANOVA and Tukey's test ($p < 0.05$) with Statistica v6.0 (Statsoft, Inc., Tulsa, OK, USA, 1997). In some cases, the means and standard deviations were plotted.

3. Results

3.1. Isolation and Identification of Mango Phytopathogenic Fungi

From Ataulfo mangos with anthracnose, four fungal strains that presented conidia formation were isolated. These strains were inoculated on healthy Ataulfo mangoes to determine their pathogenic potential. Only one fungal strain, called MID, caused typical signs of anthracnose in inoculated mangoes. Molecular identification of the MID fungal strain by amplifying the consensus sequence of the 18S-ITS1-5.8S-ITS2-26S fragment resulted in a total of 508 nucleotides (Sequence A1 in Appendix A).

According to the information contained in the BLAST of the National Center for Biotechnology Information (NCBI), the sequence obtained from the MID strain corresponded to *C. siamense*. The most similar sequence corresponded to *C. siamense* strain HLX5, with an accession number of MN860116.1, with 100% sequence coverage and 99% sequence identity.

3.2. Antifungal Activity of Isolated Bacteria

We isolated 30 bacterial strains from mangoes; all isolates were Gram-negative rods. Using the dual growth technique in culture plates, it was determined that only seven bacterial strains (B1 to B7) had an antifungal effect against *C. siamense* (Figure 1). As seen in Figure 1, of these bacterial strains, three (B1, B2, and B3) showed a marked antifungal effect against *C. siamense*. These three bacterial strains also showed antifungal effects against *A. alternata* in the culture medium (Figure 2).

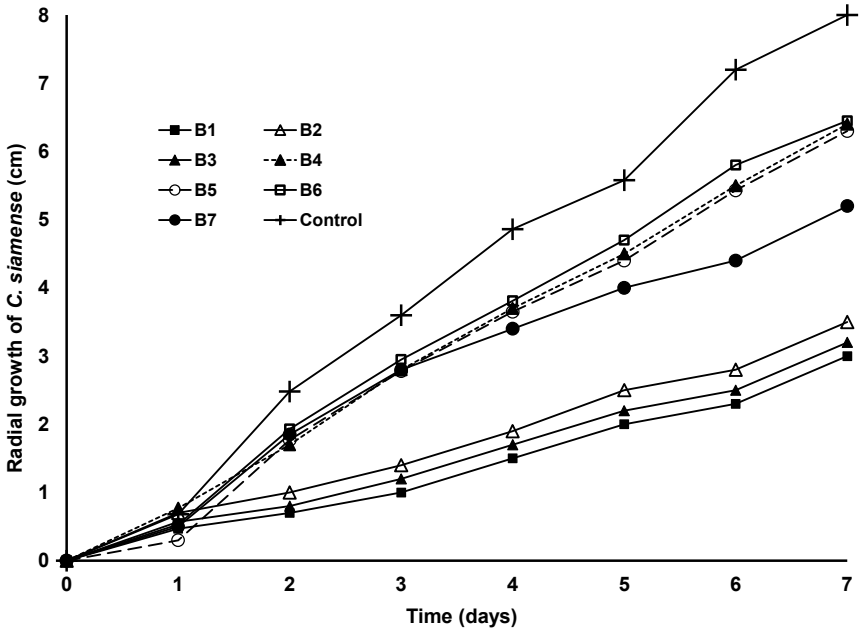

Figure 1. Antifungal effect of seven bacterial strains (B1 to B7) against *C. siamense*.

Identification of Bacterial Strains with Antifungal Activity

Of the seven bacterial strains (B1 to B7) that showed antifungal activity in in vitro tests, four (B4, B5, B6, and B7) were identified by biochemical tests, and four were of the

species *Serratia marcescens*. These were identified only with biochemical tests because they had a moderate antifungal effect in in vitro studies.

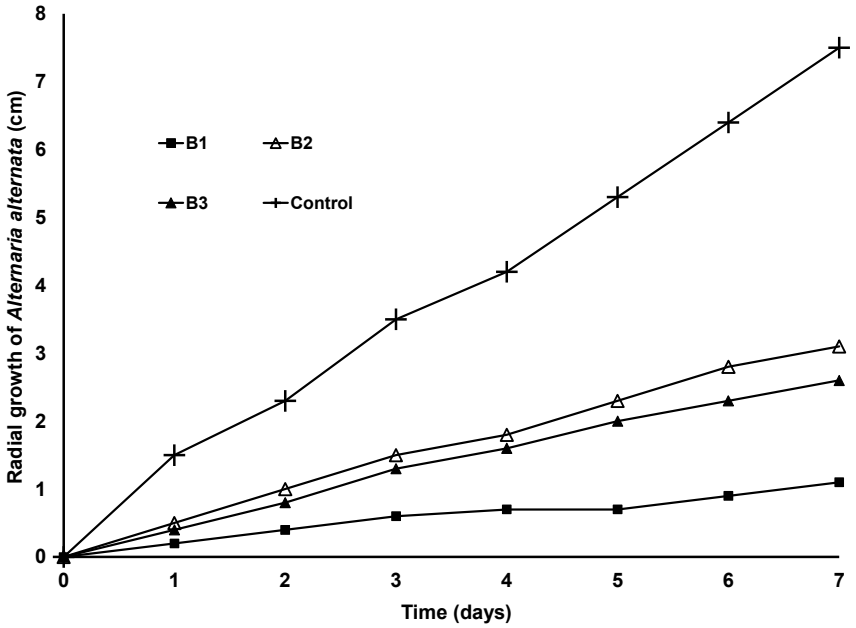

Figure 2. Antifungal effect of 3 bacterial strains against *A. alternata* in culture medium.

The other three strains (B1, B2, and B3) with the greatest antifungal effect in in vitro studies were molecularly identified by the 16s ribosomal RNA gene. The sequence most similar to the B1 strain, according to the BLAST search of the National Center for Biotechnology Information (NCBI), was *Serratia marcescens*, with 100% sequence coverage and 99% identity, sequence of the 16S fragment of strain B1 with a total of 1453 nucleotides (Sequence A2).

In the case of strain B2, the most similar sequence according to the BLAST search corresponded to *Serratia nematodiphila*, with 100% sequence coverage and 99% identity (Sequence A3).

Finally, in the case of strain B3, the most similar sequence according to the BLAST search corresponded to *S. marcescens* (Sequence A4).

3.3. Antifungal Effect of S. marcescens and S. nematodiphila Strains against the Conidia of Colletotrichum and Alternaria

In general, marked inhibition was observed in the germination of *C. siamense* and *A. alternata* conidia at around 90 and 82% with the three *Serratia* strains, for 6 and 24 h, respectively (Table 1).

Table 1. Antifungal effect of *Serratia marcescens* B1 and B3 and *S. nematodiphila* B2 strains against conidia germination of *C. siamense* and *A. alternata*.

Time (Hours)	Percentage (%) of Conidia Germination of *C. siamense* and *A. alternata*									
	Cont1C	**Cont2C**	**Cont1A**	**Cont2A**	**C + SmB1**	**C + SmB3**	**C + SnB2**	**A + SmB1**	**A + SmB3**	**A + SnB2**
0	0	0	0	0	0	0	0	0	0	0
6	41 ± 6	0	34 ± 6	0	4 ± 2	6 ± 2	4 ± 1	3 ± 2	4 ± 2	4 ± 1
12	85 ± 6	0	75 ± 6	0	13 ± 3	16 ± 5	16 ± 4	10 ± 2	13 ± 2	15 ± 3
18	93 ± 5	0	87 ± 5	0	15 ± 4	17 ± 4	18 ± 3	14 ± 3	16 ± 3	17 ± 4
24	96 ± 3	0	93 ± 4	0	16 ± 5	17 ± 3	19 ± 3	15 ± 3	16 ± 3	16 ± 3

Cont1C = Luria Bertani (LB) medium + *C. siamense*, Cont2C = sterile tap water (STW) + *C. siamense*, Cont1A = LB medium + *A. alternata*, Cont2A = STW + *A. alternata*, C + SmB1 = *C. siamense* + *S. marcescens* B1 culture, C + SmB3 = *C. siamense* + *S. marcescens* B3 culture, C + SnB2 = *C. siamense* + *S. nematodiphila* B2 culture, A + SmB1 = *A. alternata* + *S. marcescens* B1 culture, A + SmB3 = *A. alternata* + *S. marcescens* B3 culture, A + SnB2 = *A. alternata* + *S. nematodiphila* B2 culture.

3.4. Chitinolytic, Glucanolytic and Cellulolytic Activity

The three strains of *Serratia* showed chitinolytic activity on the culture medium with translucent zones for *S. marcescens* B1 and B3 and *S. nematodiphila* B2 (Table 2). Laminarin is a low molecular weight β-glucan storage polysaccharide present in brown algae. Laminarin is frequently used to determine the production of glucanases by microorganisms. In our study, all three *Serratia* strains caused depolymerisation of laminarin, indicating glucanase production (Table 2). All three *Serratia* strains showed evidence of glucanase production (Table 2). The three strains hydrolysed carboxy methyl cellulose in the culture medium (Table 2). The three *Serratia* strains produced prodigiosin at an average concentration of 1.8 to 2.5 $A_{534\,mL}{}^{-1}$ OD_{600} unit (Table 2).

Table 2. Production of compounds with antifungal activity by *S. marcescens* and *S. nematodiphila* strains.

Strain	Chitinolytic Activity (mm)	Glucanolytic Activity	Cellulolytic Activity	Prodigiosin Production
S. marcescens B1	(28 ± 2) [1]	+	+	2.505 ± 0.062 *
S. marcescens B3	(23 ± 2)	+	+	2.123 ± 0.100
S. nematodiphila B2	(24 ± 1)	+	+	1.883 ± 0.099

[1] Average of hydrolysis zone ± SD of three replicates, + = positive activity, * relative prodigiosin concentration per cell ($A_{534\,mL}{}^{-1}$ OD_{600} unit).

3.5. Antagonistic Effects of S. marcescens and S. nematodiphila on Mango

C. siamense was able to grow and cause anthracnose in mangoes infected with 10^2 conidia, both in fruit with lesions and in lesions inoculated with conidia of *C siamense*, as well as in those fruit with undamaged surfaces and inoculated with *C siamense* (Figures 3 and 4).

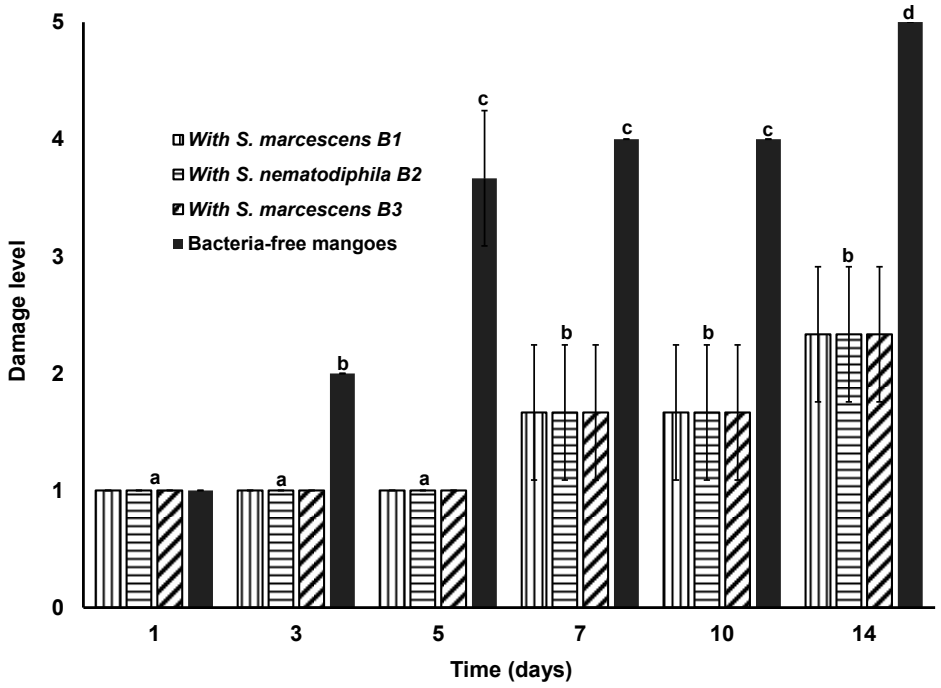

Figure 3. Mangoes with lesions inoculated with *C. siamense* and treated with strains of *Serratia*. The damage level means, 1: between 0 to 1% of damage; 2: between 1 to 5% of damage; 3: between 6 to 9% of damage; 4: between 10 to 49% of damage and 5: between 50 to 100% of damage. Different letters in the columns on the different days of treatment mean a statistically significant difference ($p < 0.05$).

In addition, 10^2 conidia of *A. alternata* also caused anthracnose in mangoes (Figure 5). However, when the mangoes were treated simultaneously with *C. siamense* or *A. alternata* and with each of the three strains of Serratia, the appearance of anthracnose signs in the

mangoes was delayed, and the progression of *C. siamense* or *A. alternata* infection was slower (Figures 3–5). It should be noted that the mangoes used as negative controls did not show spots or anthracnose during the 14 days of the study.

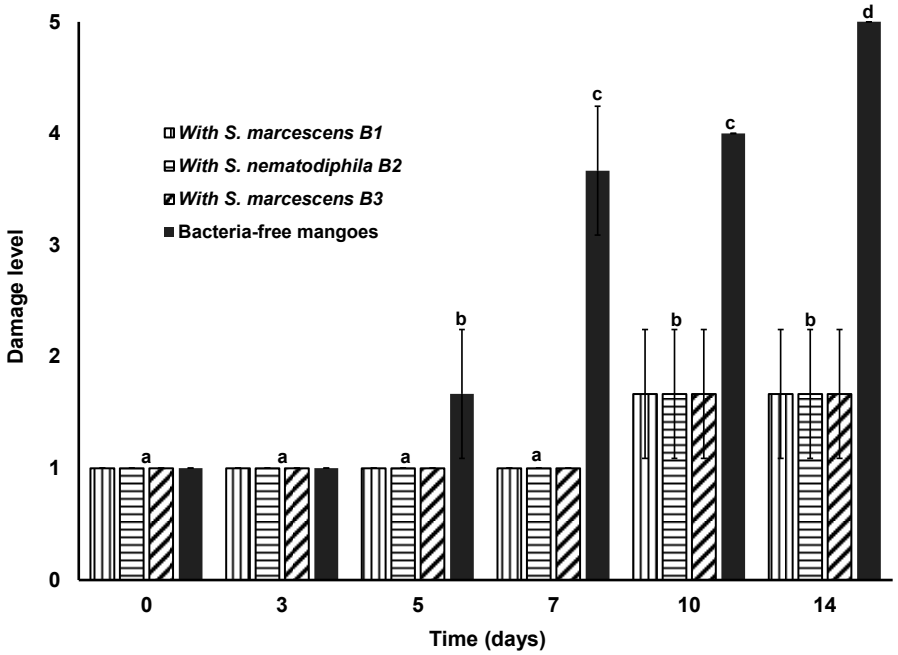

Figure 4. Mangoes with undamaged surfaces inoculated with *C. siamense* and treated with strains of *Serratia*. The damage level means, 1: between 0 to 1% of damage; 2: between 1 to 5% of damage; 3: between 6 to 9% of damage; 4: between 10 to 49% of damage and 5: between 50 to 100% of damage. Different letters in the columns on the different days of treatment mean a statistically significant difference ($p < 0.05$).

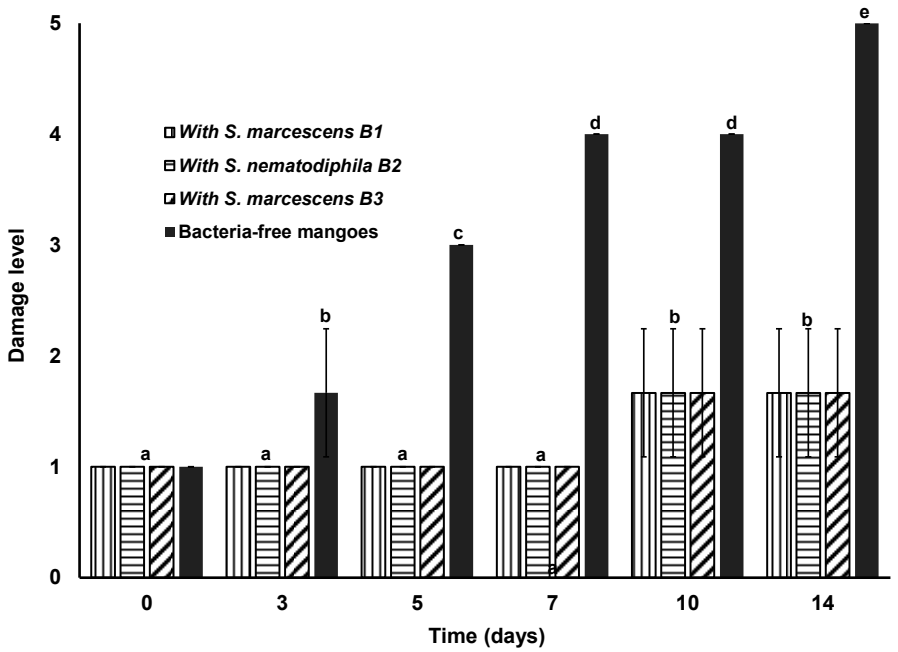

Figure 5. Mangoes inoculated with *A. alternata* and treated with strains of *Serratia.* The damage level means, 1: between 0 to 1% of damage; 2: between 1 to 5% of damage; 3: between 6 to 9% of damage; 4: between 10 to 49% of damage and 5: between 50 to 100% of damage. Different letters in the columns on the different days of treatment mean a statistically significant difference ($p < 0.05$).

3.6. Hemolytic Activity

The test for hemolytic activity on blood-supplemented TSA plates revealed that the isolate *S. marcescens* (B1 and B2 strains) lacked hemolytic activity since no transparent zone formation occurred around the bacterial colonies.

4. Discussion

Although *C. siamense* is one of the species that causes anthracnose in mangoes in different parts of the world [24,25], in Mexico, this species has been little studied as a causal agent of anthracnose. There is only one recently published article in which *C. siamense* was isolated from mangoes with anthracnose grown in Mexico [5]. Our results confirm that *C. siamense* is a species that causes anthracnose in mangoes grown in Mexico. Furthermore, the results suggest that *C. siamense* is more widespread in Mexico than previously thought and causes disease in mango fruit. In this case, further studies are necessary in Mexico to determine the real distribution of *C. siamense* and its impact on mango crops and other fruit. In addition, there is limited information available in the scientific literature on the antagonistic effects of bacteria on *C. siamense* and *A. alternata*. Recently, Xu et al. [13] described the in vitro antagonistic effect of a *Bacillus subtilis* strain on *C. siamense*. In another study, Fantinel et al. [12] reported that *Bacillus thuringiensis* var. Kurstak had a moderate antimicrobial effect on *C. siamense* in in vitro studies. The antagonistic effects of endophytic fungi on *C. siamense* have also been reported, for example, for fungi of the genera *Chaetomium*, *Fusarium*, *Phomopsis*, *Cladosporium*, *Periconia*, *Curvularia*, *Leptosphaeria*, and *Penicillium* [26].

In our study, two strains of *S. marcescens* and one of *S. nematodiphila* were identified, with remarkable antifungal effects in vitro against *C. siamense*. It has been reported that the genus *Serratia* can produce different chemical compounds with bactericidal effects [27]. However, there are no reports available in the scientific literature on the antifungal effect of *Serratia* on the genus *Colletotrichum*. Therefore, our study describes the antifungal effect of *Serratia* species on *C. siamense* for the first time (Table 1). Our results agree with those reported by Gutiérrez-Román et al. [18], who reported a high inhibition in the germination of *C. gloeosporioides* conidia by different strains of *S. marcescens*. In our study, the three *Serratia* strains had a prominent antifungal effect against *C. siamense* and *A. alternata*. The observed antifungal effect may be due to different agents, such as enzymes (chitinase, for example) or chemical compounds, e.g., prodigiosin. Due to this, it was determined if the *Serratia* strains produced any enzymes or chemical compounds that could be responsible for the observed inhibitory effect.

The three *Serratia* strains that we isolated in this study showed chitinolytic, glucanolytic, and cellulolytic activity (Table 2). Previous studies have reported the chitinolytic activity of strains of *S. marcescens* and strains of *S. nematodiphila* [28,29]. In different studies, it has been reported that one of the mechanisms by which some bacteria inhibit the growth or activity of fungi is by the production of chitinases [30]. Currently, to control phytopathogenic fungi, chitinolytic bacteria are used [30]. Chitinolytic bacteria of the genus *Serratia* are well known for their chitinase production [11]. Their ability to produce these hydrolytic enzymes could be considered an important factor for contributing significantly as an effective biocontrol agent [11]. Consequently, having bacterial strains that produce chitinases represents an alternative for the prevention and control of phytopathogenic fungi. However, as mentioned above, there are limited reports on the use of *Serratia* species as a biological control agent or on its antifungal activity, and no studies have investigated the antagonistic effects of species of the genus *Serratia* against *C. siamense*.

One of the main components of the wall of phytopathogenic fungi is the glucan; between 50 and 60% of the fungal wall is glucan [31]. Having bacteria that produce enzymes that lyse the glucan in the wall of phytopathogenic fungi becomes a viable biocontrol resource. Different authors have reported the isolation of glucanase-producing *Serratia* strains [32]. However, the isolation of non-glucanase-producing strains has also been reported [32]. This shows that *Serratia* strains may have different inhibition strategies

or mechanisms against phytopathogenic fungi. There are many reports on the cellulolytic capacity of *S. marcescens* [33]; however, there is limited information on the cellulolytic activity of *S. nematodiphila*. In this case, our study contributes to the increase in information on the cellulolytic activity of *S. nematodiphila* strains.

Prodigiosin is a chemical compound with antibacterial and antifungal effects [34]. It is a secondary metabolite produced by some bacteria of the genera *Serratia*, *Pseudomonas*, *Vibrio*, *Alteromonas*, *Rugamonas*, *Streptoverticillium*, and *Streptomyces* [34]. However, *Serratia marcescens* is the species that is most frequently related to the production of prodigiosin and is the most studied bacterium on this topic [18]. Although it has been reported that other species of the genus *Serratia* can produce prodigiosin, such as *S. plymuthica* [33] and *S. nematodiphila* [35], reports on strains of *Serratia* other than *S. marcescens* that can produce prodigiosin are limited. However, there are also strains of *S. marcescens* that do not produce prodigiosin [36]. As mentioned, in our studies, both *S. marcescens* and *S. nematodiphila* produced prodigiosin (Table 2). As observed, *S. marcescens* B1, *S. marcescens* B3, and *S nematodiphila* B2, isolated from healthy mangoes in this study, were capable of producing chitinases, glucanases, cellulases, and prodigiosin. This strongly suggests that these three *Serratia* strains have at least four different strategies or mechanisms to inhibit phytopathogenic fungi, such as *C. siamense* and *A. alternata*.

As it could be observed, the three *Serratia* strains that were tested presented an antifungal effect against *C. siamense* and *A. alternata* (Figures 3–5). This clearly shows that the three *Serratia* strains examined showed a remarkable effect against *C. siamense* and *A. alternata* in mangoes, regardless of the type of inoculation or contamination of the mangoes (Figures 3 and 4).

Currently, there are no reports on the minimum inoculum level required of *C. siamense* and *A. alternata* conidia to cause infection in mangoes. However, in the present study, an inoculum concentration of approximately 10^2 conidia was sufficient to cause infection in mango. In addition, the first signs of anthracnose became evident on the third day after inoculation (Figures 3 and 5), suggesting that the strains of *C. siamense* used in the study (and that were isolated from mangoes with anthracnose) and *A. alternata* have high virulence. In different studies, other species, such as *C. gloeosporioides*, have been reported to cause anthracnose signs in contaminated mangoes six days after inoculation of mangoes with conidia [9]. In our study, signs of anthracnose became evident on the third day after inoculation, which suggests greater virulence of *C siamense* than *C. gloeosporioides*. In fact, it has recently been reported that strains of *C. siamense* isolated from mango in Mexico were more virulent than other strains of different species of the genus *Colletotrichum* that were also isolated from mangoes in Mexico, including *C. gloeosporioides* [5].

As previously mentioned, there is currently no information available on the antifungal effect of the genus *Serratia* on the genus *Colletotrichum* or *A. alternata*. However, there are some reports of the inhibitory effect of *Serratia* on other fungal genera. For example, Guo et al. [10] reported the antifungal activity of *S. marcescens* against *Fusarium proliferatum*; the authors observed a strong inhibition of conidia germination and mycelial growth. Furthermore, they observed that *S. marcescens* suppressed fumonisin synthesis. Guo et al. [10] also reported that *S. marcescens* strain SerEW01 could synthesize chitinases that degrade the cell wall of the fungal mycelium. In another study, Zarei et al. [37] reported that *S. marcescens* inhibited the growth of *Rhizoctonia solani*, *Bipolaris* sp., *Alternaria raphanin*, and *Alternaria brassicicola* through the expression of a chitinase.

In our study, the inhibitory effect of the three strains of *Serratia* can be attributed to chitinolytic, glucanolytic, and cellulolytic activity and prodigiosin production. However, it would be appropriate to explore other possible mechanisms of inhibition of these three *Serratia* strains on *C. siamense* and *A. alternata*, as well as to evaluate the incorporation of these strains of *Serratia* from pre-harvest mangoes.

It is important to note that *S. marcescens* has been related to cases of disease in some people, which is why it is considered an opportunistic pathogen. Therefore, it is essential to appraise the risk of every potential biocontrol bacterium to avoid using potential human

pathogens as agricultural inputs. Pathogenic strains of *S. marcescens* are reported to produce hemolysins [38]. In our study, the *S. marcescens* strains did not present hemolytic activity, which strongly suggests that they are not pathogenic bacteria. However, additional testing is needed to determine that *S. marcescens* strains do not have virulence genes and they are safe for use.

5. Conclusions

To the best of our knowledge, this is the first report showing that an inoculum concentration of 10^2 conidia of *C. siamense* and *A. alternata* can initiate infection of mango fruit, causing anthracnose. It is also the first report showing that *Serratia* strains have an antifungal effect on *C. siamense* and *A. alternata*, both in the growing medium and in mango. In addition, it is the first report of the isolation of a strain of *S. nematodiphila* that simultaneously produces chitinases, glucanases, cellulases, and prodigiosin. In addition, the *S. marcescens* strains did not show hemolytic activity which is a general feature of pathogenic *S. marcescens* strains. However, additional testing is needed to determine that *S. marcescens* strains are safe for use. Therefore, after considering the safety and biocontrol potential of *Serratia* strains, it may be suggested that the three *Serratia* strains isolated in this study (*S. marcescens* B1, *S. nematodiphila* B2, and *S. marcescens* B3) can potentially be used in the biological control of anthracnose caused by *C. siamense* and *A. alternata* on mango.

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Appendix A

Sequence A1. Nucleotide sequence of the identified *Colletotrichum siamense*. According to the information contained in the BLAST of the National Center for Biotechnology Information (NCBI), the sequence obtained from the MID strain corresponded to *Colletotrichum siamense*. The most similar sequence corresponded to *C. siamense* strain HLX5, with an accession number of MN860116.1, with 100% sequence coverage and 99% sequence identity.

ATTACTGAGTTTACGCTCTACAACCCTTTGTGAACATACCTATAACTGTTGCTTCGGC
GGGTAGGGTCTCCGCGACCCTCCCGGCCTCCCGCCTCCGGGCGGGTCGGCGCCCGC
CGGAGGATAACCAAACTCTGATTTAACGACGTTTCTTCTGAGTGGTACAAGCAAATA
ATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAA
ATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCAC
ATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCA
AGCTCTGCTTGGTGTTGGGGCCCTACAGCTGATGTAGGCCCTCAAAGGTAGTGGCG
GACCCTCTCGGAGCCTCCTTTGCGTAGTAACTTTACGTCTCGCACTGGGATCCGGA
GGGACTCTTGCCGTAAAACCCCCCAATTTTCCAAAGGTTGACCTCGGATCAGGT.

Sequence A2. Nucleotide sequence of the identified *Serratia marcescens*. The sequence most similar to the B1 strain, according to the BLAST search of the National Center for Biotechnology Information (NCBI), was with an accession number of CP053572.1 with

100% sequence coverage and 99% identity (sequence of the 16S fragment of strain B1 with a total of 1453 nucleotides).

TGGCGGCAGGCCTAACACATGCAAGTCGAGCGGTAGCACAGGGGAGCTTGCTCCC
TGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGG
GGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAGAGGGG
GACCTTCGGGCCTCTTGCCATCAGATGTGCCCAGATGGGATTAGTGTGGCTCACCT
AGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGAC
ACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAA
GCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTT
TCAGCGAGGAGGAAGGTGGTGAACTTAATACGTTCATCAATTGACGTTACTCGCA
GAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAA
GCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGA
TGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTTGAAACTGGCAAGCTAGAGT
CTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGA
GGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGA
AAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATG
TCGATTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATC
GACCGCCTGG.GGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGC
CCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACC
TACTCTTGACATCCAGAGAACTTAGCAGAGATGCTTTGGTGCCTTCGGGAACTCT
GAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGT
CCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTCGGCCGGGAACTCA
AAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCAT
GGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCATATACAAAGAGAAGCGA
CCTCGCGAGAGCAAGCGGACCTCATAAAGTATGTCGTAGTCCGGATTGGAGTCTGC
AACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGCTACGGTGA
ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAA
AGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTTACCACTTTGTGATTCATGACTG
GGG.

Sequence A3. Nucleotide sequence of the identified *Serratia nematodiphila*. In the case of strain B2, the most similar sequence according to the BLAST search corresponded to *Serratia nematodiphila*, with an accession number of KP318499.1 with 100% sequence coverage and 99% identity.

AGMTTGYTSCCCGGGTGACGAGCGSCGGACGGGTGAGYAATGTCTAGGRAAACTGC
CTGAAGCTTGCTCCCCGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAA
CTGCCTGATGGAGRGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGC
AAGACCAAAGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACG
TCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCAGATGTGCCCAGAT
GGGATTAGCTAGTAGGTGGGGGGGGACCTTCGGGCCTCTTGCCATCAGATGTGCCC
AGATGGGATTAGCTAGTAGGTGGGGTAATGGCTCACCTAGGCGACGATCCCTAGCT
GGTCTGAGAGGATGACCAGCCACACTGGGTAATGGCTCACCTAGGCGACGATCCC
TAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACT
CCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCAACTGAGACACGGTC
CAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTG
ATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTGCAA
GCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTT
TCAGCGAGGAGGAAGGTGGTGAGCTTAATACGYTCATCAATTGACGTTACTCGCA
GAAGAACAGCGAGGAGGAAGGTGGTGAGCTTAATACGTTCATCAATTGACGTTAC
TCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGG
TGCAAGCGTTAATCGGAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACG
GAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTG
TTAAGTCAGATGTGAAATCCCCGGGCTATTACTGGGCGTAAAGCGCACGCAGGCG
GTTTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTTGAAA

CTGGCAAGCTAGAGTCTCGTAGAGGGGGGTAGAATTCCAACCTGGGAACTGCATT
TGAAACTGGCAAGCTAGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGT
GAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCCAGGTGTA
GCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGG
ACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC
CTGTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTA
GATACCCTGGTAGTCCACGCTGTAAACGATGTCGATTTGGAGGTTGTGCCCTTGAG
GCGTGGCTTCCGGGTAGTCCACGCTGTAAACGATGTCGATTTGGAGGTTGTGCCCT
TGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGACCGCCTGGGGAGTACGGCC
GCAAGGTTAAAACTCAAATGAAAGCTAACGCGTTAAATCGACCGCCTGGGGAGTA
CGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGA
GCATGTGGTTTAATTCGATGCAACGCGAAGAACTTGACGGGGGCCCGCACAAGCG
GTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACAT
CCAGAGAACTTWCCAGAGATGCWTTGGTGCCTTCGGGAACTCCTTACCTACTCTT
GACATCCAGAGAACTTTCCAGAGATGCATTGGTGCCTTCGGGAACTCTGAGACAG
GTGCTSCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGGTTAAGTCCCTGAG
ACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCG
CAACGAGCGCAACCATATCCTTTGCTGCCAGCGGTCCGTCGGACTCAAGGAGACG
CAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTCGGCCGGGAACTCAAAGG
AGACTGCCAGTGATAAACTGGAGGAAG.

Sequence A4. Nucleotide sequence of the identified *Serratia marcescens*. In the case of strain B3, the most similar sequence according to the BLAST search corresponded to *S. marcescens* strain S14, with an accession number of MK346258.1 with 100% sequence coverage and 99% identity.

TGGGTGACGTKYGGGGACGATGAGCAACGTCAGGAAAACTGCCTGATGGAGGGGG
ATATGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAG
GGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAG
GGGGACCTTCGACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAA
AGAGGGGGACCTTCGGGCCTCTTGCCATCAGATGTGCCCAGATGGGATTAGCTAGT
AGGTGGGGTAATGGCTCACGGCCTCTTGCCATCAGATGTGCCCAGATGGGATTAGC
TAGTAGGTGGGGTAATGGCTCACCTAGGCGACGATCCCTAGCTGGKCTCTAGGCGA
CGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGTCC
MGAMTYCYTACGGGARGSAGCRSKGGGRAWATTGCACAWGGGSGCAGCCTGATGT
CCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTG
ATGCAKCMTGSCGCGKGKGTGAAGAGGCTTCSGGTGCAGCCATGCCGCGTGTGTG
AAGAAGGCCTTCGGGT.

References

1. Dean, R.; Van Kan, J.A.; Pretorius, Z.A.; Hammond-Kosack, K.E.; Di Pietro, A.; Spanu, P.D.; Rudd, J.J.; Dickman, M.; Kahmann, R.; Ellis, J.; et al. The top 10 fungal pathogens in molecular plant pathology. *Mol. Plant Pathol.* **2012**, *13*, 414–430. [CrossRef] [PubMed]
2. Awang, Y.; Ghani, M.A.A.; Sijam, K.; Mohamad, R.B. Effect of calcium chloride on anthracnose disease and postharvest quality of red-flesh dragon fruit (*Hylocereus polyrhizus*). *Afr. J. Microbiol. Res.* **2011**, *5*, 5250–5259. [CrossRef]
3. Siddiqui, Y.; Asgar, A. *Colletotrichum gloeosporioides* (Anthracnose). In *Postharvest Decay: Control Strategies*; Chapter 11; Bautista-Baños, S., Ed.; Academic Press: Cambridge, MA, USA; Elsevier Inc.: London, UK, 2014; pp. 337–371. [CrossRef]
4. Díaz-Medina, A.R.; Arboleda-Zapata, T.; Ríos-Osorio, L.A. Biological control strategies used for the management of antracnosis caused by *Colletotrichum gloeosporioides* in mango fruits: A systematic review. *Trop. Subtrop. Agroecosyst.* **2019**, *22*, 595–611.
5. Tovar-Pedraza, J.M.; Mora-Aguilera, J.A.; Nava-Díaz, C.; Lima, N.B.; Michereff, S.J.; Sandoval-Islas, J.S.; Leyva-Mir, S.G. Distribution and pathogenicity of *Colletotrichum* species associated with mango anthracnose in Mexico. *Plant Dis.* **2020**, *104*, 137–146. [CrossRef]
6. Köhler, H.R.; Triebskorn, R. Wildlife ecotoxicology of pesticides: Can we track effects to the population level and beyond? *Science* **2013**, *341*, 759–765. [CrossRef] [PubMed]
7. Zhan, Y.; Zhang, M. Spatial and temporal patterns of pesticide use on California almonds and associated risks to the surrounding environment. *Sci. Total Environ.* **2014**, *472*, 517–529. [CrossRef] [PubMed]
8. Touré, Y.; Ongena, M.; Jacques, P.; Guiro, A.; Thonart, P. Role of lipopeptides produced by *Bacillus subtilis* GA1 in the reduction of grey mould disease caused by *Botrytis cinerea* on apple. *J. Appl. Microbiol.* **2004**, *96*, 1151–1160. [CrossRef]

9. Kefialew, Y.; Ayalew, A. Postharvest biological control of anthracnose (*Colletotrichum gloeosporioides*) on mango (*Mangifera indica*). *Postharvest Biol. Technol.* **2008**, *50*, 8–11. [CrossRef]
10. Guo, Z.; Zhang, X.; Wu, J.; Yu, J.; Xu, M.; Chen, D.; Zhang, Z.; Li, X.; Chi, Y.; Wan, S. In vitro inhibitory effect of the bacterium *Serratia marcescens* on *Fusarium proliferatum* growth and fumonisins production. *Biol. Control* **2020**, *19*, 202–219. [CrossRef]
11. Zarei, M.; Aminzadeh, S.; Zolgharnein, H.; Safahieh, A.; Ghoroghi, A.; Motallebi, A.; Daliri, M.; Sahebghadam-Lotfi, A. *Serratia marcescens* B4A chitinase product optimization using Taguchi approach. *Iran. J. Biotechnol.* **2010**, *8*, 252–262.
12. Fantinel, V.S.; Muniz, M.F.B.; Poletto, T.; Dutra, A.F.; Krahn, J.T.; Favaretto, R.F.; Sarzi, J.S. Biocontrole in vitro de *Colletotrichum siamense* utilizando *Trichoderma* spp. e *Bacillus thuringiensis* var. *kurstaki*. *Rev. Ciência Agríc.* **2018**, *16*, 43–50. [CrossRef]
13. Xu, J.X.; Li, Z.Y.; Lv, X.; Yan, H.; Zhou, G.Y.; Cao, L.X.; He, Y.H. Isolation and characterization of *Bacillus subtilis* strain 1-L-29, an endophytic bacteria from *Camellia oleifera* with antimicrobial activity and efficient plant-root colonization. *PLoS ONE* **2020**, *15*, e0232096. [CrossRef] [PubMed]
14. Živković, S.; Stevanović, M.; Đurović, S.; Ristić, D.; Stošić, S. Antifungal activity of chitosan against *Alternaria alternata* and *Colletotrichum gloeosporioides*. *Pestic. Phytomed.* **2018**, *33*, 197–204. [CrossRef]
15. White, T.J.; Bruns, T.; Lee, S.; Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*; Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., Eds.; Academic Press Inc.: New York, NY, USA, 1990; pp. 315–322. [CrossRef]
16. Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A.; Kumar, S. Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* **2013**, *30*, 2725–2729. [CrossRef]
17. Saha, D.; Purkayastha, G.D.; Ghosh, A.; Isha, M.; Saha, A. Isolation and characterization of two new *Bacillus subtilis* strains from rhizosphere of eggplant as potential biocontrol agents. *J. Plant Pathol.* **2012**, *94*, 109–127. [CrossRef]
18. Gutiérrez-Román, M.I.; Holguín-Meléndez, F.; Bello-Mendoza, R.; Guillén-Navarro, K.; Dunn, M.F.; Huerta-Palacios, G. Production of prodigiosin and chitinases by tropical *Serratia marcescens* strains with potential to control plant pathogens. *World J. Microbiol. Biotechnol.* **2012**, *28*, 145–153. [CrossRef]
19. Renwick, A.; Campbell, R.; Coe, S. Assessment of in vivo screening systems for potential biocontrol agents of *Gaeumannomyces graminis*. *Plant Pathol.* **1991**, *40*, 524–532. [CrossRef]
20. Ashwini, N.; Srividya, S. Potentiality of *Bacillus subtilis* as biocontrol agent for management of anthracnose disease of chilli caused by *Colletotrichum gloeosporioides* OGC1. *3 Biotech* **2014**, *4*, 127–136. [CrossRef]
21. Slater, H.; Crow, M.; Everson, L.; Salmond, G.P. Phosphate availability regulates biosynthesis of two antibiotics, prodigiosin and carbapenem, in *Serratia* via both quorum-sensing-dependent and-independent pathways. *Mol. Microbiol.* **2003**, *47*, 303–320. [CrossRef]
22. Corkidi, G.; Balderas-Ruíz, K.A.; Taboada, B.; Serrano-Carreón, L.; Galindo, E. Assessing mango anthracnose using a new three-dimensional image-analysis technique to quantify lesions on fruit. *Plant Pathol.* **2006**, *55*, 250–257. [CrossRef]
23. Gerhardt, P.; Murray, R.; Wood, W.; Krieg, N. *Methods for General and Molecular Bacteriology*; American Society of Microbiology: Washington, DC, USA, 1994; pp. 21–42.
24. Lima, N.B.; Batista, M.V.D.A.; De Morais, M.A.; Barbosa, M.A.G.; Michereff, S.J.; Hyde, K.D.; Câmara, M.P.S. Five *Colletotrichum* species are responsible for mango anthracnose in northeastern Brazil. *Fungal Divers.* **2013**, *61*, 75–88. [CrossRef]
25. Sharma, G.; Kumar, N.; Weir, B.S.; Hyde, K.D.; Shenoy, B.D. The ApMat marker can resolve *Colletotrichum* species: A case study with *Mangifera indica*. *Fungal Divers.* **2013**, *61*, 117–138. [CrossRef]
26. Du, W.; Yao, Z.; Li, J.; Sun, C.; Xia, J.; Wang, B.; Ren, L. Diversity and antimicrobial activity of endophytic fungi isolated from *Securinega suffruticosa* in the Yellow River Delta. *PLoS ONE* **2020**, *15*, e0229589. [CrossRef]
27. Lapenda, J.C.; Silva, P.A.; Vicalvi, M.C.; Sena, K.X.F.R.; Nascimiento, S.C. Antimicrobial activity of prodigiosin isolated from *Serratia marcescens* UFPEDA. *World J. Microbiol. Biotechnol.* **2015**, *31*, 399–406. [CrossRef] [PubMed]
28. Basharat, Z.; Tanveer, F.; Yasmin, A.; Shinwari, Z.K.; He, T.; Tong, Y. Genome of *Serratia nematodiphila* MB307 offers unique insights into its diverse traits. *Genome* **2018**, *61*, 469–476. [CrossRef] [PubMed]
29. Han, K.I.; Patnaik, B.B.; Cho, A.R.; Lim, H.K.; Lee, J.M.; Jang, Y.G.; Jeong, Y.S.; Yoo, T.K.; Lee, G.S.; Han, M.D. Characterization of chitinase-producing *Serratia* and *Bacillus* strains isolated from insects. *Entomol. Res.* **2014**, *44*, 109–120. [CrossRef]
30. Veliz, E.A.; Martínez-Hidalgo, P.; Hirsch, A.M. Chitinase-producing bacteria and their role in biocontrol. *AIMS Microbiol.* **2017**, *3*, 689–705. [CrossRef] [PubMed]
31. Bowman, S.M.; Free, S.J. The structure and synthesis of the fungal cell wall. *Bioessays* **2006**, *28*, 799–808. [CrossRef]
32. Kalbe, C.; Marten, P.; Berg, G. Strains of the genus *Serratia* as beneficial rhizobacteria of oilseed rape with antifungal properties. *Microbiol. Res.* **1996**, *151*, 433–439. [CrossRef]
33. Sethi, S.; Datta, A.; Gupta, B.L.; Gupta, S. Optimization of cellulase production from bacteria isolated from soil. *Int. Sch. Res. Notices* **2013**, *2013*, 985685. [CrossRef]
34. Darshan, N.; Manonmani, H.K. Prodigiosin and its potential applications. *J. Food Sci. Technol.* **2015**, *52*, 5393–5407. [CrossRef] [PubMed]
35. Manas, N.H.A.; Yee, C.L.; Tesfamariam, Y.M.; Zulkharnain, A.; Mahmud, H.; Mahmod, D.S.A.; Fuzi, S.F.Z.M.; Azelee, N.I.W. Effects of oil substrate supplementation on production of prodigiosin by *Serratia nematodiphila* for dye-sensitized solar cell. *J. Biotechnol.* **2020**, *317*, 16–26. [CrossRef] [PubMed]
36. Grimont, P.A.; Grimont, F.; Starr, M.P. *Serratia* species isolated from plants. *Curr. Microbiol.* **1981**, *5*, 317–322. [CrossRef]

37. Zarei, M.; Aminzadeh, S.; Zolgharnein, H.; Safahieh, A.; Daliri, M.; Noghabi, A.; Ghoroghi, A.; Motallebi, A. Characterization of a chitinase with antifungal activity from a native *Serratia marcescens* B4A. *Braz. J. Microbiol.* **2011**, *42*, 1017–1029. [CrossRef]
38. Kurz, C.L.; Chauvet, S.; Andres, E.; Aurouze, M.; Vallet, I.; Michel, G.P.; Uh, M.; Celli, J.; Filloux, A.; De Bentzmann, S.; et al. Virulence factors of the human opportunistic pathogen Serratia marcescens identified by in vivo screening. *EMBO J.* **2003**, *22*, 1451–1724. [CrossRef]