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Postharvest Application of *Bacillus amyloliquefaciens* PMB04 Fermentation Broth Reduces Anthracnose Occurrence in Mango Fruit

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Abstract: Anthracnose is a destructive postharvest disease of mango fruit, especially after ripening. The application of antagonistic *Bacillus* spp. strains is one of many promising control strategies. *B. amyloliquefaciens* (PMB04) exhibits strong antagonistic activity against anthracnose in strawberry fruit, yet has not been explored for anthracnose control in mango fruit. Among seven tested rhizobacterial strains, PMB04 exhibited the strongest inhibition against *Colletotrichum gloeosporioides* (Cg) growth. PMB04 demonstrated better inhibitory activity against Cg mycelial growth than the fungicides pyraclostrobin and difenoconazole. A PMB04 fermentation broth (PMB04FB) was prepared and tested, and showed superior efficacy in the reduction of anthracnose disease severity in mangoes compared to a PMB04 suspension, difenoconazole, or pyraclostrobin. We further found PMB04FB increased the mortality ratio of Cg conidia. Our study demonstrates that treatment with PMB04FB is an effective control method for anthracnose in mango and represents a promising strategy for postharvest mango processing.

Keywords: agricultural management; antagonistic activity; biological control; fermentation broth; fungicides

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1. Introduction

The mango (*Mangifera indica* L.) is an economically important fruit tree often planted in tropical and subtropical countries. According to the FAO, the cultivated area of mangoes has increased each year from 2015 to 2019 [1]. In Taiwan, the annual planting area reached more than 10,000 hectares in the past decade, and "Irwin" is one of the most productive cultivars [2]. Mangoes are subjected to many diseases that affect the yield and quality of the fruit [3–8]. Anthracnose disease, caused by *Colletotrichum* spp., is one of the most devastating diseases on various cultivars of mango during pre- and post-harvest production worldwide [9]. To date, many strategies have been developed to control anthracnose in mango fruit. Common strategies for controlling postharvest diseases in mango fruit include using chemical fungicides before harvest and hot water treatment after harvest [10,11]. In Taiwan, many fungicides are recommended during growing seasons, such as thiram, azoxystrobin, trifloxystrobin, fluazinam, prochloraz, difenoconazole, metconazole, pyraclostrobin, and tebuconazole. However, no fungicides are currently recommended for post-harvest treatment [12]. In most fungicides, the mode of action (MOA) is inhibition of the respiration of pathogens (FRAC code: 11) or inhibition of the sterol biosynthesis in the membrane (FRAC code: 3) [13]. The MOA of these fungicides is highly specific to the

inhibition of the pathogen; however, due to the health concerns, alternative treatments must be developed to reduce the occurrence of mango anthracnose. Therefore, the application of antagonistic microorganisms to control mango anthracnose after harvest is a promising strategy. Previously, many reports have demonstrated that postharvest treatment with antagonistic bacteria is successful in reducing the occurrence of anthracnose in mango fruit [14–19]. However, reports on the application of fermentation broth with antagonistic microorganisms for postharvest fruit processing have seldom been discussed. In this study, we assess the antagonistic and biocontrol efficacy of *B. amyloliquefaciens* PMB04, as well as a fermentation broth prepared from the PMB04 strain (PMB04FB) against *C. gloeosporioides* (Cg) isolated from infected mango fruit. We evaluate the antagonistic activity and biocontrol efficacy of PMB04, PMB04FB, and two fungicides (difenoconazole and pyraclostrobin) for comparison, in addition to the combined treatment of PMB04FB with difenoconazole and pyraclostrobin for controlling mango fruit anthracnose. Our results provide evidence that PMB04FB treatment is an effective anthracnose control strategy for postharvest mangoes.

2. Materials and Methods

2.1. Growth Conditions and Suspension Preparation of Microorganisms

The pathogen causing anthracnose was isolated from infected mango fruit and confirmed as *Colletotrichum gloeosporioides* isolate PMBMCG-B2. Before the experiment, the isolate was cultured on a potato dextrose agar (PDA) plate at 28 °C for 7 days. To maintain the pathogen, a piece of mycelium with a diameter of 1 cm was cut from a culture medium with fungal hyphae and transferred to a new PDA plate. To obtain the spore suspension as an inoculum, the plates of PMBMCGB2 were flooded with sterilized distilled water and spores were harvested to adjust to 1×10^5 spores mL^{-1}. Seven *Bacillus* spp. strains isolated from a soil sample from fields of vegetable crops (PMB03, PMB04, PMB09, PMB11, PMB13, PMB20, and PMB25) were used in this study [20]. All *Bacillus* spp. strains were cultured on a nutrient broth agar (NA) plate and incubated at 28 °C for 48 h. A bacterial suspension was prepared by flooding the bacterial cells from the culture on the NA plate with sterilized distilled water and adjusting its OD_{600} to 0.3 (approximately 10^8 CFU mL^{-1}) for further experiments.

2.2. Inhibitory Assay of Bacillus spp. *Strains and Fungicides against Colletotrichum Gloeosporioides*

To evaluate the antagonistic effect of rhizobacterial strains, an assay on the inhibitory effect against the *C. gloeosporioides* isolate PMBMCG-B2 was performed as previously described [21]. First, a 0.8 cm diameter mycelial disc was placed in the middle, and 20 µL of bacterial suspensions of *Bacillus* spp. strains were applied to the glass ring 2 cm away from the middle of the PDA-NA dual medium and incubated at 28 °C for 4 days. To test the inhibitory effect of fungicides against *C. gloeosporioides*, two commonly used fungicides in the pre-harvest treatment of mangoes, difenoconazole and pyraclostrobin (Lanlix, Taiwan), were also analyzed in this study. Similarly, 20 µL of difenoconazole (16.6 mg ai mL^{-1}) or pyraclostrobin (7.8 mg ai mL^{-1}) dilution based on the recommended concentration was spotted in the glass ring on PDA-NA dual medium and incubated at 28 °C for 4 days. The inhibitory ratio was calculated as follows: inhibitory ratio (%) = RC (mm) − RT (mm)/RC (mm) × 100. RC indicates the radial growth of the mycelium in the treatment with sterilized water as the negative control, and RT indicates the radial growth in the treatment with the bacterial suspension of each rhizobacterial strain.

2.3. Control Efficacy Assay of Postharvest Treatments on Mango Fruit to Anthracnose Disease

To assess whether the *B. amyloliquefaciens* strain PMB04 and the two fungicides were able to control mango anthracnose, inoculation of the harvested fruit was carried out. The plant materials used in this study were mango fruit (*Mangifera indica* L. "Irwin") within 24 h after harvest, which were kept at room temperature before the assay. Before inoculation, the mango fruits were first wiped with a paper towel containing 75% alcohol.

The surfaces of the sterilized fruit was soaked in the PMB04 bacterial suspension or dilution of each fungicide for 1 min and air-dried for 30 min. Then, 10 μL of a *C. gloeosporioides* spore suspension at 1×10^5 conidia mL^{-1} was applied to the fruit by dipping without mechanical injuries, and the treated fruit were incubated in a plastic box at 30 °C for 7 days. The disease indexes of the fruit were divided into 5 scales (0 indicated healthy fruit; 1 indicated that the average diameters of symptoms were under 3.0 mm, 2 indicated 3.1–6.0 mm average diameters of symptoms, 3 indicated 6.1–9.0 mm average diameters of symptoms, 4 indicated 9.1–12.0 mm average diameters of symptoms, and 5 indicated average diameters of symptoms of over 12.0 mm). The disease incidence of each treatment was calculated with five fruit as one repeat, and four repeats were carried out for all treatments. The disease severity was calculated using the following formula: $[(0 \times N0 + 1 \times N1 + 2 \times N2 + 3 \times N3 + 4 \times N4 + 5 \times N5/(5 \times N)] \times 100\%$.

2.4. Preparation of Liquid Fermentation Broth of Bacillus amyloliquefaciens PMB04

The liquid fermentation broth of *B. amyloliquefaciens* PMB04 was used to perform the control efficacy assay. To prepare the fermentation broth, the 2% (*v*/*v*) overnight culture of PMB04 in Luria–Bertani (LB) broth was transferred into 400 mL of fresh LB broth and incubated at 37 °C for 6 h. The culture was then added into a 30 L automatic sterilized fermentation tank (BTF-B30L, Biotop Process & Equipment Inc., Nantou County, Taiwan) containing 20 L of sterilized fermentation medium composed of 3% granulated sugar and 1% yeast powder (Sunright, New Taipei City, Taiwan). The fermentation was performed at 37 °C under 120 rpm for 5 days, and the final fermentation broth (PMB04FB) was stored at 4 °C for further experiments.

2.5. Inhibition Assay of PMB04FB on C. gloeosporioides Conidia

A 200-fold dilution of PMB04FB and bacterial suspension were used to assess the germination and mortality ratios of *C. gloeosporioide* conidia. The germination ratio assay was performed by applying 100 μL of dilution to 200 μL of 0.1% glucose containing 10^5 conidia. The mixture was cultured in a 25 °C growth chamber and assessed at 12 h post-treatment. The mortality ratio assay was carried out by staining the mixture with 10 μL of 0.04% trypan blue (Sigma, St. Louis, MO, USA) for 45 min. There were three replicates of each treatment, with twenty conidia serving as one replicate. To evaluate the status of apoptosis on conidia, a 200-fold dilution of PMB04FB was centrifuged under 10,000× *g* for 10 min at 4 °C. Then, the supernatant was filtered with a 0.22 μm filter, 100 μL of the filtrate was applied to 200 μL of the conidia suspension as described above, and the mixture was strained with FITC-Annexin V and Propidium Iodide according to the manual (Takara Bio USA, Inc. San Jose, CA, USA). The stained conidia were observed under a fluorescent microscope (Leica Microsystems GmbH, Wetziar, Germany) at 465–495 nm/515–555 nm (excitation/emission) and 565–595 nm/615–655 nm to evaluate the initiation of apoptosis and cell death, respectively.

2.6. Control Efficacy Assay of Combined Fermentation Broth and Fungicide against Anthracnose Disease

To determine whether the fermentation broth was effective in reducing the occurrence of anthracnose in the postharvest treatment of the fungicides, the fermentation broth was mixed with different fungicides at full and half dose in this assay. Before preparing the mixture, a 200-fold dilution of PMB04FB was prepared as the fermentation broth treatment. Difenoconazole was then added into the diluted fermentation broth or sterilized water to obtain a mixture with final concentrations of 16.6 mg ai mL^{-1} (1 × DA) and 8.3 mg ai mL^{-1} (1/2 × DA) difenoconazole. Similarly, 1 × PS and $\frac{1}{2}$ × PS were prepared with pyraclostrobin at 7.8 mg ai mL^{-1} and 3.9 mg ai mL^{-1} of the final concentration, respectively. The disease severity was calculated with five fruit as one repeat, and four repeats were carried out for all treatments.

2.7. Inhibitory Effect of Fungicides on the Growth of Bacillus amyloliquefaciens PMB04

To analyze whether the fungicides used in this study affected the growth of *B. amyloliquefaciens* PMB04, the change in the OD value in the liquid medium was used for evaluation. Before the assay, the overnight culture of PMB04 in NB broth was transferred into 5 mL of fresh NB broth and incubated at 37 °C for 8 h as the initial culture. Subsequently, 1 mL of the culture was added to 50 mL of fresh NB broth containing 1 × DA, $\frac{1}{2}$ × DA, 1 × PS, or $\frac{1}{2}$ × PS. After a 24 h incubation at 37 °C under 200 rpm, the OD values at 600 nm were measured to evaluate the inhibitory effect of PMB04 on bacterial growth. Each treatment was replicated five times.

2.8. Statistical Analysis

Statistical analysis was performed using SPSS Statistics software for Windows, version 25 (IBM Corp, Armonk, NY, USA). Analysis of variance (ANOVA) was used to assess differences between the treatments for all the assays to obtain *F* values. Post hoc tests (Tukey's HSD) were performed to compare means between treatments to obtain *p* values.

3. Results

3.1. Inhibitory Effect of Bacillus spp. *Strains and Fungicides against Colletotrichum gloeosporioides*

To test the inhibitory effects of seven *Bacillus* spp. strains and two fungicides, the inhibitory zones against mycelium growth of *C. gloeosporioides* were measured. The results revealed that PMB03, PMB04, and PMB09 produced inhibitory zones on the mycelial growth of *C. gloeosporioides* PMBMCG-B2 with inhibition ratios of 14.7%, 37.5%, and 21.7%, respectively. Among the inhibitory rates of the strains, the inhibitory rate of PMB04 was significantly higher than those of PMB03 and PMB09 (Figure 1A). We assessed PMB04 and two fungicides, difenoconazole and pyraclostrobin, for the inhibition of PMBMCG-B2 mycelium growth. The inhibitory ratios of PMB04 (35.4%) and pyraclostrobin (33.3%) were comparable to one another and significantly higher than those of difenoconazole (11.2%) (Figure 1B).

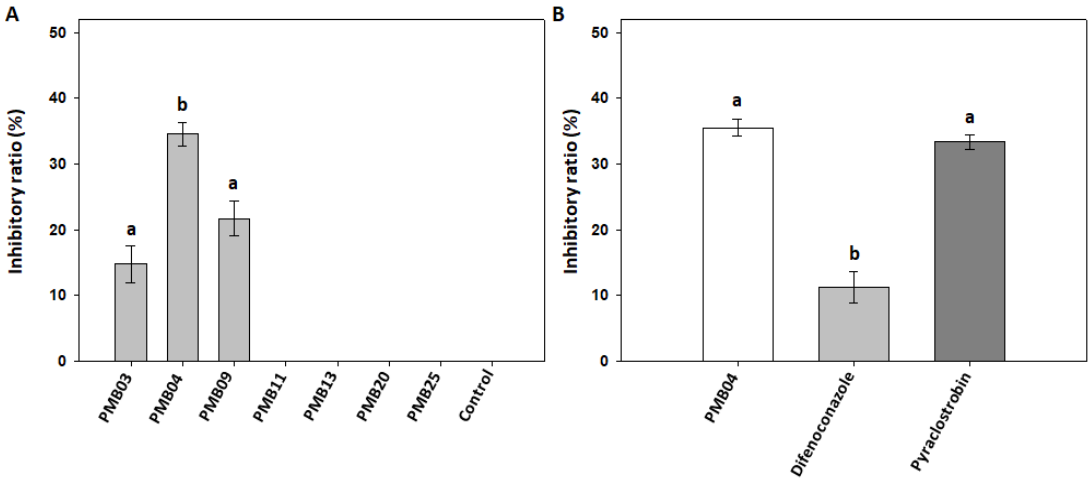

Figure 1. Inhibitory effects of *Bacillus amyloliquefaciens* strains and fungicides against mycelial growth of *Colletotrichum gloeosporioidese*. (**A**) shows the mycelial growth inhibition rate caused by the *B. amyloliquefaciens* strains on PDA-NA plates. The inhibition rates were calculated as described in the materials and methods. Sterilized water was used as a negative control. (**B**) reveals the inhibitory rate against mycelial growth caused by *B. amyloliquefaciens* PMB04, difenoconazole, and pyraclostrobin. In the assay, 20 µL of difenoconazole and pyraclostrobin at 16.6 mg ai mL^{-1} and 7.8 mg ai mL^{-1}, respectively, were applied to the paper disc. Data represent mean ± SD. The different letters above the columns indicate significant differences based on Tukey's HSD test (The *F* value for panels (**A**,**B**) were 16.7 and 64.5, respectively; $p < 0.05$).

3.2. Anthracnose in Mango Fruit Controlled by Bacterial Suspension of *Bacillus amyloliquefaciens* PMB04

The effect of the *B. amyloliquefaciens* PMB04 bacterial suspension on the post-harvest control of anthracnose in mango fruit was analyzed. The results showed that the treatment of mango fruit with a bacterial suspension of *B. amyloliquefaciens* PMB04 reduced anthracnose disease incidence to 70.0% at 7 days post-inoculation, compared with control inoculation of PMBMCG-B2 alone (90.0%) (Figure 2A). Compared with the disease severity of mango anthracnose caused by the control PMBMCG-B2 alone (58.0%), the disease severity decreased to 37% following treatment with the *B. amyloliquefaciens* PMB04 bacterial suspension (Figure 2B). In the observation of symptoms, the *B. amyloliquefaciens* PMB04 bacterial suspension treatment resulted in only mild necrosis symptoms on diseased fruit (Figure 2C).

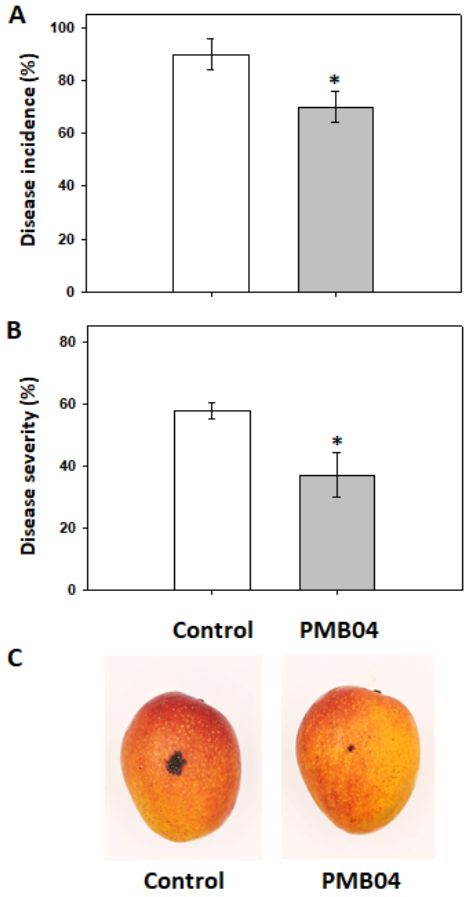

Figure 2. Application of *Bacillus amyloliquefaciens* PMB04 bacterial suspension against anthracnose disease in mango fruit. (**A**,**B**) show the disease incidence and disease severities at 7 days post-inoculation. The surfaces of sterilized fruit were soaked in the bacterial suspension of *B. amyloliquefaciens* PMB04 for 1 min as the treatment. The control was performed with sterilized water as a negative control. A spore suspension of *Colletotrichum gloeosporioides* was then spotted on the surface of the fruit for inoculation. Data represent mean ± SD. The asterisks indicate a significant difference compared with the control treatment, as assessed using a *t*-test ($p < 0.05$). (**C**) shows the symptom appearances of different treatments on the fruit.

3.3. The Inhibitory Effect of PMB04FB on Germination and Mortality of *C. gloeosporioides* Conidia

Conidia germination and mortality ratios were used as indicators to determine whether PMB04FB increases antifungal activity. The PMB04 bacterial suspension reduced the conidia germination ratio (66.7%) compared to the control (93.3%). PMB04FB further reduced the conidia germination ratio to 5.0% (Figure 3A). Both the bacterial suspension and PMB04FB

caused cell death of conidia, with PMB04FB causing 55.0% cell death (Figure 3B). In the analysis of the apoptotic state of conidia treated with PMB04FB filtrate, the fluorescent signal of intracellular nucleic acid was observed at 12 h post-treatment, while the fluorescent signal of phosphatidylserine on the outer side of the membrane was not observed (Figure 3C).

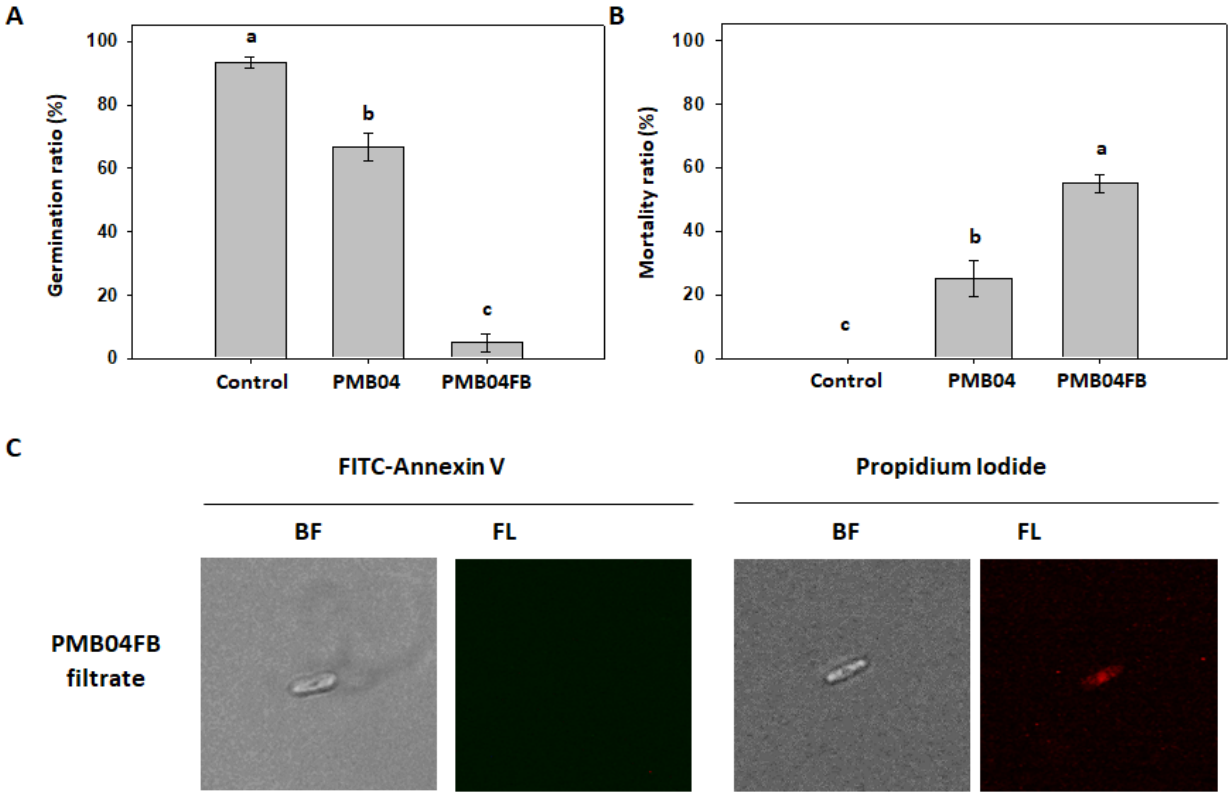

Figure 3. *B. amyloliquefaciens* PMB04 inhibition of *Colletotrichum gloeosporioides* conidia. (**A**,**B**) show the germination and mortality ratios of the bacterial suspension (PMB04) and 200-fold dilution of PMB04 fermentation broth (PMB04FB) on conidia at 12 h post treatment, respectively. Sterilized water was used as a negative control. Data represent mean ± SD, and different letters indicate significant differences by treatment based on Tukey's HSD test (the *F* value for germination ratio and mortality ratio were 54.0 and 201.0, respectively. $p < 0.05$). (**C**) shows the treated conidia under bright field (BF) and fluorescence (FL). The conidia were stained with FITC-annexin V and propidium iodide in the FL images.

3.4. Anthracnose in Mango Fruit Controlled by Fermentation Broth of Bacillus amyloliquefaciens PMB04 and Fungicides

To compare the effects of PMB04FB and fungicides on anthracnose occurrence, mango fruits were soaked in different treatments before PMBMCG-B2 inoculation. The PMB04 bacterial suspension, PMB04FB, difenoconazole, and pyraclostrobin effectively reduced the development of anthracnose symptoms (Figure 4A). No symptoms were observed in most fruits treated with PMB04FB. Compared to the disease incidence of mango anthracnose caused by PMBMCG-B2 without treatment (86.6%), the disease severity following treatment was reduced to 55.0% with PMB04 bacterial suspension, 13.3% with PMB04FB, 44.4% with difenoconazole, and 48.5% with pyraclostrobin (Figure 4B). Similarly, compared to the disease severity of mango anthracnose caused by PMBMCG-B2 without treatment (63.7%), the disease severity following treatment was reduced to 37.5% with PMB04 bacterial suspension, 15.0% with PMB04FB, 31.6% with difenoconazole, and 29.0% with pyraclostrobin (Figure 4C). PMB04FB demonstrated the highest control efficacy in terms of disease incidence and severity among all treatments (Figure 4).

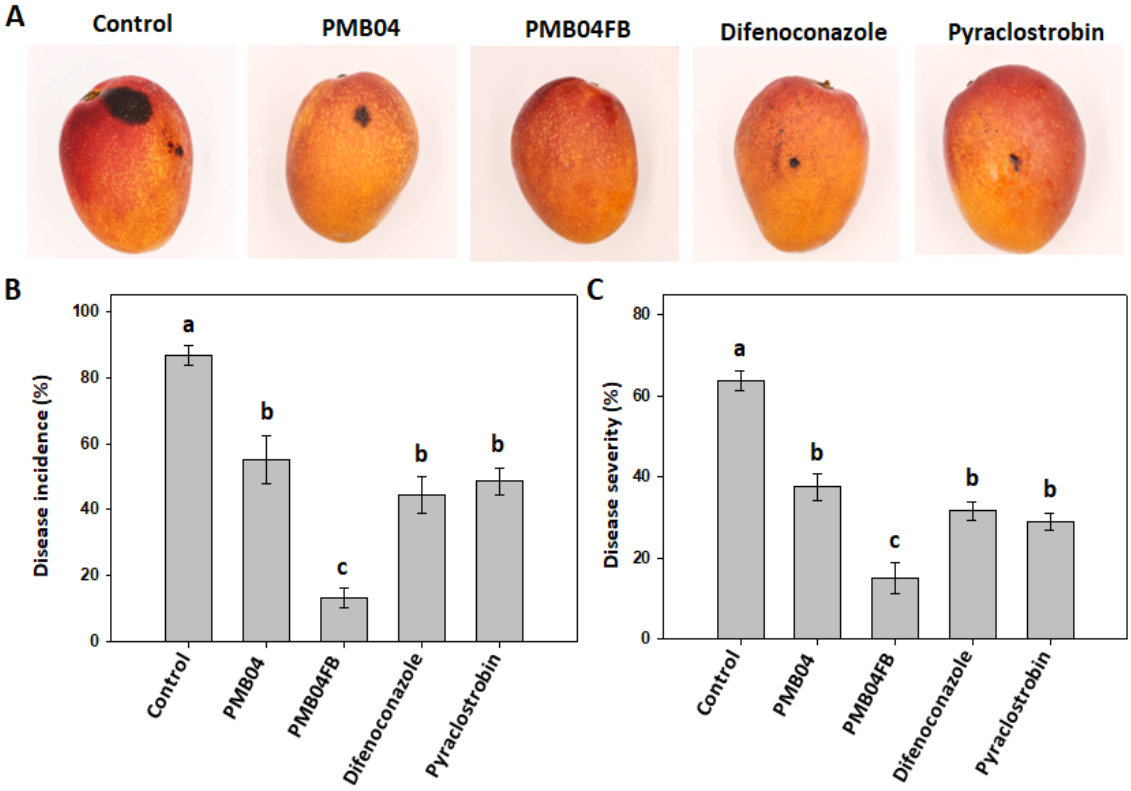

Figure 4. Application of *Bacillus amyloliquefaciens* PMB04 fermentation broth and fungicides against anthracnose disease in mango fruit. (**A**) shows the symptom appearances of the different treatments on fruit at 7 days post-inoculation. The surfaces of sterilized fruit were soaked in the 200-fold dilution of *B. amyloliquefaciens* PMB04 fermentation broth (PMB04FB), 16.6 mg ai mL^{-1} difenoconazole, or 7.8 mg ai mL^{-1} pyraclostrobin for 1 min as the treatment. The control was performed with sterilized water as a negative control. A spore suspension of *Colletotrichum gloeosporioides* was then spotted on the surface of the fruit for inoculation. (**B**,**C**) show the disease incidence and disease severity at 7 days post-inoculation, respectively. Data represent mean ± SD. Different letters above the columns indicate significant differences based on Tukey's HSD test (The *F* value for disease incidence and disease severity were 46.0 and 43.8, respectively. $p < 0.05$).

3.5. Control of Anthracnose with Combined Fermentation Broth and Fungicide

To uncover whether the combined treatment of fermentation broth and fungicides improved the control efficacy against anthracnose in mango fruit after harvest, we mixed full and half doses of the recommended concentration of fungicide with the fermentation broth and analyzed the disease outcomes. The results showed that PMB04FB treatment significantly reduced the disease severity of anthracnose from 63.7% to 15.0%. In the combined treatment of PMB04FB with pyraclostrobin, the disease severity decreased to 18.0% and 32.0% in the treatments with the full and half dose, respectively. The full and half doses of difenoconazole combined with PMB04FB reduced the disease severity to 36.8% and 46.0%, respectively (Figure 5A). Among these treatments, PMB04FB alone and PMB04FB combined with the full dose of pyraclostrobin yielded the best control efficacy against anthracnose. In most of the combined treatments with difenoconazole and pyraclostrobin, the disease severity was higher than that in the treatment with PMB04FB alone. The observations of symptom development revealed that most fruits were symptomless when treated with PMB04FB alone and PMB04FB combined with a full dose of pyraclostrobin on the 7th day post-inoculation. However, most of the fruit treated with PMB04FB combined with fungicides presented only mild symptoms (Figure 5B).

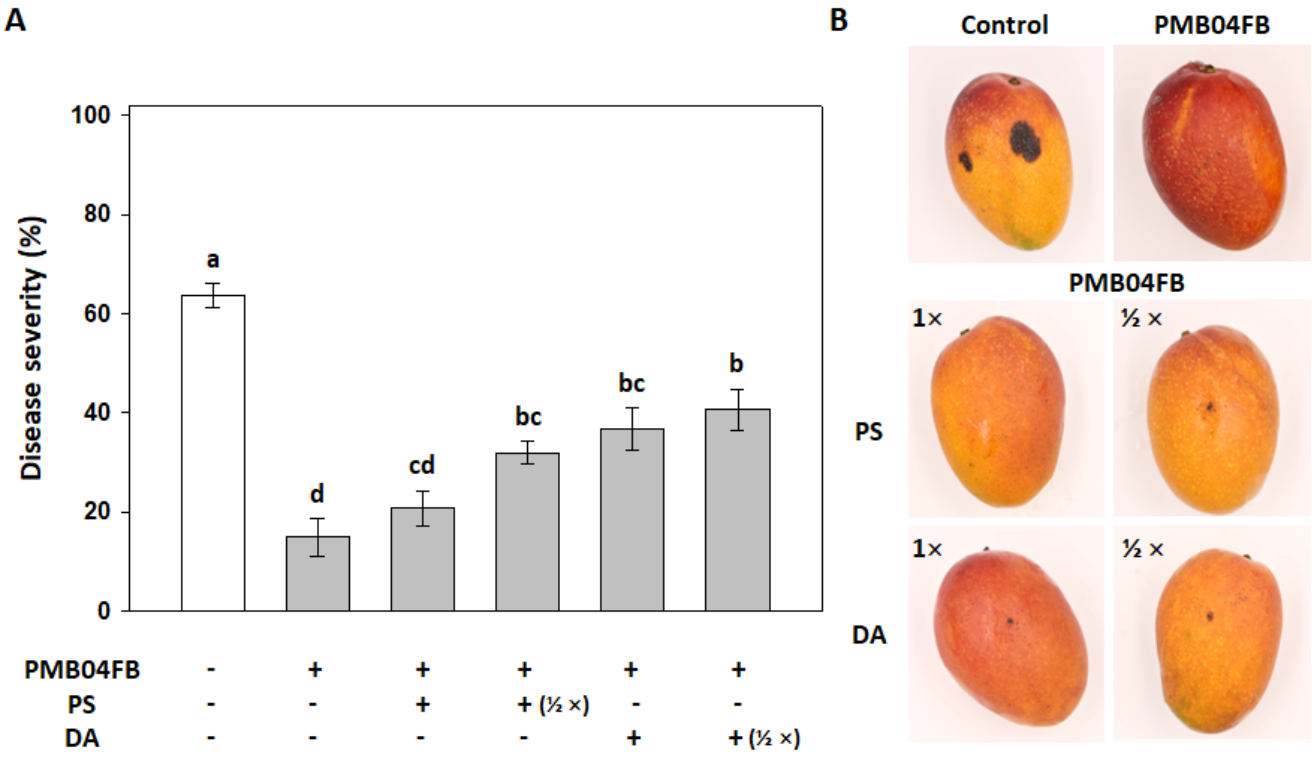

Figure 5. Control of combined *Bacillus amyloliquefaciens* PMB04 fermentation broth and fungicides against anthracnose disease in mango fruit. (**A**) shows the disease severity at 7 days post-inoculation. The surfaces of sterilized fruit were soaked in the 200-fold dilution of *B. amyloliquefaciens* PMB04 fermentation broth (PMB04FB) containing a full (1×) or half dose (1/2×) of pyraclostrobin (PS) or difenoconazole (DA) for 1 min as the treatment. The symbols "+" and "−" indicate the inclusion and exclusion, respectively, of the treatments. A negative control was performed with sterilized water alone. A spore suspension of *Colletotrichum gloeosporioides* was then spotted on the surface of the fruit for inoculation. Data represent mean ± SD. Different letters above the columns indicate significant differences based on Tukey's HSD test ($F = 36.9$; $p < 0.05$). (**B**) displays the symptom appearances of the different treatments on fruit.

3.6. Growth Inhibition of Bacillus amyloliquefaciens PMB04 by Fungicides

To test whether the combined treatments of difenoconazole and pyraclostrobin affected the growth of *B. amyloliquefaciens* PMB04, a growth evaluation was carried out in nutrient broth by applying the fungicides at different dosages. The results showed that both difenoconazole and pyraclostrobin significantly inhibited the growth of *B. amyloliquefaciens* PMB04 in nutrient broth, and its growth was also significantly inhibited with only half of the fungicide dose (Figure 6).

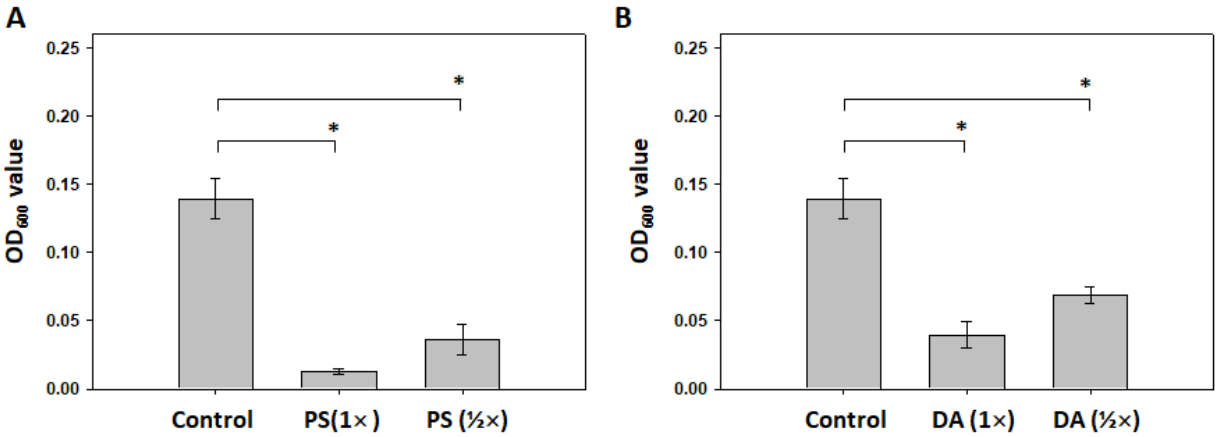

Figure 6. Inhibitory effect of fungicides against the growth of *Bacillus amyloliquefaciens* PMB04. To perform the assay, the overnight culture of PMB04 was transferred into 5 mL of fresh NB broth containing a full (1×) or half dose (1/2×) of pyraclostrobin (PS) or difenoconazole (DA). The OD values of bacterial culture at 600 nm were measured at 24 h post-treatment to check the growth of *B. amyloliquefaciens* PMB04. (**A**,**B**) show the OD values under treatment with PS and DA, respectively. Sterilized water was used as a negative control. Data represent mean ± SD, and the asterisks indicate significant differences compared with the control treatment assessed using a *t*-test ($p < 0.05$).

4. Discussion

Mango is an economically important fruit crop in many tropical and subtropical countries, but production is limited by anthracnose disease caused by *Colletotrichum* spp. [9]. With the aim of preventing anthracnose disease occurrence in postharvest mangoes, we explored whether *Bacillus* spp. could directly and effectively inhibit Cg survival. Of the *Bacillus* spp. screened, PMB04, which we previously found to control the occurrence of anthracnose in strawberry fruit [21], showed the strongest antagonistic ability (Figure 1A). *Bacillus* spp. produce various biologically active compounds that inhibit the growth of various pathogens [22–24]. It is possible that the differences in the compounds produced by *Bacillus* spp. are responsible for their differences in inhibitory efficacy against Cg, which may explain why PMB04 produces biologically active compounds that are effective against multiple Cg strains.

Multiple reports have revealed that the number of bacteria and the production of important compounds produced by *B. amyloliquefaciens* can be increased by applying fermentation technology [25–27]. In this study, a liquid PMB04 fermentation formula reduced anthracnose disease incidence and severity in mangoes (Figure 4). It is speculated that the fermentation process may increase the antagonistic compounds produced by PMB04. PMB04FB effectively inhibited Cg spore germination and caused Cg conidia shrinkage and deformation. From the results of Annexin-V staining, we speculate that the cell death caused by PMB04FB was not related to apoptosis. Since fermentation technologies can increase the amount and type of biological compounds produced, the specific antagonistic substance produced during the PMB04 fermentation process to inhibit Cg conidia germination and increase Cg conidia mortality is still unknown.

Fungicides, such as difenoconazole and pyraclostrobin, are viewed as simple, effective treatments against anthracnose during the growing season [28,29]. However, there is no consensus on the application of biocontrol agents in combination with fungicides. Although all treatment combinations significantly reduced the anthracnose disease severity compared to the control, the combined treatment of PMB04FB with difenoconazole or pyraclostrobin did not enhance anthracnose control in mangoes. It is possible that difenoconazole and pyraclostrobin compromise PMB04, therefore reducing the overall efficacy of PMB04FB against anthracnose. Nevertheless, since the MOAs for difenoconazole and pyraclostrobin are specific to the inhibition of sterol biosynthesis and respiration of fungi and are recommended for application two weeks before harvest, it would be worthwhile to

explore the combined effect of chemical fungicide application during the growing season with PMB04FB for postharvest mango fruits.

We demonstrated that the postharvest treatment of mango fruit with a PMB04 fermentation broth (PMB04FB) effectively reduced the occurrence of anthracnose. The application of the developed PMB04FB is a promising strategy for postharvest processing and management of anthracnose to maintain crop quality and avoid postharvest losses in mangoes.

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