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Effect of lactic acid bacteria and *Bacillus* on anthracnose disease in postharvest papaya fruit

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Abstract. *Thi QVC, Dung TQ, Huynh NTN, Truc NT, Thuy NP. 2023. Effect of lactic acid bacteria and* Bacillus *on anthracnose disease in postharvest papaya fruit. Biodiversitas 24: 5883-5894.* Anthracnose disease of papaya fruit caused by *Colletotrichum* (isolate TD1), identified by 5.8S rRNA sequence homology, is a main obstacle in papaya fruit, influencing fruit quality and minimizing shelf life. Therefore, to diminish the disease and sustain the fruit quality, the impact of Lactic Acid Bacteria (LAB) and *Bacillus* on the growth of anthracnose disease and fruit quality was investigated *in vitro* and *in vivo*. Furthermore, 13 LAB and 12 *Bacillus* isolates were collected from traditional fermented vegetables and papaya rhizospheric soils. The results showed that 13 LAB isolates and 6 isolates of *Bacillus* were inhibitory against *Colletotrichum* in an *in vitro* test. Two isolates of LDC11 and BHL21 with the highest antifungal activity were selected to evaluate their effect on *Colletotrichum* growth and papaya fruit quality under *in vivo* conditions. These findings indicated that the isolates LDC11 and BHL21 at a density of 10^6 CFU/mL reduced the disease incidence and severity. The LAB and *Bacillus* treated papaya fruit increased a number of parameters, such as weight loss, TSS, and L*, a*, and b* values. However, vitamin C content, TA, and fruit firmness were reduced compared to the control. The research shows a potential applications of LAB and *Bacillus* in the postharvest preservation of papaya fruit. To our knowledge, this is the first study to apply *Bacillus* and LAB bacteria to control diseases in postharvest papaya in Vietnam.

Keywords: Anthracnose, *Colletotrichum*, lactic acid bacteria, postharvest disease

INTRODUCTION

Papaya (*Carica papaya* L.) is a crucial fruit in many tropical and subtropical countries worldwide. Papaya fruit contains vitamins A, E, and C and antioxidant compounds (Alara et al. 2022). However, this fruit is susceptible to fungal diseases, especially anthracnose caused by *Colletotrichum* spp. during the postharvest stage (Gabrekiristos and Dagnew 2020). The fungus that attacks fruit usually forms brown spots on the peel, penetrating the fruit flesh and causing it to rot, become perishable, and suffer damage during storage (Saini et al. 2017). According to Hodges et al. (2011), microbial attack is responsible for approximately 30% of postharvest losses of fruits and vegetables in developing nations. Significant losses can be attributable to fungal invasions during storage and transit (Liu et al. 2013).

Until now, farmers mainly used fungicides to prevent and treat fungal diseases on fruit, including anthracnose caused by *Colletotrichum* spp. in pre- and postharvest papaya fruit (Qadri et al. 2020). The abuse of chemical drugs pollutes the environment, affects consumers' health, and reduces the value of exported agricultural products (Igbedioh et al. 1991). Heat treatment and antifungal drugs are the two most widely used ways to prevent postharvest fungal diseases (Feliziani and Romanazzi 2013). The quality of preserved fruit is not uniform and is frequently harmed by heat because managing heat dispersion during processing is a challenging and unresolved problem for heat treatment methods. On the contrary, using antifungal drugs poses health risks to consumers, environmental pollution, and many pathogens develop pesticide resistance (Vitiello et al. 2023). Furthermore, natural foods devoid of chemical residues are also demanded by consumers (Valencia-Chamorro et al. 2011). As a result, numerous studies have been conducted to look for alternative preservation solutions to minimize harmful effects during storage and preserve food quality (Saucedo-Pompa et al. 2007; Saucedo-Pompa et al. 2009).

In recent years, the trend of postharvest preservation of agricultural products by biological means, such as the use of microorganisms and/or their metabolic products to avoid spoilage and lengthen the shelf food life, has attracted the attention of many scientists (Zubrod et al. 2019). The use of edible coatings, particularly on highly perishable products, is one of the suggested strategies for extending the shelf life of fruit (Yadav et al. 2022). There have also been reports of the biological control of postharvest infections using antagonistic agents, such as bacteria or yeasts, that are inhibit to pathogenic microbes (Liu et al. 2013; Buchholz et al. 2018; Verma et al. 2022). Many previous research results have shown the inhibit influence of LAB and *Bacillus* on many fungal diseases on postharvest fruits (Ghosh et al. 2015; Neelima et al. 2016;

Lastochkina et al. 2019). According to Belkacem-Hanfi et al. (2014), LAB species from stored wheat samples showed high inhibitory effects against *Aspergillus carbonarius*. In a study by Cavaglieri et al. (2005) revealed that ten *Bacillus* strains could inhibit *Fusarium verticillioides* growth, a soil-borne pathogen in maize. The study by De Simone et al. (2021) showed that the cell-free supernatants of two *Lactiplantibacillus plantarum* strains contrasted the growth of *Botrytis cinerea*, responsible for the cause of fruit and vegetable spoilage phenomena in postharvest kiwi fruits. Until now, however, little information has been noticed about the isolation and antifungal activity of LAB and *Bacillus* against *Colletotrichum* spp., which seriously causes anthracnose disease in postharvest fruits in Vietnam. In general, most previous studies have focused on determining the antifungal activity of LAB and *Bacillus* under *in vitro* conditions, while the control of fungal diseases and postharvest quality of papaya fruit have not been studied. Therefore, this goal was to isolate and evaluate the influence of LAB and *Bacillus* on the *Colletotrichum* growth and quality of postharvest papaya fruits.

MATERIALS AND METHODS

Sampling, isolation, and identification of *Colletotrichum* spp.

Colletotrichum spp. was isolated from anthracnose papaya fruit with typical symptoms such as a large, sunken circular wound with a dark brown to black color. The infected papaya fruits were collected from different regions of Vinh Long Province, the Mekong Delta, Vietnam. The fungi were isolated according to the tissue isolation method of Cai et al. (2009). In brief, the diseased papaya fruit was cleaned with a running tap and distilled water many times. Next, the diseased parts were cut into 1 x 1 cm pieces and sterilized with 70% ethanol for 90 sec. The samples were then rinsed three times with sterile distilled water. After that, these segments were placed aseptically in a potato dextrose agar plate (PDA: 200 g/L potato, 20 g/L glucose, and 20 g/L agar, pH = 7.0). Finally, the dishes were incubated at 30ºC for 3-5 days. The fungus *Colletotrichum* was identified based on morphological characteristics (Barnett and Barry 1972), PCR technique, and sequencing of the 5.8S gene fragment with primers ITS1: 5′-TCC GTA GGT GAA CCT GCG G-3′ and ITS4: 5′-TCC TCC GCT TAT TGA TAT GC-3′ (White et al. 1990). PCR products were purified and sequenced by Macrogen Company, Korea (www.macrogen.com).

The isolated fungi were tested the pathogenicity by inoculating experiments on healthy fruits (Jimenez et al. 1993; Marin et al. 1996). In brief, healthy and uninjured fruits were washed with running water many times. The fruits were then sterilized on their surfaces using 70% ethanol and rinsed thrice with sterile distilled water. Next, the fruits were inoculated with the fungus *Collectotrichum* at a 10^5 spore/mL concentration. The inoculated fruits were kept with wet tissue to maintain humidity and incubated in a dark place. Finally, the pathogen was reisolated and identified from the diseased portion to prove Koch's postulates.

Sample collection, isolation, and identification of LAB and *Bacillus*

LAB isolates were isolated from fermented vegetables and purchased at traditional markets in Vinh Long province of the Mekong Delta, Vietnam. LAB was isolated, according to Magnusson et al. (2003). Briefly, 10 mL or 10 g of samples were added to the 90 mL sterile water. Then, the mixture was serially diluted to 10^{-6}. Finally, the sample from each dilution was spread on MRS (De Man et al. 1960) with the following compositions: 20 g/L glucose, 10 g/L peptone, 2 g/L ammonium citrate, 5 g/L yeast extract, 10 g/L meat extract, 5 g/L sodium acetate, 0.05 g/L manganese sulfate tetrahydrate, 5 g/L dipotassium phosphate, and 0.1 g/L magnesium sulfate heptahydrate (pH = 6.5) and incubated at 37ºC for 24-48 hours.

Bacillus bacteria was isolated from papaya rhizospheric soils in Vinh Long province of the Mekong Delta, Vietnam. Bacteria were isolated, according to Ashwini and Srividya (2014). Briefly, 10 g rhizospheric soils were diluted in 90 mL sterile water and heated at 80°C for 20 min. Then, the mixture was serially diluted to 10^{-6}. A sample from each dilution was then streaked on nutrient agar (NA) with the following compositions: 0.5 g/L peptone, 0.5 g/L sodium chloride, and 0.2 g/L yeast extract (pH = 7.0) and incubated at 37°C for 24 h.

LAB and *Bacillus* were identified with morphological and biochemical characteristics such as Gram staining and spore staining, oxidase, and catalase reactions (Harrigan and McCance 1976) and identified by PCR reaction and 16S rRNA gene fragment sequencing with primers 27F: 5'-AGA GTT TGA TCM TGC TCA G-3' and 1492R: 5'-TAC GGY TAC CTT GTT ACG ACT T-3' (Heuer et al. 1997).

Antifungal activity of isolated bacterial isolates against *Colletotrichum*

The mycelium-and-spore antagonistic method was used to test the antifungal activity of LAB and *Bacillus* against the fungus *Colletotrichum*.

Antifungal activity of LAB isolates against *Colletotrichum*

The dual culture method (Lahlali et al. 2020) evaluated mycelial growth inhibition with minor modifications. Briefly, bacteria were inoculated into two 2 cm lines on PDA agar plates. Next, using a cork borer, a mycelial plug (0.5 x 0.5 mm) was cut and placed within bacterial bands in the center of the plate. The inhibitory activity was recorded after five to seven days of incubating at 30°C. Plates with only mycelial plugs were used as a negative control.

The dual culture overlay method was used to detect the spore inhibitory activity of LAB against *Colletotrichum* (Magnusson and Schnürer 2001). LAB isolates were inoculated in two parallel lines (the length of line is 2 cm) on MRS agar plates and anaerobically incubated at 30°C for 24 h. The plates were overlaid with 2 mL of PDA medium (0.7% agar) supplemented with *Colletotrichum*'s spores at 10^6 spores/mL density. The plates were incubated

at 37°C for 48 hours. The inhibition zone diameter (d) was recorded and scaled as follows: d ≤ 2 mm: weak activity; 21 mm ≤ d ≤ 40 mm: average inhibition; 41 mm ≤ d ≤ 60 mm: strong inhibition; and d > 61 mm: very strong activity (Muhialdin et al. 2018).

Antifungal activity of *Bacillus* isolates against *Colletotrichum*.

The mycelium inhibitory activity of *Bacillus* against *Colletotrichum* was tested by the dual culture method described above. In this study, the agar well diffusion method was used to evaluate the spore inhibitory activity of *Bacillus* (Dhanasekaran et al. 2012). In brief, *Bacillus* bacteria were cultured in nutrient broth (NB) medium for 24 h. Then, the culture solution was centrifuged at 10,000 rpm, at 4°C for 10 min, and collected the supernatant. The fungal suspension (100 µL) with *Colletotrichum*'s spores was spread onto the agar surface at 10^6 spores/mL density. Next, a 5 mm cork borer was used to perforate the well on PDA agar plates. Then, the supernatant (80 µL) was added to the wells, and the plate was incubated at 30°C for 24 h.

Effects of LAB and *Bacillus* on the growth of anthracnose and quality of postharvest papaya fruits

The effect of LAB and *Bacillus* on anthracnose disease and postharvest quality of papaya fruits was carried out according to Yadravi et al. (2022) with some modifications. The papaya fruit weighs around 500-800 g and is harvested at the green ripe stage with 1-2 yellow streaks on the surface (8-9 months after fruiting). Fruits were selected with uniform size, shape, and ripeness without any signs of mechanical wounding, insect damage, or disease for this experiment. LAB was cultured in MRS, while *Bacillus* were cultured in NB medium and incubated at 30°C for 24 h. Bacterial density was then determined by spectrophotometric measurements at 600 nm (Kim et al. 2021), where $OD_{600\ nm}$ = 1.0 corresponds to a bacterial density of 10^8 CFU/mL. Finally, based on the survey results, the papaya fruits were immersed in a solution of LAB and *Bacillus* for one minute with the following treatments:

+ Control treatment: papaya fruit was not treated with bacteria.

+ LAB treatment: papaya fruit was treated with isolate LDC11 at a 10^6 CFU/mL density.

+ *Bacillus* treatment: papaya fruit was treated with isolate BHL21 at a 10^6 CFU/mL density.

The treatments were arranged in a completely randomized design (CRD), and each treatment was repeated thrice with 44 fruits/treatment. Papaya fruit is stored at a temperature of 25°C until 12 days. Samples will be collected periodically every 3 days during storage to analyze the following parameters:

Disease incidence (%): the fruits are observed for the disease symptoms, and the number of infected fruits is recorded on days 0, 3, 6, 9, and 12 during the study (McMillan 1986).

The disease severity: the extent of infection is scored using a 0-4 scale (Hofman et al. 1997) as follows: 0 = No infection, 0.1-25.0% fruit surface infected = 1, 25.1-50.0% fruit surface infected=2, 50.1-75.0% fruit surface infected = 3 and 4 = >76.0% fruit surface infected.

Weight loss: a 4-odd electronic is used as balance; papaya fruits are weighed at the start of the experiment (0 days) and every 3 days interval of storage (12 days) (Khaliq et al. 2015).

Total soluble solids (TSS): According to Zhang et al. (2008), TSS is calculated by determining each fruit's refractive index using a handheld refractometer (Atago Master 20T, Japan), and the findings are represented as percentages (%).

Titratable acidity (TA): The total acid content of the juice is measured by titrating with 0.1 N NaOH (Shi et al. 2018).

Vitamin C: According to Roe et al. (1948), the vitamin C content is determined; the results represented in mg of ascorbic acid per 100 g fresh weight.

The fruit firmness: the samples are analyzed using a texture analyzer (Food Technology TMS-Pro, USA). The firmness of the flesh on two opposite sides of each fruit was measured and recorded as N (newtons). For each fruit, the firmness is determined by averaging three readings.

Fruit color: A colorimeter is used, and the peel's color is determined (Hunter Lab, MH-C800 4500L, USA). This is determined at three locations on the fruit: L* (range from 0 to 100 for white) and a* (positive values for red, negative values for green) lightness levels are calculated.

Statistical analysis

The data were processed using Microsoft Excel and Statgraphic 16.1 software. The BLASTn tool was used to compare the similarity of LAB and *Bacillus* with fungal strains in the NCBI database. A neighbor-joining phylogenetic tree was constructed using the MEGA 5.1 software and the Kimura-2 model with 1,000 replications. One-way analysis of variance (ANOVA) and Tukey's multiple range tests were used to examine the treatment differences with a 95% confidence level.

RESULTS AND DISCUSSION

Isolation and identification of *Colletotrichum*

In this study, two isolates of *Colletotrichum* (namely, isolates TD1 and TD2) were isolated on a PDA medium from anthracnose-diseased papaya fruits (Figure 1.A). The findings revealed that the fungal colonies of two isolated fungal isolates reached 60-70 mm in diameter (Figure 1.B) after 7 days, and the thick mycelium was white. After 10 days of culture, an orange concentric circle was formed inside the mycelium, and oil droplets were mixed in the mycelium (Figure 1.C). Under the microscope, the results showed that the mycelium had no septum, and the spores of the two fungal isolates were cylindrical, one pointed and the other round (Figure 1.D). The mycelium and spore morphological characteristics of the fungus *Colletotrichum* in this study are consistent with the fungus *Colletotrichum* isolated from dragon fruit (Pei et al. 2020) and papaya fruit (Marquez-Zequera et al. 2018).

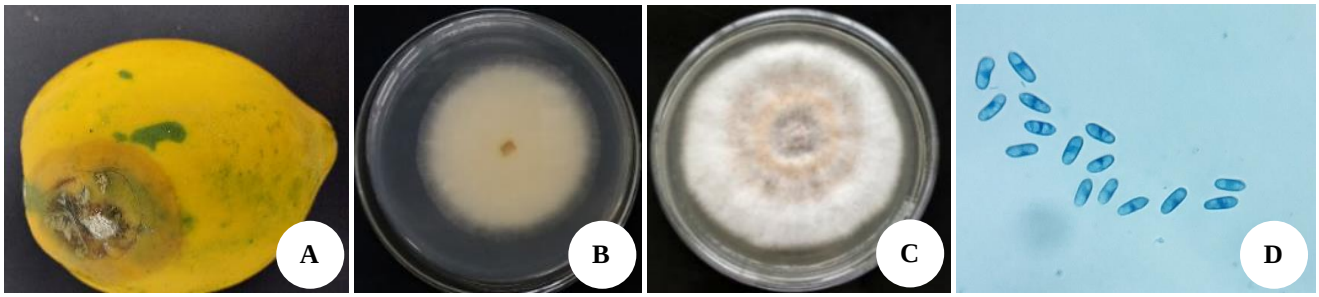

Figure 1. Isolation of the fungus *Colletotrichum.* A. Infected papaya fruit, B-C. Colony of *Colletotrichum*, D. Microscopic appearance

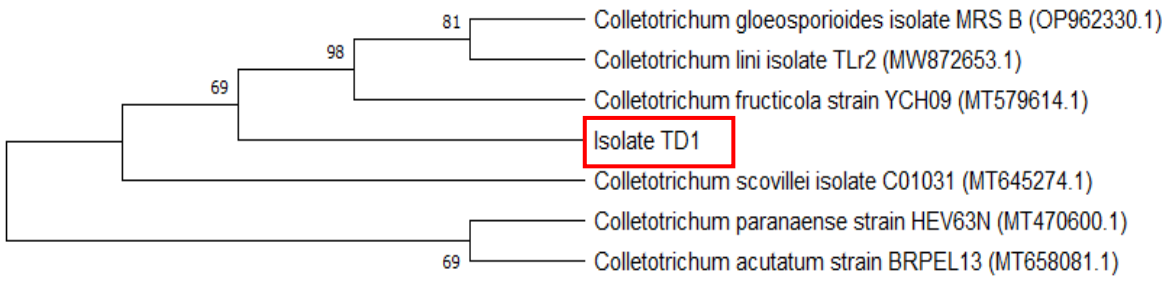

Figure 2. Phylogenetic tree of *Collectotrichum* isolate TD1 based on ITS regions of ribosomal DNA sequences (bootstrap values are given at branching points)

Figure 3. Isolation of LAB from traditional fermented vegetables. Note: A. The LAB's colony isolated on MRS medium, B. The clearance zone appeared around the bacterial colonies, C. Gram stain image of LAB (100X)

The infection showed that two fungal isolates gave positive results, and symptoms appeared on fruits within 3-5 days after inoculation. These features are similar to the symptoms of anthracnose on papaya fruit (Kimaru et al. 2018) and avocado fruit (Aktaruzzaman et al. 2018). Reisolation and identification were made from artificially inoculated papaya fruits that showed typical signs of lesions, and reisolated fungi were similar to those of the original ones. In addition, the infection results revealed that the isolate TD1 has stronger virulence than the isolate TD2. This isolate was selected for sequencing and performing the following experiments. Sequencing results showed that the fungal isolate TD1 was 99.25% homologous to *C. fructicola* YCH09 (MT579614.1) in the NCBI database. The phylogenetic tree showed that isolates TD1 were distributed into separate clusters (Figure 2).

Isolation of LAB

The study isolated thirteen LAB isolates from traditional fermented vegetables. Most colonies were round (Figure 3.A), and the clearance zone appeared around the bacterial colonies when the medium was supplemented with $CaCO_3$ (Figure 3.B). In the study, all isolated bacterial isolates were Gram-positive, rod-shaped (Figure 3.C), non-spore-forming, and had negative catalase and oxidase reactions. The findings indicated that the morphological and biochemical characteristics of isolated LAB are similar to the previous results published by Arimah et al. (2014). A report by Zakaria et al. (2018) showed that 30 strains from fermented catfish had a clear zone around the colony and were gram-positive with bacilli-shaped, catalase-negative, and oxidase-negative.

Antagonistic activity of LAB against *Colletotrichum*

The results demonstrated that 13 LAB isolates inhibited the germination of spores and mycelium elongation of *Colletotrichum*, of which 4 isolates (30.7%) had strong antagonistic activity, 6 isolates (46.1%) had moderate antagonistic activity, and 3 isolates (30.7%) showed weak antifungal activity (Figure 4). The findings also revealed

that isolate LDC11 has the highest antagonistic activity against *Colletotrichum*, with a 62.8% inhibition of fungal growth. Recent studies have shown that LAB inhibits fungi because they produce certain substances that inhibit the growth of spoilage molds on fruits and foods (Lindgren and Dobrogosz 1990). El-Mabrok et al. (2012) reported that 2 species of *L. plantarum* and *L. paracasei* strongly prohibited the germination of *C. gloeosporioides*, which infects chili seeds. The research of Cheong et al. (2014) collected 897 strains of LAB, of which 12 strains (1.3%) indicated inhibitory activity against *Penicillium commune*, the common cheese spoilage mold, inhibiting its growth by more than 60%. Lipińska et al. (2016) investigated the inhibitory activity of several LAB species; they discovered that *L. pentosus* and *L. plantarum* strongly inhibit the mycelial growth of *F. latenicum*, *A. niger*, *Alternaria alternata*, and *A. brassicicola*. Barrios-Roblero et al. (2019) isolated 10 LAB strains from 2 fermented beverage samples (Tepache and Te). By this investigation, all strains prohibited spore germination by at least 60% and mycelium growth by 100%. The recent study by Steglińska et al. (2022) revealed that most LAB isolates inhibit a wide range of antifungal activity spectra, consisting of 10 phytopathogens: *C. coccodes*, *Pectobacterium carotovorum*, *Streptomyces scabiei*, *A. solani*, *A. tenuissima*, *A. alternata*, *Phoma exigua*, *Rhizoctonia solani*, *F. oxysporum*, and *F. sambucinum*.

Sequencing results showed that the isolate LDC1 and LDC11 were 99.69 and 100% similar to *Lactiplantibacillus plantarum* strain KB-25 (MT378128.1) and *Lactiplantibacillus plantarum* strain 1929 (MT597746.1) in the GeneBank. The phylogenetic tree showed that 2 isolates LDC1 and LDC11 were distributed into the same clusters (Figure 5).

Isolation of *Bacillus*

Moreover. 12 *Bacillus* isolates were collected from 3 papaya rhizosphere soil samples. The results showed that the bacterial colonies were usually milky white with wrinkled large, smooth small, and smooth large colonies (Figure 6.A). The findings showed that the isolates were gram-positive, long rod-shaped (Figure 6.B), spore-forming (Figure 6.C), catalase- and oxidase-positive. The current study indicated that the morphological and biochemical characteristics of isolated *Bacillus* agree with the previous results of Ashwini and Srividya (2014). From the rhizosphere of *Coffea arabica* L., the research of Kejela et al. (2016) showed that all isolated *Bacillus* species were gram-positive, catalase-positive, spore-forming, rod-shaped, and able to survive at 80°C.

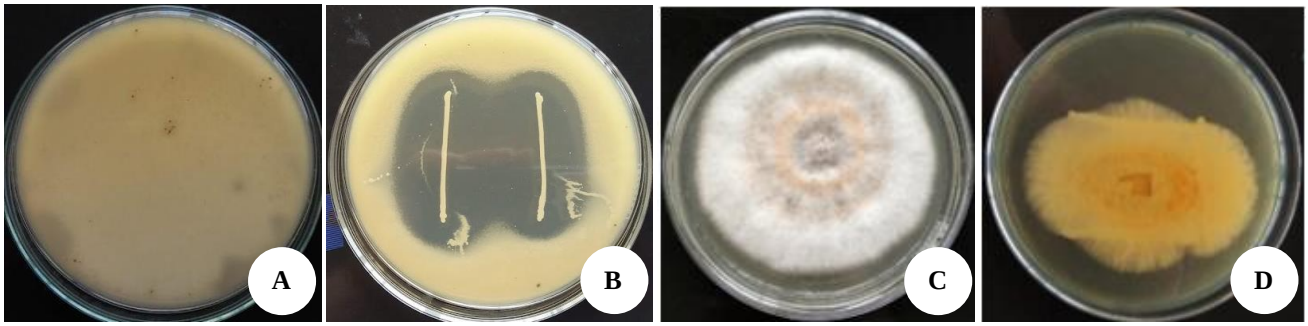

Figure 4. Antagonistic activity of LAB against *Colletotrichum* (isolate TD1). Note: Spore antagonistic activity of LAB against *Colletotrichum* (isolate TD1) at day 3: (a). Control, and (b). Isolate LDC11; mycelial antagonistic activity of LAB against *Colletotrichum* (isolate TD1) at day 3: (c). Control, and (d). Isolate LDC11

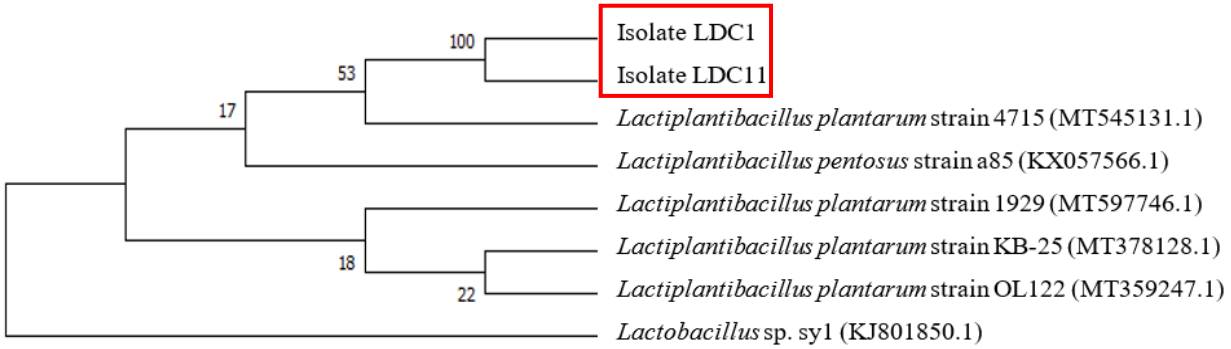

Figure 5. Phylogenetic tree of two isolates LDC1 and LDC11, based on 16S rRNA sequences (bootstrap values are given at branching points)

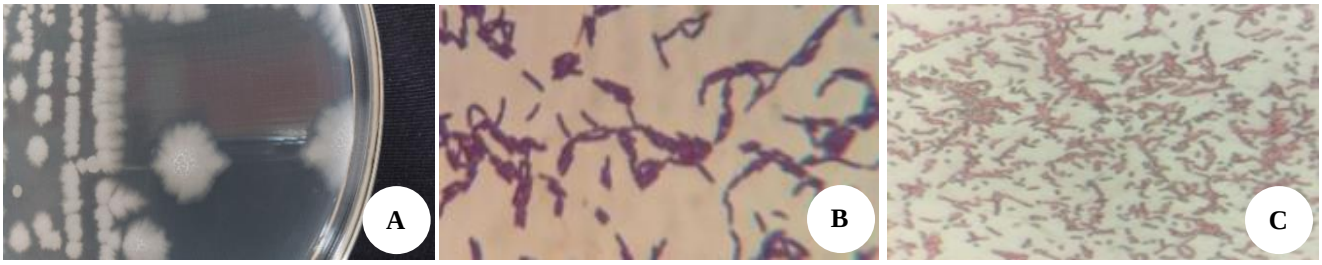

Figure 6. *Bacillus* isolated from papaya rhizospheric soils. Note: A. *Bacillus*' colony on NA medium (isolate BHL21), B. Gram stain image of isolate BHL21 (100X), C. Spore stain image of isolate BHL21 (100x)

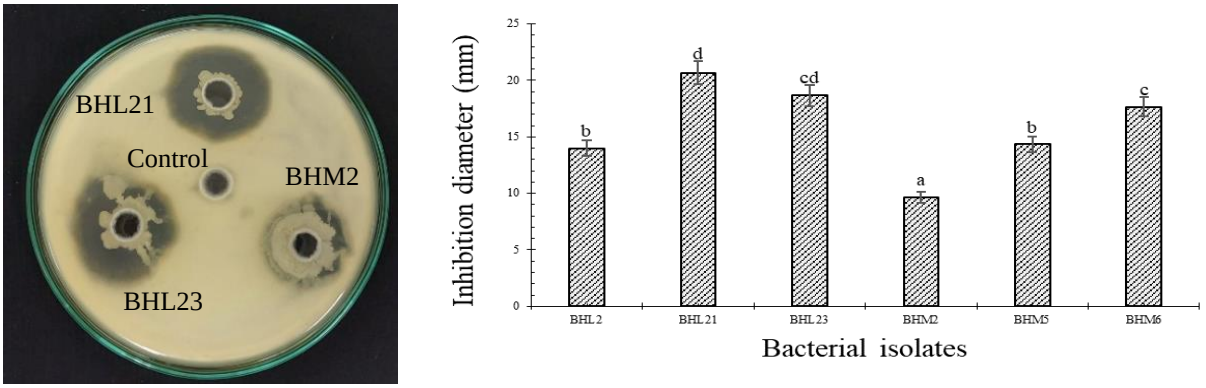

Figure 7. Mycelial inhibitory activity of *Bacillus* against *Colletotrichum* (isolate TD1). Note: Vertical bars represent SE, means followed by different letters are statistically significant ($p<0.05$)

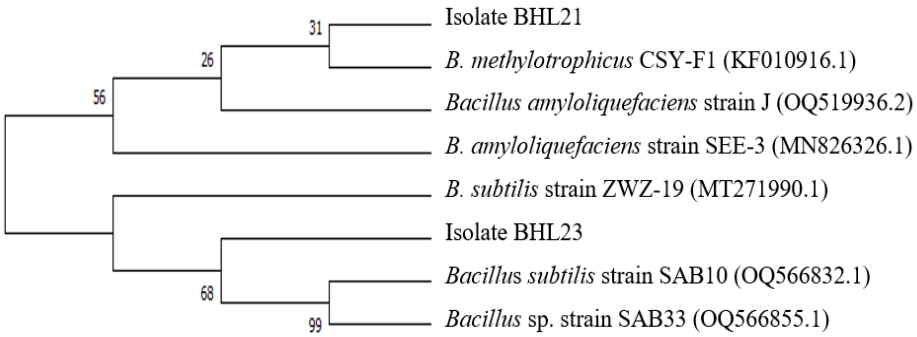

Figure 8. Phylogenetic tree of two isolates BHL21 and BHL23, based on 16S rRNA sequences (bootstrap values are given at branching points)

Antagonistic activity of *Bacillus* against *Colletotrichum* (isolate TD1)

The findings showed that 6/12 *Bacillus* isolates had spore and mycelial antagonistic activity against *Colletotrichum* of which 2 isolates, BHL21 and BHL23, exhibited the highest activity with inhibition diameters of 20 mm and 18 mm, respectively (Figure 7). The results of Hailmi et al. (2017) showed that *Bacillus* sp. (UniSZA-DA) strongly inhibited the growth of *C. gloeosporioides*, which causes anthracnose on papaya grown in Malaysia, by an average of 58.89 ± 2.72% after 7 days of incubation for the control. Research by Gao et al. (2018) showed *B. subtilis* CF-3 exhibited antifungal activity against fungal diseases on peaches and litchi, such as *Botrytis cinerea*, *C. gloeosporioides*, *Penicillium expansum*, *Monilinia fructicola*, and *Alternaria alternata*. Similarly, the results of Girish and Prabhavathi (2019) showed that *B. amyloliquefaciens* (MTCC 10439), *B. cereus* (MTCC 9017) effectively inhibited the growth of the fungi *C. gloeosporioides* and *C. carica papayae and* showed a zone of inhibition or reduction of mycelium growth compared with the control after 10 days of incubation by the dual culture method.

Sequencing results showed that isolates BHL21 and BHL23 were 99.63 and 99.09% homologous to *B. methylotrophicus* CSY-F1 (KF010916.1) and *Bacillus amyloliquefaciens* strain J (OQ519936.2*)* in the NCBI

database. The phylogenetic tree showed that 2 isolates BHL21 and BHL23 were distributed into 2 distinct clusters (Figure 8).

Effect of isolates LDC11 and BHL21 on disease incidence in papaya fruit

The research showed that papaya fruit treated with two isolates of LDC11 and BHL21 reduced the disease incidence and significantly differed from the control (Figure 9.A). By day 3, the two bacterial treatments did not show any infection symptoms, but in the control group, disease signs appeared (7.4%). By day 6, both LAB- and *Bacillus* treatments had a disease incidence of 28.57%, a statistically significant difference compared to the control, which had a disease incidence of 47.61%. At storage day 9, the LDC11 treatment had an incidence of 53.33 and 60% for the BHL21 treatment, which was statistically different from the control (the incidence was 100%). At the end of the storage day, all treatments had an infection incidence of 100%. Gamagae et al. (2003) reported that yeast treatment (*Candida oleophila*) could reduce the anthracnose disease caused by *Colletotrichum gloeosporioides* on papaya (*C. papaya*) fruits in storage. In addition, *Trichosporon asahii* treatment was to inhibit the growth of *C. gloeosporioides* on papaya *in vitro* and *in vivo* test (Hassan et al. 2021). The findings are in agreement with Chavez-Diaz et al. (2019), who reported that cell suspensions (CS) and 'cell–free' supernatants (CFE) from *B. subtilis*, *B. subtilis*, and *B. licheniformis* were very effective in controlling soft rot caused by *Rhizopus stolonifer* in blackberry fruits.

Effect of isolates LDC11 and BHL21 on the disease severity in papaya fruit

The results showed that the disease severity increased in all the treatments during the 12 days of storage. However, disease severity in the two bacterial treatments was significantly ($P<0.05$) lower compared to the control treatment during the storage period (Figure 9.B). By day 3, the disease severity was only observed in the control (13.88%). On days 6 and 9, the highest disease severity was recorded in the control treatment (the disease severity in these periods was 52.38 and 63.33%, respectively). At the end of storage day 12, the highest disease severity was found in the control treatment (94.44%), which was statistically significant ($P<0.05$) compared with the fruits subjected to LAB (80.55%) and *Bacillus* treatment (72.22%). This result is because LAB and *Bacillus* treatment may produce the antifugal compounds (proteinaceous substances, organic acids, and hydrogen peroxide) or lytic enzymes, nutrient and space competition, signal interference and induced systemic resistance (ISR) in plants (Chen et al. 2020; Oirdi et al. 2021) that could be inhibit the growth of fungal mycelium. Therefore, LAB and *Bacillus* treatment could reduce the disease severity at day 12 in compared with the control treatment. Previous studies showed that *Bacillus amyloliquefaciens* treatment had significantly reduce anthracnose disease (disease incidence and severity) on papaya fruit (Osman et al. 2011). In addion, on tomatoes, Hamed et al. (2011) demonstrated the inhibitory effectiveness of using LAB isolated from milk and yogurt to control *Fusarium oxysporum*. The efficiency of utilizing *B. subtilis* CF-3 isolated from yogurt in fighting fungal diseases on peaches and litchi was also demonstrated by research by Gao et al. (2018). These authors demonstrated that *B. cinerea*, *Monilinia fructicola*, *C. gloeosporioides*, *Penicillium expansum*, and *A. alternata* were all susceptible to *B. subtilis* CF-3's antifungal effects in this study.

Weight loss

The results showed that the weight loss of LAB- and *Bacillus*-treated papaya fruits increased during the storage period (Figure 10.A). However, the weight loss for the fruits subjected to the treatment with LAB and *Bacillus* was significantly ($P<0.05$) lower than the control (weight loss at days 3, 6, 9, and 12 were 1.8, 4.5, 6.9, and 9.2%, respectively). The findings align with the study conducted by Kaarunya et al. (2022), who reported that the weight loss of tomato fruit treated with LAB was lower than that of tomato fruit not treated with LAB.

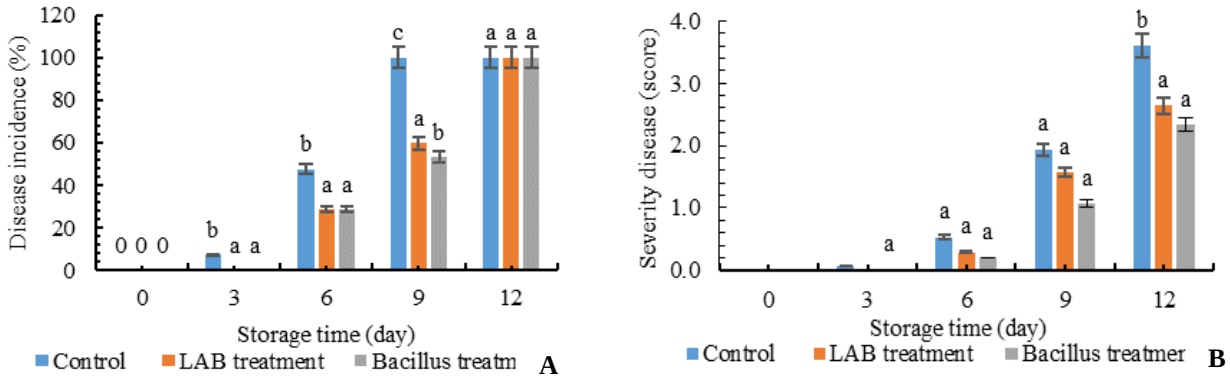

Figure 9. Disease incidence and severity of disease in papaya fruits during storage. Note: Vertical bars represent SE, means followed by different letters are statistically significant ($p<0.05$)

Vitamin C content

The results showed that the vitamin C concentration declined steadily with storage time. In two bacterial treatments, vitamin C was higher than in the control. However, there was no significant difference (P>0.05) between the bacteria-treated-treatment and the control from storage days 0 to 12 (Figure 10.B). At 12 days of storage, the vitamin C content in the control treatment decreased by 85.41%, significantly higher than the two bacteria treatments of 51.27 and 63.66%, respectively. Research by Wang et al. (2010) indicated that *B. subtilis* EXWB1-inoculated melon retained high levels of vitamin C.

Total Soluble Solid (TSS)

The findings showed that the TSS increased in all treatments during the storage days, and the TSS in bacteria-treated fruits was lower than the control treatment (Figure 11.A). However, no statistically significant difference **(**P>0.05**)** in TSS was found between papaya fruits subjected to the bacteria and the control from day 0 to day 12 (Figure 11.A). The TSS in the control treatment was 7.5 °Brix at day 0, which increased until it reached 9.8 °Brix on the last day of storage. TSS in LAB treatment increased from 7.6 at day 0 to 9.4 °Brix at storage day 12. In *Bacillus* treatment, meanwhile, TSS increased from 7.5 at day 0 to 10 °Brix at storage day 12. This result conflicts with a study by Wang et al. (2021), who found that strawberry fruit treated with *B. halotolerans* KLBC XJ-5 sustained higher TSS contents than the control group. Nevertheless, after 4 days at 22°C, there were no statistically significant differences in fruit TSS between the two groups.

Total titratable acid content

The research showed that TA decreased in all treatments during the storage days (Figure 11.B). From storage days 0 to 6, TA in papaya fruits subjected to bacteria was not significantly higher (P>0.05) compared to the control. Meanwhile, TA in the control treatment was not significantly higher than that of the bacteria-supplemented treatments from storage days 9 to 12 (Figure 11.B). According to the study of Wang et al. (2021), strawberry fruits treated with *B. halotolerans* KLBC XJ-5 retained higher TA contents than those in the control group. However, after 4 days at 22°C, there were no statistically significant differences in fruit TA between the two groups.

Firmness

The study showed that the firmness of the three treatments decreased during the storage period (Figure 11.C). It can be seen that there was no significantly difference fruit's firmness between the control and *Bacillus* treatment from day 0 to 12. While, the first 3 days of the storage period, no difference in firmness between the control and LAB treatment. However, from day 6 to 9, the papaya fruit treated with LAB had a lower firmness than the control but in the end of storage, the firmness of LAB treatment had higher than the control. Therefore, throughout this study, it was shown that fruit's firmness was maintained during storage due to two isolates that inhibited the growth of *Collectotrichum*. Pingping et al. (2017) supposed that one of the most crucial factors in assessing whether biocontrol agents can be utilized in the actual postharvest process is the impact of fruit quality. The findings align with the previous study by Wang et al. (2010), who demonstrated that melon fruits treated with *B. subtili*s EXWBl were also firmer than those treated with chlorine dioxide or a preservative for 10 days at 25°C. The research of Yuan et al. (2022) revealed that *B. velezensis* strain P2-1 had no discernible impact on the firmness of apple fruits.

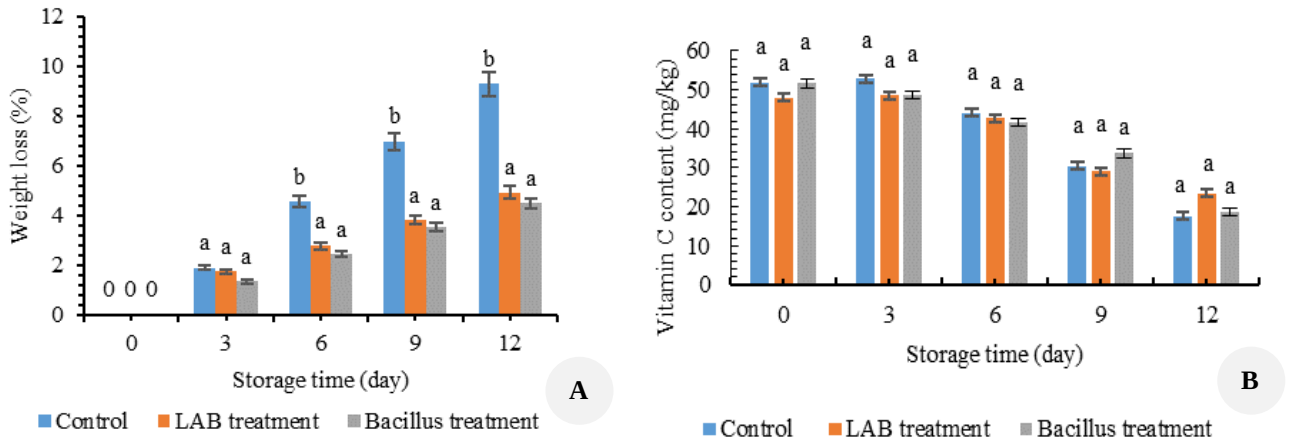

Figure 10. Weight loss and vitamin C content of papaya fruit during storage. Note: Vertical bars represent SE, means followed by different letters are statistically significant (p<0.05)

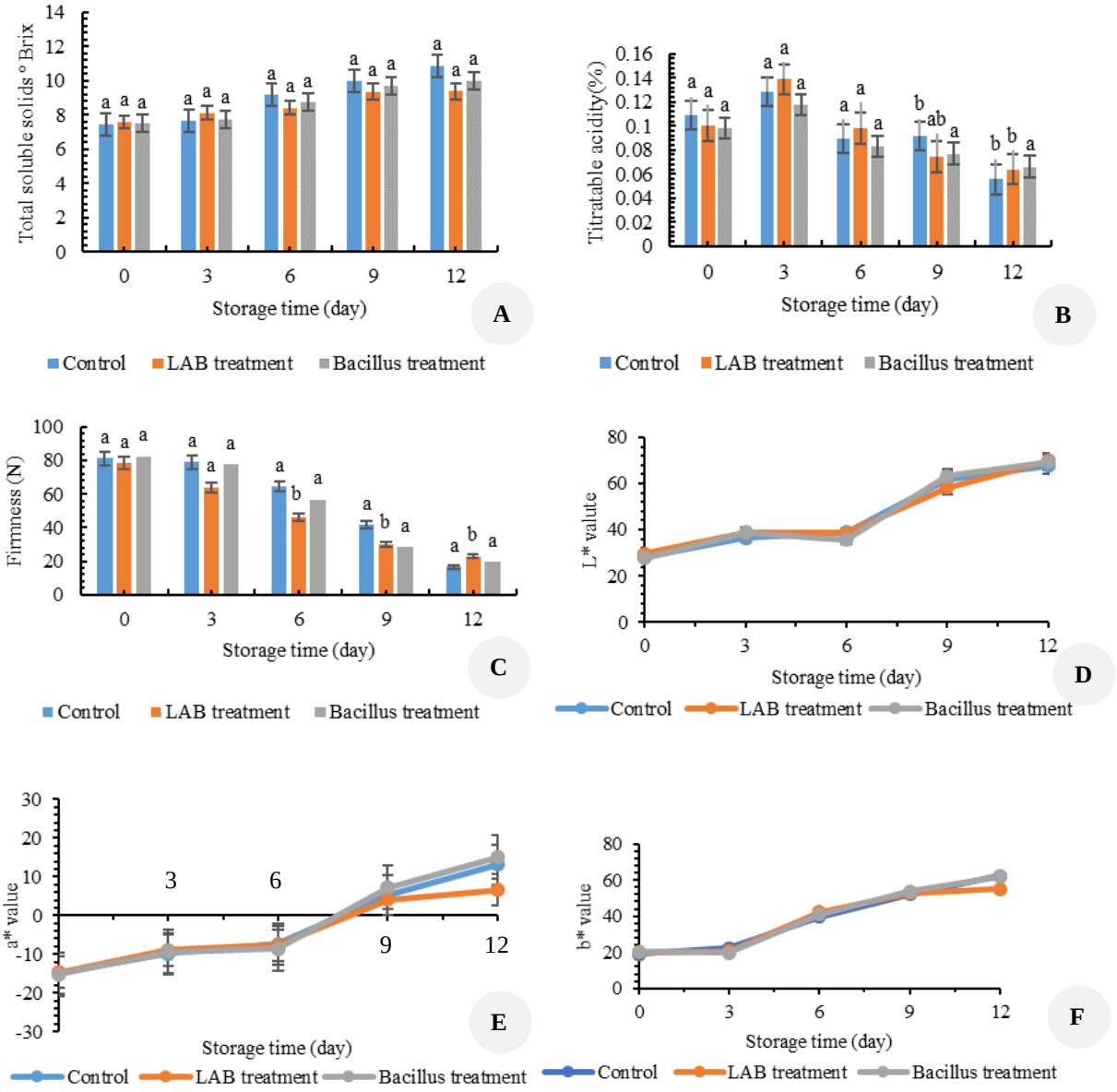

Figure 11. Effect of isolates LDC11 and BHL21 on TSS, TA, and color fruit during the storage period. Note: Vertical bars represent SE, means followed by different letters are statistically significant ($p<0.05$)

Fruit color

The results showed that L* values increased in all three treatments during storage. Although the L* value increased the most in the control treatment, the difference was not statistically significant ($P>0.05$) compared with the two bacteria treatments (Figure 11.D). In this study, the a* values in all three treatments increased, but the a* value in the control treatment increased more; the difference was not statistically significant ($P>0.05$) when compared to the two treatments with bacterial treatment (Figure 11.E). The a* value at day 12 in the controls (LAB- and *Bacillus*-treatment) was 13.21, 6.62, and 15.08, respectively. Meanwhile, the observations showed that the b* values in the three treatments increased, but the b* values in the control treatments increased even more. However, the difference was not statistically significant ($P>0.05$) compared with the two bacteria treatments (Figure 11.F). At day 12, the b* values of the controls, LAB- and *Bacillus*-treatment, were 62.41, 54.98, and 61.96, respectively. This result in agreement with Osman et al. (2011) who reported that *Bacillus amyloliquefaciens* treatment had no effect on the color of postharvest papaya fruit in storage. In this work, two BHL21 and LDC11 isolates had to reduce the anthracnose disease in postharvest papaya fruit and extending the sheflife. However, no significantly impact on the postharvest papaya fruit quality during storage.

In conclusion, the study isolated and identified LAB and *Bacillus* isolates from traditional fermented vegetables and papaya rhizospheric soils with antagonistic activity against *Colletotrichum* spp. In particular, two isolates, LDC11 and BHL21, have the highest antagonistic ability against *Colletotrichum*. At a 10^6 CFU/mL concentration, two isolates, LDC11 and BHL21, reduced disease incidence and severity and weight loss in comparison to the control. However, the LAB and *Bacillus*-treated papaya fruit did not affect the fruit quality such as color changes, firmness, TSS, TA and vitamin C content during storage. Therefore, to improve the quality of postharvest papaya fruits, the effect of isolates BHL21 and LDC11 combined with further treatment such as edible coating or modified atmosphere packaging should be investigated in future researchs.

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REFERENCES

Aktaruzzaman M, Afroz T, Lee Y-G, Kim B-S. 2018. Post-harvest anthracnose of papaya caused by *Colletotrichum truncatum* in Korea. Eur J Plant Pathol 150: 259-265. DOI: 10.1007/s10658-017-1265-y.

Alara OR, Abdurahman NH, Alara JA. 2020. *Carica papaya*: Comprehensive overview of the nutritional values, phytochemicals and pharmacological activities. Adv Trad Med 22: 17-47. DOI: 10.1007/s13596-020-00481-3.

Arimah BD, Ogunlowo OP, Adebayo MA, Jesumirhewe C. 2014. Identification of lactic acid bacteria isolated from selected Nigerian foods and comparison of their bacteriocins activities. Intl J Pharm Clin Res 6 (2): 929-937.

Ashwini N, Srividya S. 2014. Potentiality of *Bacillus subtilis* as biocontrol agent for management of anthracnose disease of chilli caused by *Colletotrichum qloeosporioides* OGC1. 3 Biotech 4 (2): 127-136. DOI: 10.1007/s13205-013-0134-4.

Barnett HL, Hunter BB. 1972. Illustrated genera of imperfect fungi. Illustrated genera of imperfect fungi 188-191.

Barrios-Roblero C, Rosas-Quijano R, Salvador-Figueroa M, Gálvez-López D, Vázquez-Ovando A. 2019. Antifungal lactic acid bacteria isolated from fermented beverages with activity against *Colletotrichum gloeosporioides*. Food Biosci 29: 47-54. DOI: 10.1016/j.fbio.2019.03.008.

Belkacem-Hanfi N, Fhoula I, Semmar N, Guesmi A, Perraud-Gaime I, Ouzari H-I, Boudabous A, Roussos S. 2014. Lactic acid bacteria against postharvest moulds and ochratoxin A isolated from stored wheat. Biol Control 76: 52-59. DOI: 10.1016/j.biocontrol.2014.05.001.

Buchholz F, Kostić T, Sessitsch A, Mitter B. 2018. The potential of plant microbiota in reducing postharvest food loss. Microb Biotechnol 11 (6): 971-975. DOI: 10.1111/1751-7915.13252.

Cai L, Hyde KD, Taylor PWJ, Weir B, Waller J, Abang MM, Zhang JZ, Yang YL, Phoulivong S, Liu ZY, Shivas RG. 2009. A polyphasic approach for studying *Colletotrichum*. Fungal Divers 39 (1): 183-204.

Cavaglieri L, Orlando J, Rodriguez MI, Chulze S, Etcheverry M. 2005. Biocontrol of *Bacillus subtilis* against *Fusarium verticillioides* in vitro and at the maize root level. Res Microbiol 156 (5-6): 748-754. DOI: 10.1016/j.resmic.2005.03.001.

Chavez-Diaz IF, Mena-Violante HG, Hernandez-Lauzardo AN, Oyoque-Salcedo G, Oregel-Zamudio E and Angoa-Perez MV. 2019. Post-harvest control of *Rhizopus stolonifer* on blackberry (*Rubus fruticosus*) by blackberry native crop bacteria. Revista de la Facultad de Ciencias Agrarias UNCuyo 51 (2): 306-317.

Chen K, Tian Z, He H, Long C, Jiang F. 2020. *Bacillus* species as potential biocontrol agents against citrus diseases. Biol Control 151: 104419. DOI: 10.1016/j.biocontrol.2020.104419.

Cheong EYL, Sandhu A, Jayabalan J, Le TTK, Nhiep NT, Ho HTM, Zwielehner J, Bansal N, Turner MS. 2014. Isolation of lactic acid bacteria with antifungal activity against the common cheese spoilage mould *Penicillium commune* and their potential as biopreservatives in cheese. Food Control 46: 91-97. DOI: 10.1016/j.foodcont.2014.05.011.

De Man JC, Rogosa D, Sharpe ME. 1960. A medium for the cultivation of lactobacilli. J Appl Microbiol 23 (1): 130-135. DOI: 10.1111/j.1365-2672.1960.tb00188.x.

De Simone N, Capozzi V, de Chiara MLV, Amodio ML, Brahimi S, Colelli G, Drider D, Spano G, Russo P. 2021. Screening of lactic acid bacteria for the bio-control of *Botrytis cinerea* and the potential of *Lactiplantibacillus plantarum* for eco-friendly preservation of fresh-cut kiwifruit. Microorganisms 9 (4): 773. DOI: 10.3390/microorganisms9040773.

Dhanasekaran D, Panneerselvam A, Thajuddin N. 2012. Applications of actinobacterial fungicides in agriculture and medicine. INTECH Open Access Publisher. DOI: 10.5772/25549.

El-Mabrok ASW, Hassan Z, Mokhtar AM, Aween MM. 2012. Efficacy of *Lactobacillus plantarum* C5 cells and their supernatant against *Colletotrichum gloeosporioides* on germination rate of chilli seeds. Res J Biol Sci 7 (4): 159-164. DOI: 10.3923/rjbsci.2012.159.164.

Feliziani E, Romanazzi G. 2013. Preharvest application of synthetic fungicides and alternative treatments to control postharvest decay of fruit. Stewart Postharvest Rev 9 (3) 1-6. DOI:10.2212/spr.2013.3.3.

Gabrekiristos E, Dagnew A. 2020. A newly emerging disease of papaya in Ethiopia: Black spot (*Asperisporium caricae*) disease and management options. J Plant Pathol Microbiol 11: 488. DOI: 10.35248/2157-7471.20.11.488.

Gamagae SU, Sivakumar D, Wijeratnam RSW, Wijesundera RLC. 2003. Use of sodium bicarbonate and *Candida oleophila* to control anthracnose in papaya during storage. Crop Prot 22 (5): 775-779. DOI: 10.1016/S0261-2194(03)00046-2.

Gao H, Li P, Xu X, Zeng Q, Guan W. 2018. Research on volatile organic compounds from *Bacillus subtilis* CF-3: Biocontrol effects on fruit fungal pathogens and dynamic changes during fermentation. Front Microbiol 9: 456. DOI: 10.3389/fmicb.2018.00456.

Ghosh R, Barman S, Mukhopadhyay A, Mandal NC. 2015. Biological control of fruit-rot of jackfruit by rhizobacteria and food grade lactic acid bacteria. Biol Control 83: 29-36. DOI: 10.1016/j.biocontrol.2014.12.020.

Girish K, Prabhavathi HR. 2019. Antifungal activity of bacteria against the phytopathogens of papaya (*Carica papaya* L.). EurAsian J Biosci 13 (1): 83-91.

Hailmi MS, Wahida WN, Badaluddin NA, Abdullah TA, Aziz ZFA, Kadir J. 2017. Potential of *Pseudomonas* sp. (UniSZA-MKB10) and *Bacillus* spp. (UniSZA-BK3, UniSZA-BK4 and UniSZA-DA) as biological control agent for controlling anthracnose disease of *Carica papaya* L. J Agrobiotechnol 8 (2): 64-76.

Hamed HA, Moustafa YA, Abdel-Aziz SM. 2011. In vivo efficacy of lactic acid bacteria in biological control against *Fusarium oxysporum* for protection of tomato plant. Life Sci 8 (4): 462-468.

Hassan H, Mohamed MTM, Yusoff SF, Hata EM, Tajidin NE. 2021. Selecting antagonistic yeast for postharvest biocontrol of *Colletotrichum gloeosporioides* in papaya fruit and possible mechanisms involved. MDPI (Agronomy) 11: 760-717. DOI: 10.3390/agronomy11040760.

Harrigan WF, McCance ME. 1976. Laboratory methods in food and dairy microbiology. Academic Press Inc., London.

Heuer H, Krsek M, Baker P, Smalla K, Wellington E. 1997. Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. Appl Environ Microbiol 63 (8): 3233-3241. DOI: 10.1128/aem.63.8.3233-3241.1997.

Hodges RJ, Buzby JC, Bennett B. 2011. Postharvest losses and waste in developed and less developed countries: Opportunities to improve resource use. J Agric Sci 149: 37-45. DOI: 10.1017/S0021859610000936.

Hofman PJ, Smith LG, Joyce DC, Johnson GI, Meiburg GF. 1997. Bagging of mango (*Mangifera indica* cv.Keitt') fruit influences fruit quality and mineral composition. Postharvest Biol Technol 12 (1): 83-91. DOI: 10.1016/S0925-5214(97)00039-2.

Igbedioh SO. 1991. Effects of agricultural pesticides on humans, animals, and higher plants in developing countries. Arch Environ Health: An Intl J 46 (4): 218-224. DOI: 10.1080/00039896.1991.9937452.

Jimenez M, Logrieco A, Bottalico A. 1993. Occurrence and pathogenicity of *Fusarium* species in banana fruits. J Phytopathol 137 (3): 214-220. DOI: 10.1111/j.1439-0434.1993.tb01341.x.

Kaarunya A, Meenatchi R, Vignesh S. 2022. Effect of lactic acid bacteria and propolis extract on the control of postharvest decay in tomato and its quality attribute changes. Pharm Innov J 11 (8): 521-529.

Kejela T, Thakkar VR, Thakor P. 2016. *Bacillus species* (BT42) isolated from *Coffea arabica* L. rhizosphere antagonizes *Colletotrichum gloeosporioides* and *Fusarium oxysporum* and also exhibits multiple plant growth promoting activity. BMC Microbiol 16: 1-13. DOI: 10.1186/s12866-016-0897-y.

Khaliq G, Mohamed MTM, Ali A, Ding P, Ghazali HM. 2015. Effect of gum arabic coating combined with calcium chloride on physico-chemical and qualitative properties of mango (*Mangifera indica* L.) fruit during low temperature storage. Sci Hortic 190: 187-194. DOI: 10.1016/j.scienta.2015.04.020.

Kim K-T, Kim J-W, Kim S-I, Kim S, Nguyen TH, Kang C-H. 2021. Antioxidant and anti-inflammatory effect and probiotic properties of lactic acid bacteria isolated from canine and feline feces. Microorganisms 9 (9): 1971. DOI: 10.3390/microorganisms9091971.

Kimaru SK, Monda E, Cheruiyot RC, Mbaka J, Alakonya A. 2018. Morphological and molecular identification of the causal agent of anthracnose disease of avocado in Kenya. Intl J Microbiol 2018: 4568520. DOI: 10.1155/2018/4568520.

Lahlali R, Mchachti O, Radouane N, Ezrari S, Belabess Z, Khayi S, Mentag R, Tahiri A, Barka EA. 2020. The potential of novel bacterial isolates from natural soil for the control of brown rot disease (*Monilinia fructigena*) on apple fruits. Agronomy 10 (11): 1814. DOI: 10.3390/agronomy10111814.

Lastochkina O, Seifikalhor M, Aliniaeifard S, Baymiev A, Pusenkova L, Garipova S, Kulabuhova D, Maksimov I. 2019. *Bacillus* Spp.: Efficient biotic strategy to control postharvest diseases of fruits and vegetables. Plants 8 (4): 97. DOI: 10.3390/plants8040097.

Lindgren SE, Dobrogosz WJ. 1990. Antagonistic activities of lactic acid bacteria in food and feed fermentations. FEMS Microbiol Rev 7 (1-2): 149-163. DOI: 10.1111/j.1574-6968.1990.tb04885.x.

Lipińska L, Klewicki R, Klewicka E, Kołodziejczyk K, Sójka M, Nowak A. 2016. Antifungal activity of *Lactobacillus* sp. bacteria in the presence of xylitol and galactosyl-xylitol. BioMed Res Intl 2016: 5897486. DOI: 10.1155/2016/5897486.

Liu J, Sui Y, Wisniewski M, Droby S, Liu Y. 2013. Review: Utilization of antagonistic yeasts to manage postharvest fungal diseases of fruit. Intl J Food Microbiol 167 (2): 153-160. DOI: 10.1016/j.ijfoodmicro.2013.09.004.

Magnusson J, Schnürer J. 2001. *Lactobacillus coryniformis subsp. coryniformis* strain Si3 produces a broad-spectrum proteinaceous antifungal compound. Appl Environ Microbiol 67 (1): 1-5. DOI: 10.1128/AEM.67.1.1-5.2001.

Magnusson J, Ström K, Roos S, Sjögren J, Schnürer J. 2003. Broad and complex antifungal activity among environmental isolates of lactic acid bacteria. FEMS Microbiol Lett 219 (1): 129-135. DOI: 10.1016/S0378-1097(02)01207-7.

Marin DH, Sutton TB, Blankenship SM, Swallow WH. 1996. Pathogenicity of fungi associated with crown rot of bananas in Latin America on Grande Naine and disease-resistant hybrid bananas. Plant Dis (USA) 80 (5): 525-528. DOI: 10.1094/PD-80-0525.

Marquez-Zequera I, Cruz-Lachica I, Ley-Lopez N, Carrillo-Facio JA, Osuna-Garcia LA, Garcia-Estrada RS. 2018. First report of *Carica papaya* fruit anthracnose caused by *Colletotrichum fructicola* in Mexico. Plant Dis 102 (12): 2649-2649. DOI: 10.1094/PDIS-05-18-0736-PDN.

McMillan Jr RT. 1986. *Guignardia citricarpa* a cause of black spot on mango foliage in Florida[1]. J Phytopathol 117 (3): 260-264. DOI: 10.1111/j.1439-0434.1986.tb00940.x.

Muhialdin BJ, Hassan Z, Saari N. 2018. In vitro antifungal activity of lactic acid bacteria low molecular peptides against spoilage fungi of bakery products. Ann Microbiol 68 (9): 557-567. DOI: 10.1007/s13213-018-1363-x.

Neelima R. 2016. Anuja and Sanjay. Probiotic *Lactobacillus* as biocontrol agent in postharvest diseases of banana and papaya fruits. Intl J Curr Res 8 (5): 31388-31392.

Oirdi SE, Lakhlifi T, Bahar AA, Yatim M, Rachid Z, Belhaj A. 2021. Isolation and identification of *Lactobacillus plantarum 4F*, a strain with high antifungal activity, fungicidal effect, and biopreservation properties of food. J Food Process Preserv 55 (6): 1-11. DOI: 10.1111/jfpp.15517.

Osman MS, Sivakumar D, Korsten L. 2011. Effect of biocontrol agent *Bacillus amyloliquefaciens* and 1-methyl cyclopropene on the control of postharvest diseases and maintenance of fruit quality. Crop Prot 30: 173-178. DOI: 10.1016/j.cropro.2010.09.014.

Pei S, Liu R, Gao H, Chen H, Wu W, Fang X, Han Y. 2020. Inhibitory effect and possible mechanism of carvacrol against *Colletotrichum fructicola*. Postharvest Biol Technol 163: 111126. DOI: 10.1016/j.postharvbio.2020.111126.

Pingping S, Jianchao C, Xiaohui J, Wenhui W. 2017. Isolation and characterization of *Bacillus amyloliquefaciens* L-1 for biocontrol of pear ring rot. Hortic Plant J 3 (5): 183-189. DOI: 10.1016/j.hpj.2017.10.004.

Qadri R, Azam M, Khan I, Yang Y, Ejaz S, Akram MT, Khan MA. 2020. Conventional and modern technologies for the management of postharvest diseases. In: Haq IU, Ijaz S (eds.) Plant Disease Management Strategies for Sustainable Agriculture through Traditional and Modern Approaches. Springer Nature. DOI: 10.1007/978-3-030-35955-3.

Roe JH, Mills MB, Oesterling MJ, Damron CM. 1948. The determination of diketo l-gulonic acid, dehydro-l-ascorbic acid, and l-ascorbic acid in the same tissue extract by the 2, 4-dinitrophenylhydrazine method. J Biol Chem 174 (1): 201-208. DOI: 10.1016/S0021-9258(18)57387-7.

Saini TJ, Gupta SG, Anandalakshmi R. 2017. First report of papaya anthracnose caused by *Colletotrichum* salsolae in India. New Dis Rep 35: 27-27. DOI:10.5197/j.2044-0588.2017.035.027.

Saucedo-Pompa S, Jasso-Cantu D, Ventura-Sobrevilla J, Saenz-Galindo A, Rodriguez-Herrera R and Aguilar CN. 2007. Effect of candelillawax with natural antioxidants on the shelf life quality of fresh cut fruits. J Food Qual 30 (5): 823-836. DOI: 10.1111/j.1745-4557.2007.00165.x.

Saucedo-Pompa S, Rojas-Molina R, Aguilera-Carbó AF, Saenz-Galindo A, Garza HDL, Jasso-Cantú D and Aguilar CN. 2009. Edible film based on candelilla wax to improve the shelf life and quality of avocado. Food Res Intl 42 (4): 511-515. DOI: 10.1016/j.foodres.2009.02.017.

Shi Z, Wang F, Lu Y, Deng J. 2018. Combination of chitosan and salicylic acid to control postharvest green mold caused by *Penicillium digitatum* in grapefruit fruit. Sci Hortic 233: 54-60. DOI: 10.1016/j.scienta.2018.01.039.

Steglińska A, Kołtuniak A, Motyl I, Berłowska J, Czyżowska A, Cieciura-Włoch W, Okrasa M, Kręgiel D, Gutarowska B. 2022. Lactic acid bacteria as biocontrol agents against potato (*Solanum tuberosum* L.) pathogens. Appl Sci 12 (15): 7763. DOI: 10.3390/app12157763.

Valencia-Chamorro SA, Palou L, Del Río MA, Pérez-Gago MB. 2011. Antimicrobial edible films and coatings for fresh and minimally processed fruits and vegetables: A review. Crit Rev Food Sci Nutr 51 (9): 872-900. DOI: 10.1080/10408398.2010.485705.

Verma S, Azevedo LCB, Pandey J, Khusharia S, Kumari M, Kumar D, Kaushalendra, Bhardwaj N, Teotia P, Kumar A. 2022. Microbial intervention: An approach to combat the postharvest pathogens of fruits. Plants 11 (24): 3452. DOI: 10.3390/plants11243452.

Vitiello A, Ferrara F, Boccellino M, Ponzo A, Cimmino C, Comberiati E, Zovi A, Clemente S, Sabbatucci M. 2023. Antifungal drug resistance: An emergent health threat. Biomedicines 11 (4): 1063. DOI: 10.3390/biomedicines11041063.

Wang F, Xiao J, Zhang Y, Li R, Liu L, Deng J. 2021. Biocontrol ability and action mechanism of *Bacillus halotolerans* against *Botrytis cinerea* causing grey mould in postharvest strawberry fruit. Postharvest Biol Technol 174: 111456. DOI: 10.1016/j.postharvbio.2020.111456.

Wang Y, Xu Z, Zhu P, Liu Y, Zhang Z, Mastuda Y, Xu L. 2010. Postharvest biological control of melon pathogens using *Bacillus subtilis* EXWB1. J Plant Pathol 92 (3): 645-652.

White TJ, Bruns T, Lee SJWT, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protoc: Guide Methods Appl 18 (1): 315-322. DOI: 10.1016/B978-0-12-372180-8.50042-1.

Yadav A, Kumar N, Upadhyay A, Sethi S, Singh A. 2022. Edible coating as postharvest management strategy for shelf-life extension of fresh tomato (*Solanum lycopersicum* L.): An overview. J Food Sci 87 (6): 2256-2290. DOI: 10.1111/1750-3841.16145.

Yadravi RN, Rudresh DL, Jagadeesh SL, Ambika DS. 2022. Effect of antagonistic microorganisms on extension of shelf-life and physiochemical properties of papaya (*Carica papaya*) var. Red Lady. Intl J Environ Clim Chang 12 (11): 2629-2634. DOI: 10.9734/ijecc/2022/v12i1131268.

Yuan H, Shi B, Wang L, Huang T, Zhou Z, Hou H, Tu H. 2022. Isolation and characterization of *Bacillus velezensis* strain P2-1 for biocontrol of apple postharvest decay caused by *Botryosphaeria dothidea*. Front Microbiol 12: 4148. DOI: 10.3389/fmicb.2021.808938.

Zakaria SF, Lani MN, Seng CT, Ahmad F, Ahmad KM, Hassan Z. 2018. Antifungal activity of lactic acid bacteria isolated from fermented catfish (*Clarias gariepinus*) as biocontrol of *Sclerotium rolfsii* infecting chili plants. Malays Appl Biol 47 (4): 117-126.

Zhang H, Wang L, Dong Y, Jiang S, Zhang H, Zheng X. 2008. Control of postharvest pear diseases using *Rhodotorula glutinis* and its effects on postharvest quality parameters. Intl J Food Microbiol 126 (1-2): 167-171. DOI: 10.1016/j.ijfoodmicro.2008.05.018.

Zubrod JP, Bundschuh M, Arts G, Brühl CA, Imfeld G, Knäbel A, Payraudeau S, Rasmussen JJ, Rohr J, Scharmüller A, Smalling K, Stehle S, Schulz R, Schäfer RB. 2019. Fungicides: An overlooked pesticide class? Environ Sci Technol 53 (7): 3347-3365. DOI: 10.1021/acs.est.8b04392.