Paenibacillus brasiliensis YS-1: A Potential Biocontrol Agent to Retard Xinyu Tangerine Senescence

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Abstract: The Xinyu tangerine (Citrus reticulata Blanco) is a non-climacteric fruit that is widely cultivated and consumed in China but highly susceptible to fungal infections. Antagonistic microorganisms can control postharvest diseases and extend the storage life of citrus fruits. However, little work has been done to investigate the effects of applying Paenibacillus brasiliensis YS-1 by immersion to enhance the cold storability of Xinyu tangerines. Fruits were soaked with P. brasiliensis YS-1 fermented filtrates for 10 min and in sterile water as the control. The decay incidence, weight loss, nutrient content, respiration rate, malondialdehyde (MDA) content, and defensive enzymes activities in citrus fruit were measured during cold storage at 5 ± 0.5 °C. The results showed that P. brasiliensis YS-1 treatment significantly reduced postharvest decay and effectively maintained the nutritional quality compared to the control under cold storage. The weight loss, respiration rate, and MDA content were lower in P. brasiliensis YS-1-treated fruits than the control fruits, indicating that P. brasiliensis YS-1 treatment increased the activities of superoxide dismutase (SOD), peroxidase (POD), polyphenol oxidase (PPO), and phenylalanine ammonia-lyase (PAL). According to the results, a postharvest application of P. brasiliensis YS-1 can control the postharvest decay and maintain fruit quality, as well as increase the defensive enzyme activity, so as to achieve the purpose of retarding postharvest senescence in citrus fruit.

Keywords: Paenibacillus brasiliensis YS-1; Citrus reticulata Blanco; postharvest senescence; storability

1. Introduction

Citrus reticulata Blanco is a widely cultivated fruit that is consumed in China because it has rich juicy contents, a delicious taste, it is easy to peel, has fewer dregs, a higher yield, and abundant nutrients, such as vitamin C, flavonoids, anthocyanins, and various other antioxidants [1–3]. Among mandarin varieties, Xinyu tangerines (Citrus reticulata Blanco) belong to the loose-skinned citrus group and are listed in the national geographic indication for the protection of products in China due to its equitable color, high juice content, fresh flavor, and promotion of human health [2]. However, it is a perishable citrus fruit, with a short postharvest storage life of 30 d at room temperature [4]. The majority of fresh mandarins ripen in early October to late November, and with their tender peel, postharvest diseases (green mold, blue mold, sour rot, and stem-end rot) caused by fungal pathogens such as Penicillium digitatum Sacc., P. italicum Wehmer, Geotrichum citri-aurantii E.E. Butler, and Diaporthe citri (Faw.) Wolf contribute to significant and heavy economic losses during harvest, storage, transportation, and even marketing [5–9]. A pathogenic fungal infection is an important factor that affects the
nutritional value and storage life of harvested citrus fruits. Therefore, it is a matter of great urgency to reduce the postharvest fungal rots of mandarins and other horticultural fruits.

Traditionally, the postharvest fungal decay of citrus fruits is chiefly controlled by a variety of synthetic fungicides including imazalil, pyrimethanil, thiabendazole, and polyhexamethylene guanidine, which have been commonly used to ensure a stable supply of fruit at markets to cope with an ever-increasing demand [10,11]. However, the widespread use of synthetic fungicides has become a global health concern due to their potential undesirable risk to human health, resistant strains, and environmental contamination [12,13]. Keeping in view these environmental and health issues, controlling postharvest fungal diseases using various biologically degradable compounds sourced from antagonist microorganisms shows a great potential as an alternative to synthetic preservatives to enhance the storage life of citrus fruit.

In recent decades, the use of antagonistic microorganisms and/or their secondary metabolites has been reported to have great potential as a promising alternative to synthetic fungicides for controlling postharvest diseases in citrus and several other horticultural crops [12,14]. Many antagonistic microorganisms such as *Aureobasidium pullulans* strain ACBL-77 [15], *Bacillus amyloliquefaciens* BUZ-14 [16], *B. subtilis* ABS-S14 [17], *Kloeckera apiculata* 34-9 [18], lactic acid bacteria [19], *Pseudomonas fluorescens* ZX [20], *Rhodosporidium paludigenum* Fell and Tallman [21], and *Yarrowia lipolytica* W29 [22] have been reported and considered as promising biological control agents (BCAs) for the postharvest disease control of citrus fruit. Biocontrol bacteria *Paenibacillus brasilensis* YS-1 is a soil-born Gram-positive bacteria and phylogenetically resembles *P. polymyx* [23,24], while *P. brasilensis* displays a strong antimicrobial activity against various phytopathogenic fungi in vitro, including *Penicillium italicum* Wehmer which frequently causes blue mold disease in citrus fruit [23,25]. Additionally, *P. brasilensis* has been reported to exhibit antagonist activities against many human pathogenic fungal strains, such as *Cryptococcus* spp., *Candida albicans* sorotype B, *Fonsecaea pedrosoi* (Brumpt), *Histoplasma capsulatum* var. duboisii and *Fusarium moniliforme* Sheldon LGM-2 [26], and it has high security in terms of posing no threat to humans and the environment, and is generally recognized as safe (GRAS) [24].

Previously, our lab successfully isolated a biocontrol bacterium *P. brasilensis* YS-1 from the soil of a kumquat (*Fortunella japonica* Swingle) root, which was identified by 16S rDNA sequencing combined with the Biolog microbial identification system [23]. One of main antifungal compounds was identified as cytosine, which was analyzed by mass spectrometry and nuclear magnetic resonance (1H-NMR, 13C-NMR, and 2D-NMR) [27]. However, the effects of postharvest treatments with *P. brasilensis* YS-1 on the postharvest storability and fruit quality of Xinyu tangerines have not been evaluated yet. In the current study, the effects of *P. brasilensis* YS-1 dipping treatment on the fruit quality, senescence-associated material contents, and defensive enzymes activities in harvested Xinyu tangerines were investigated. Furthermore, the study also focused on developing a safe and effective BCA to enhance postharvest storability and prolong the storage life of Xinyu tangerines.

### 2. Materials and Methods

#### 2.1. Fruit and Bacterial Strain

Healthy Xinyu tangerines (Pengjia No. 39) were picked at commercial maturity from an orchard in Yushui District of Xinyu City, China, and were promptly transported to the laboratory within 3 h. The fruits were sorted based on the uniformity of size and color, and the defected fruits with any mechanical wounds or diseases were removed.

The antagonistic bacterium *P. brasilensis* YS-1 was isolated from the soil of a kumquat (*Fortunella japonica* Swingle) root, and identified by 16S rDNA sequencing [23]. This strain was maintained on potato-dextrose agar (PDA) and embedded in 30% glycerol at ~80 °C for conservation. *Paenibacillus brasilensis* YS-1 fermented filtrates: *P. brasilensis* YS-1 was cultured in Luria-Bertani liquid medium (10 g/L of tryptone, 3 g/L of beef extract, 20 g/L of glucose, 5 g/L of NaCl) at 27 °C for
24 h with 180 r/min. Then, 2.0% of the fermented filtrate was migrated to liquid fermentation medium (60 g/L of soluble starch, 10 g/L of yeast extract, 6 g/L of NaCl, 2 g/L of MgSO$_4$, 2 g/L of K$_2$HPO$_4$·3H$_2$O) at 27 °C for 48 h with 180 r/min, and centrifuged (8000× g, 20 min; 5804R, Eppendorf, Hamburg, Germany). The supernatant was collected and heated for 20 min in a boiling water bath, and then centrifuged to obtain YS-1 fermented filtrates.

2.2. Sample Treatments

A total of 1000 selected fruits were washed with sterile water and divided into two groups (500 fruits per group). The *P. brasilensis* YS-1 group was dipped in a fermented filtrate suspension of *P. brasilensis* YS-1 for 10 min, and the control group was immersed in sterile water for 10 min. After being air dried at room temperature, all fruits were individually film packaged and stored at 5 ± 0.5 °C with a relative humidity (RH) of 80–90%. During 60 d of cold storage, the *P. brasilensis* YS-1-treated and control fruits were evaluated for fruit decay and sampled every 10 d to analyze the physicochemical indicators.

2.3. Evaluation of Fruit Decay and Weight Loss

The percentage of fruit decay incidence and weight loss were measured according to the method described by Chen et al. [28] with certain modifications. The same 100 Xinyu tangerines were taken out to evaluate the fruit decay at intervals of 10 d during cold storage at 5 °C. Fruits with any indication of fungal rot were defined as decayed fruits. The fruit weight was measured every 10 d by an AX224ZH digital weighing balance (± 0.0001 g, Ohaus Co., Ltd., Parsippany, NJ, USA) and compared with the harvested weight.

2.4. Analysis of the Total Soluble Solids, Total Sugar, Titratable Acidity, and Vitamin C Contents in Fruit Pulp

Ten grams of juice was extracted from 10 fruits in each replicate and was centrifuged at 8000× g for 15 min. The supernatant was collected to determine the contents of total soluble solids (TSS), total sugar, titratable acidity (TA), and vitamin C (VC) following the procedures defined by Chen et al. [2]. The TSS content (%) was determined using a RA-250WE Brix-meter (Atago, Tokyo, Japan). The total sugar content was assayed using the anthrone colorimetric method. Both the TA and VC contents in the supernatant were determined by a titration with 0.1 MNaOH (pH 8.0) and 2, 6-dichlorophenol indophenols, respectively. The results are expressed as a percentage (%) of the citric acid on a fresh weight basis and milligrams of ascorbic acid per 100 g of juice (mg 100 g$^{-1}$). Each analysis was carried out in three replicates with 10 fruits per replicate.

2.5. Determination of the Respiration Rate and Malondialdehyde Content

The fruit respiration rate of 10 fruits from the *P. brasilensis* YS-1-treated and control groups was determined according to the method described by Chen et al. [2,28]. The respiration rate was measured by a GHX-3051H fruit and vegetable breathing apparatus (Jingmi Scientific LLC., Shanghai, China), from the CO$_2$ production and the results are expressed as mg/(kg·h).

The malondialdehyde (MDA) content in the *P. brasilensis* YS-1-treated and control groups was determined according to the thiobarbituric acid (TBA) method described by Mahunu et al. [14] with some minor modifications. The pericarp tissues of 10 fruits were ground in a MM 400 grinder (Retsch GmbH., Arzberg, Germany), and 3.0 g of powder was homogenized in 15 mL of 10% (w/v) TBA. After centrifugation (12,000× g at 4 °C, 20 min), 2 mL of the supernatant was mixed with 2 mL of 0.67% (w/v) TBA, and immersed in a boiling water bath for 30 min. Then, it was cooled and centrifuged at 8000× g (5804R, Eppendorf) for 10 min. Finally, the absorbance of the solution was measured at three wavelengths (450, 532, and 600 nm) by a M5 Multiscan Spectrum microplate reader (Molecular Devices Corporation, Sunnyvale, CA, USA). The result of the MDA content is expressed as mmol/g frozen weight (FW).
2.6. Assay of the Defensive Enzyme Activity

Each sample (2.0 g) derived from the pericarp tissues of 10 fruits was homogenized with various ice-cold extraction buffers to obtain extracts for assaying the following defensive enzymes: 8 mL of a 50 mM ice-cold phosphate buffer (pH 7.8) containing 1 mM ethylene diamine tetraacetic acid (EDTA), and 2% (w/v) polyvinyl pyrrolidone (PVP) for superoxide dismutase (SOD, EC 1.15.1.1); 8 mL of a 100 mM ice-cold acetate buffer (pH 5.5) containing 1 mM polyethylene glycol (PEG), 4% (w/v) PVP, and 1% (w/v) Triton X-100 for peroxidase (POD, EC 1.11.1.7) and polyphenol oxidase (PPO, EC 1.10.3.1), and 5 mL of a 50 mM ice-cold Tris-HCl buffer (pH 8.8) containing 15 mM β-mercaptoethanol, 5 mM VC, 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 0.15% (w/v) PVP for phenylalanine ammonia-lyase (PAL, EC 4.3.1.5). After centrifugation (12,000×g at 4 °C, 30 min), the supernatants were collected and used as crude enzyme extracts for the assays.

The SOD activity was assayed using nitroblue tetrazolium (NBT) as previously described by Wan et al. [29]. One unit of enzyme activity was defined as a 50% inhibition of NBT photoreduction. Both the POD and PPO activity was determined according to the methods of guaiacol oxidation at 470 nm and catechol oxidation at 420 nm, respectively [14,30]. One unit of enzyme activity was defined as an increment of 0.01 per minute. The PAL activity was determined by using a PAL assay kit (Nanjing Jiancheng Bioengineering Inst., China) measuring the absorbance at 290 nm using the M5 microplate reader (Molecular Devices Corp., Sunnyvale, CA, USA).

2.7. Statistical Analysis

All data from the physicochemical experiments are displayed as the mean ± standard error (SE). The SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA) was used to determine the differences with the independent samples t-test (p < 0.05) for each storage time.

3. Results

3.1. Changes in the Decay Incidence and Weight Loss of Citrus Fruits after P. brasilensis YS-1 Treatment

A postharvest treatment with P. brasilensis YS-1 significantly prevented fungal infections and reduced fruit decay during cold storage. Citrus fruits treated with P. brasilensis YS-1 became infected with fungal pathogens at 40 d of storage, while visible signs of fruit rot were found on fruits in the control group at 30 d of cold storage (Figure 1). At the end of cold storage (60 d), the percentage of decay of the control group sharply rose to 7.33%, with a significant 2.20-fold increase over fruits treated with P. brasilensis YS-1.

![Figure 1. Effect of P. brasilensis YS-1 treatment on the decay rate of citrus fruits stored at 5 ± 0.5 °C for 60 d. Vertical bars represent the mean ± SE (n = 3). Letters indicate the statistical differences according to the independent samples t-test (p < 0.05) on each storage day.](image)

The percentage of weight loss increased with the extension of cold storage time (Figure 2). Compared with the non-treated control citrus fruits, a lower amplification of weight loss was observed
in the *P. brasilensis* YS-1-treated fruits during cold storage, and a significant difference between the weight loss of *P. brasilensis* YS-1-treated and control groups was observed after 30 d of cold storage.

Figure 2. Effect of *P. brasilensis* YS-1 treatment on the weight loss of citrus fruits stored at 5 ± 0.5 °C for 60 d. Vertical bars represent the mean ± SE (*n* = 10). Letters indicate statistical differences according to an independent samples *t*-test (*p < 0.05*) on each storage day.

### 3.2. Changes in the Postharvest Quality Attributes in Citrus Fruits after *P. brasilensis* YS-1 Treatment

The contents of the TSS and total sugar in the *P. brasilensis* YS-1-treated and control groups exhibited a quick increase at 30 d and 20 d, respectively, and then gradually reduced to the end of the cold storage period (Table 1). The independent samples *t*-test indicated that the contents of the TSS and total sugar in the *P. brasilensis* YS-1-treated group were significantly (*p < 0.05*) higher than that of the control fruits during the last 40 days of storage.

Table 1. Variation in the total soluble solids (TSS), total sugar, titratable acidity (TA), and vitamin C (VC) contents of citrus fruit under cold storage in relation to *P. brasilensis* YS-1 treatment.

| Storage Time (d) | Treatment          | TSS Content (%) | Total Sugar Content (%) | TA Content (%) | VC Content (mg 100 g\(^{-1}\)) |
|------------------|--------------------|-----------------|-------------------------|---------------|-------------------------------|
| 0                | Control            | 12.03 ± 0.058b  | 11.15 ± 0.182b          | 0.66 ± 0.012b | 21.42 ± 0.272a                |
|                  | *P. brasilensis* YS-1 | 12.23 ± 0.058a  | 11.88 ± 0.124a          | 0.63 ± 0.011a | 22.73 ± 0.368b                |
| 10               | Control            | 12.80 ± 0.000a  | 12.44 ± 0.295a          | 0.60 ± 0.012a | 23.95 ± 0.488b                |
|                  | *P. brasilensis* YS-1 | 12.43 ± 0.156a  | 12.18 ± 0.113b          | 0.51 ± 0.012b | 21.40 ± 0.446b                |
| 20               | Control            | 12.63 ± 0.058b  | 12.33 ± 0.055a          | 0.58 ± 0.006b | 25.81 ± 0.452a                |
|                  | *P. brasilensis* YS-1 | 12.63 ± 0.058a  | 12.33 ± 0.055a          | 0.58 ± 0.006b | 25.81 ± 0.452a                |
| 30               | Control            | 12.53 ± 0.058a  | 12.53 ± 0.058a          | 0.51 ± 0.010a | 23.59 ± 0.557a                |
|                  | *P. brasilensis* YS-1 | 12.53 ± 0.058a  | 12.53 ± 0.058a          | 0.51 ± 0.010a | 23.59 ± 0.557a                |
| 40               | Control            | 12.13 ± 0.058b  | 11.62 ± 0.273b          | 0.45 ± 0.015b | 19.24 ± 0.263b                |
|                  | *P. brasilensis* YS-1 | 12.10 ± 0.000a  | 12.10 ± 0.100a          | 0.44 ± 0.017a | 21.07 ± 0.164a                |
| 50               | Control            | 11.70 ± 0.100b  | 10.75 ± 0.176b          | 0.37 ± 0.017b | 18.38 ± 0.488b                |
|                  | *P. brasilensis* YS-1 | 11.90 ± 0.000a  | 11.90 ± 0.000a          | 0.40 ± 0.088a | 19.49 ± 0.327a                |
| 60               | Control            | 11.57 ± 0.058b  | 10.15 ± 0.224b          | 0.34 ± 0.013b | 15.92 ± 0.225b                |
|                  | *P. brasilensis* YS-1 | 11.90 ± 0.000a  | 11.90 ± 0.000a          | 0.40 ± 0.088a | 19.49 ± 0.327a                |

The TA content in the *P. brasilensis* YS-1-treated and control groups dropped gradually with a prolonged storage time (Table 1). It was found that a slower reduction of the TA content was observed in the *P. brasilensis* YS-1-treated group compared to the non-treated controls. Further statistical comparisons indicated that the TA content in the *P. brasilensis* YS-1-treated group was notably (*p < 0.05*) higher than in the control fruits from 20 d to 60 d.

Table 1 illustrates that the VC content in the *P. brasilensis* YS-1-treated and control groups increased slightly and reached the highest level at 20 d, and then dropped quickly as storage time progressed, while the *P. brasilensis* YS-1-treated group presented a significantly (*p < 0.05*) lower VC loss than the control group during the whole storage time.
3.3. Change in the Respiration Rate of Citrus Fruits after *P. brasiliensis* YS-1 Treatment

Fruit respiration is a vital factor for evaluating the postharvest quality and storability of citrus fruit and other horticultural products; the higher the fruit respiration, the more nutrients are consumed and the lower the storability of harvested fruit. After falling sharply in the first 30 d of cold storage, the respiration rate of the control group showed a noticeable increase from 30 d to the end of storage, while the *P. brasiliensis* YS-1-treated fruit exhibited a slight upward trend (Figure 3). Further comparisons demonstrated a significant difference between the respiration rate of the *P. brasiliensis* YS-1-treated and control groups after 20 d of cold storage.

![Figure 3. Effect of *P. brasiliensis* YS-1 treatment on the respiration rate of citrus fruits stored at 5 ± 0.5 °C for 60 d. Vertical bars represent the mean ± SE (n = 3). Letters indicate statistical differences according to an independent samples *t*-test (*p* < 0.05) on each storage day.](image3.png)

3.4. Change in the MDA Content of Citrus Fruits after *P. brasiliensis* YS-1 Treatment

The MDA content increased gradually regardless of treatment during the complete cold storage period. Postharvest treatment of *P. brasiliensis* YS-1 delayed the increase in MDA content. At the end of storage, the MDA content in the *P. brasiliensis* YS-1-treated fruits was about 19.4% lower than that in control fruit (Figure 4). During the last 50 d of cold storage (except for day 20), a slower increase in the MDA content was observed in the *P. brasiliensis* YS-1-treated fruit compared to the control group.

![Figure 4. Effect of *P. brasiliensis* YS-1 treatment on the malondialdehyde (MDA) content of citrus fruits stored at 5 ± 0.5 °C for 60 d. Vertical bars represent the mean ± SE (n = 3). Letters indicate statistical differences according to an independent samples *t*-test (*p* < 0.05) on each storage day.](image4.png)

3.5. Change in the Defensive Enzyme Activity of Citrus Fruit after *P. brasiliensis* YS-1 Treatment

The SOD activity in the control fruits increased during the first 20 d of cold storage and then dropped sharply in the remaining storage time (Figure 5A). The *P. brasiliensis* YS-1 treatment peaked at day 30, with the peak of SOD activity in the *P. brasiliensis* YS-1-treated fruit being significantly higher (*p* < 0.05) than that of the control fruits. During the last 40 d of cold storage, there was a significant difference in the SOD activity between the two groups.
The POD activity in the *P. brasilensis* YS-1-treated and control groups increased rapidly during the first 30 d of cold storage, followed by a decline during the subsequent storage period (Figure 5B). Compared with the control group, further comparisons indicated that a higher value of POD activity was observed in the *P. brasilensis* YS-1-treated fruits from 20 d to 50 d of cold storage.

The PPO activity in the *P. brasilensis* YS-1-treated and control fruits showed a similar tendency, rising gradually during the first 40 d of cold storage, then dropping sharply over the following days (Figure 5C). However, after 30 d of storage, the PPO activity in the *P. brasilensis* YS-1-treated fruits was higher than that of the control fruits.

The change in the PAL activity exhibited a similar trend to the POD activity during the whole storage period, in the control and *P. brasilensis* YS-1-treated groups, with PAL activity initially increasing, then peaking at 30 d, after which the activity decreased gradually, and the treatment of *P. brasilensis* YS-1 maintained a higher PAL activity compared with the control group after 20 d of storage (Figure 5D).

### 3.6. Pearson Correlation of Citrus Fruits after *P. brasilensis* YS-1 Treatment

A correlation-based approach using the Pearson coefficient was chosen to evaluate the positive and negative relationships between the decay incidence and defensive enzyme (SOD, POD, PPO and PAL) activity, VC content, respiration rate and MDA content and enzyme activity for *P. brasilensis* YS-1-treated and control citrus fruits during cold storage at 5 ± 0.5 °C for 60 d. Significant positive correlations (in red) and negative correlations (in blue) are displayed in Figure 6.

The numerical value and color intensity are proportional to the Pearson correlation coefficients. The correlation coefficient indicates that the defensive enzymes, respiration, and MDA involved in reactive oxygen species (ROS), energy, and membrane lipid metabolism in citrus fruits, are differentially regulated by postharvest treatments and cold storage, as previously demonstrated in other horticultural crops, such as kiwifruits [31], pears [30], and broccoli [32].

**Figure 5.** Effects of *P. brasilensis* YS-1 treatment on SOD (A), POD (B), PPO (C) and PAL (D) activities of citrus fruits stored at 5 ± 0.5 °C for 60 days. Vertical bars represent the mean ± SE (n = 3). Letters indicate statistical differences according to an independent samples *t*-test (*p* < 0.05) on each storage day.
YS-1 application for enhancing the postharvest preservation of citrus fruits. The results of the current study indicate that 

\( P. \) brasiliensis efficiently reduced the decay incidence and weight loss in citrus fruit, mimicking earlier studies. Lai et al. [35] and Wu et al. [36] demonstrated that a postharvest treatment with antagonistic microorganisms (Photorhabdus luminescens (Enterobacteriaceae) Hb1029 and Bacillus amyloliquefaciens subsp. LY-1) developed biofilm formations on the surface of litchi fruits since they have a latent effect in suppressing fungal infections and continuous fruit respiration. Furthermore, both in vitro and in vivo tests showed that \( P. \) brasiliensis YS-1 prominently inhibited the spore germination of \( P. \) italicum to induce blue mold development on Xinyu tangerines in our previous study [23,33]. Less decay incidence was observed in \( P. \) brasiliensis YS-1-treated citrus fruits, which might be linked to its antimicrobial potential.

The SOD activity was positively correlated with the POD activity (\( r = 0.879; p < 0.01 \)) and the VC content (\( r = 0.861; p < 0.01 \)). This result indicates that SOD, POD, and VC are the main important antioxidant enzymes or compounds responsible for scavenging excess ROS and reducing oxidative damage, and together with PPO and PAL make up a defensive system to improve fruit disease resistance. Furthermore, the SOD activity was negatively correlated with the respiration rate (\( r = -0.553; p < 0.05 \)), MDA content (\( r = -0.438; p < 0.05 \)) and decay incidence (\( r = -0.734; p < 0.01 \)).

The results show a significant positive correlation between the MDA content and decay incidence (\( r = 0.912; p < 0.01 \)), highlighting that membrane lipid peroxidation is responsible for the accumulation of MDA, which decreases fruit disease resistance and leads to the occurrence of decay rot.

4. Discussion

The potential of \( P. \) brasiliensis YS-1 as a biocontrol agent for controlling citrus postharvest disease has been well reported [25,33]. However, the current study is unique as it deals with \( P. \) brasiliensis YS-1 application for enhancing the postharvest preservation of citrus fruits. The results of the current study indicate that \( P. \) brasiliensis YS-1 could significantly reduce fruit decay incidence, maintain a high nutritional quality, suppress fruit respiration and MDA accumulation, while increasing the activities of defense-associated enzymes (SOD, POD, PPO, and PAL) in citrus fruit.

Fruit decay incidence and weight loss are two important indicators in evaluating the postharvest storability of harvested fruits [17,34]. Generally, the decay incidence and weight loss of harvested fruits increase with prolonged storage periods due to the attenuation of disease resistance and continuous fruit respiration. The data obtained in the current study show that a 2.0% fermented filtrate of \( P. \) brasiliensis YS-1 efficiently reduced the decay incidence and weight loss in citrus fruit, mimicking earlier studies. Lai et al. [35] and Wu et al. [36] demonstrated that a postharvest treatment with antagonistic microorganisms (Photorhabdus luminescens (Enterobacteriaceae) Hb1029 and Bacillus amyloliquefaciens subsp. LY-1) developed biofilm formations on the surface of litchi fruits since they have a latent effect in suppressing fungal infections and fruit respiration. Furthermore, both in vitro and in vivo tests showed that \( P. \) brasiliensis YS-1 prominently inhibited the spore germination of \( P. \) italicum to induce blue mold development on Xinyu tangerines in our previous study [23,33]. Less decay incidence was observed in \( P. \) brasiliensis YS-1-treated citrus fruits, which might be linked to its antimicrobial potential.
Therefore, given that its biocontrol capacity to reduce decay incidence and weight loss is directly linked with its antimicrobial and biofilm-forming ability on the surface of citrus fruits, it should be suggested that *P. brasilensis* YS-1 should be considered as a promising biocontrol agent to control postharvest diseases and prolong the storage-life of harvested citrus fruit.

TSS, total sugar, TA, and VC are considered as important indicators to evaluate the nutritional quality, texture, and flavor of harvested citrus fruits [2]. The TSS is one of the best quality parameters to appraise the texture and nutritional value of fresh horticultural products [29,37]. Total sugar comprises multiple monosaccharides, including glucose, fructose, maltose, sucrose, and certain hydrolysable starches [38]. The TA of sweet oranges, mandarins, tangerines, ponkans, and pummelos are generally interpreted as the percentage of citric acid present in citrus fruits, which are a major source of citric acid [38]. Fresh citrus fruit is the best source of vitamin C (VC), which has an antioxidant capacity and nutritional benefits for human health [37,39]. Additionally, VC is commonly used as a quality parameter for estimating the nutritional value of horticultural products [28,34]. In the current study, *P. brasilensis* YS-1-treated citrus fruits had higher TSS, total sugar, TA, and VC contents after 30 d of storage, indicating that the treatment of *P. brasilensis* YS-1 could potentially reduce nutrient degradation in the later storage period (Table 1). Similar results were previously reported by Habiba and colleagues [40] who noted that Kinnow mandarin (*Citrus reticulata* Blanco) treated with two epiphytic yeasts (HAB-31 and HAB-53) had higher contents of TSS, TA, and VC than the control fruit. Lai and co-workers [35] reported that the change in TSS, TA, and trehalose contents were decreased by treating litchi fruit with a suspension of *P. luminescens* Hb1029 (1.0 × 10^8 CFU mL^{-1}). A possible explanation for these results may be that *P. brasilensis* YS-1 formed a biofilm to suppress the vital activities of fruits, such as reducing the fruit respiration rate, reducing water evaporation and delaying nutrient degradation. These results reveal that the treatment of *P. brasilensis* YS-1 could significantly delay the degradation of TSS, TA, and VC, and subsequently lead to maintaining a higher nutritional quality in the pulp of harvested citrus fruits.

Fruit respiration is another important parameter that affects the deterioration of nutrients and limits the storage life of harvested citrus fruit [28,37]. It is linked with deleterious changes in organic acids and soluble carbohydrates. In our current study, the increased respiration rate was significantly slowed down by the treatment of *P. brasilensis* YS-1 compared with the non-treated citrus fruit (Figure 3), corresponding with the lower reduction of TA and TSS content (Table 1). The lower decay incidence (Figure 1), weight loss (Figure 2), and MDA accumulation (Figure 4), indicate that the potential of *P. brasilensis* YS-1 to delay postharvest fruit senescence can be linked with the inhibition of the respiration rate. The rate of fruit respiration increased in the control group, suggesting that vigorous respiration and oxidative stress probably lead to a high decay incidence in citrus fruit. Additionally, the increase in the respiration rate during storage life can be due to the loss of organic acids, especially citric acid [38]. Similarly, several studies reported the same phenomenon for pears [41], plums [39] and table grapes [42]. These findings collectively demonstrate that a postharvest treatment of *P. brasilensis* YS-1 down-regulated fruit respiration and delayed the deterioration of the nutritional quality of citrus fruits under cold storage.

Membrane damage or deterioration is a peculiar physiological feature of plant senescence, and this process is chiefly accompanied by an intensified membrane lipid peroxidation with the accumulation of MDA, a final product of cell oxidative damage [14,35,42]. In harvested citrus fruit, the MDA content gradually increased as fruit ripening and senescence progressed during postharvest storage. However, the postharvest application of *P. brasilensis* YS-1 significantly suppressed the accumulation of the MDA content in citrus fruits (Figure 4). The result clearly showed that the lower the disease resistance, the higher the decay incidence. This indicates that a treatment with *P. brasilensis* YS-1 could delay the process of membrane lipid peroxidation and hence reduce oxidative cellular damage and fruit decay, and delay postharvest senescence in citrus fruit. This is consistent with studies showing that treatment with antagonistic microorganisms such as *P. luminescens* Hb1029, *Bacillus subtilis*, *B. amyloliquefaciens* L-1...
and *R. paludigenum*, could reduce the membrane lipid peroxidation in sweet oranges [34], litchis [35], pears [43] and jujubes [44] during postharvest storage.

Both SOD and POD play crucial roles in scavenging reactive oxygen species (ROS) and reducing membrane lipid peroxidation, and hence protecting fruits from oxidative stress [31,34]. In addition, polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL) are the main defense enzymes in plant resistance physiology. PPO is a critical enzyme that participates in the oxidation of phenolic compounds, and PAL remains the key enzyme involved in the phenylpropanoid pathway. Therefore, PPO and PAL activity could be used as important indicators to assess disease resistance processes in plant tissues [17,22]. It is also reported that an enhanced disease resistance in harvested citrus fruits and other horticultural products is closely related to increased levels of SOD, POD, PPO, and PAL [17,34,41,42].

Herein, the results demonstrated that postharvest treatments of *P. brasilensis* YS-1 significantly increased the activities of SOD, POD, PPO, and PAL in citrus fruits (Figure 5). Based on these findings, it is demonstrated that the treatment of *P. brasilensis* YS-1 could significantly enhance and maintain a higher activity of defensive enzymes, such as SOD, POD, PPO and PAL in citrus peel, and suppressed fruit respiration and the accumulation of MDA content, resulting in a lower decay incidence and retarding postharvest fruit senescence of Xinyu tangerines. Similarly, other botanical preservatives, as alternatives to synthetic fungicides, have been applied to improve SOD and POD activity in Xinyu tangerines [2]. Wang and co-workers [34] also found that the co-fermentation of probiotics and Chinese herbs, as a novel liquid fermentation compound (LFC), induced the elevation of SOD, PPO, PAL, and POD activities in citrus fruits. Meanwhile, an interesting finding in the current study is that postharvest treatment with *P. brasilensis* YS-1 could markedly delay the oxidation of VC (ascorbic acid, AsA) and maintain its high level in harvested citrus fruits (Table 1). As an important nutrient, VC has potent antioxidant capacity to eliminate ROS and reduce oxidative cellular damage in citrus fruit in postharvest storage, implying that a higher VC content could delay the process of citrus fruit senescence and ultimately prolong its storage life [28,39]. Similar results were reported for different fruits (litchis, strawberries, pears, apples, and jujubes) treated with other antagonistic microorganisms, such as *P. luminescens* Hb1029 [35], *B. amyloliquefaciens* LY-1 [36], *Lactobacillus plantarum* [45], *B. amyloliquefaciens* L-1 [46], *Sporidiobolus pararoseus* Y16 [47], and *R. paludigenum* [44]. Hence, the results in our current study indicate that the treatment of *P. brasilensis* YS-1 can effectively delay postharvest senescence and prolong the storage life of citrus fruits. This is probably due to the high levels of defensive enzymes and VC content in the *P. brasilensis* YS-1-treated fruits during the middle and late stage of the storage period at 5 °C. This may be due to a higher ROS scavenging capacity, contributing to the prevention of oxidative damage that begins during fruit senescence due to membrane lipid peroxidation.

5. Conclusions

The results led us to conclude that the use of *P. brasilensis* YS-1 retarded the postharvest senescence of citrus fruit. Moreover, *P. brasilensis* YS-1-treated fruits showed a lower decay incidence, weight loss, respiration rate and MDA content, and postponed the degradation of TSS, total sugar, TA, and VC. This contributed to maintaining higher SOD, POD, PPO, and PAL activities. Taking all the data together, *P. brasilensis* YS-1 could be considered as a potential biocontrol agent for the postharvest preservation of citrus fruit. However, the applicability and commercialized production of *P. brasilensis* YS-1 need to be investigated.

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