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Full Length Research Paper

Efficacy of preharvest spraying with *Pichia guilliermondii* on postharvest decay and quality of cherry tomato fruit during storage

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This study investigated the effects of preharvest spraying with *Pichia guilliermondii* on postharvest natural decay of cherry tomato fruit, and evaluated the quality attributes during storage. Preharvest spraying with *P. guilliermondii* significantly decreased the decay index. Moreover, the efficacy of this biological control was positively correlated with spraying frequency. However, preharvest spraying with *P. guilliermondii* and spraying frequency did not significantly affect fruit quality attributes regardless of whether the cherry tomato fruit was grown or stored after harvest. This observation indicates that preharvest spraying with *P. guilliermondii* does not influence the quality attributes of the fruit. In addition, the activities of peroxidase (POD), phenylalanine ammonia-lyase (PAL), and β-1,3-glucanase increased in all *P. guilliermondii* treatments, suggesting a positive correlation with spraying frequency. The results suggest that cherry tomato fruit is capable of responding to preharvest spraying with *P. guilliermondii*, which can activate its defensive enzymes and thus induce host disease resistance.

Key words: Preharvest, *Pichia guilliermondii*, cherry tomato fruit, decay, quality.

INTRODUCTION

Cherry tomato fruit is popular in China because of its delicious taste and contribution to human health and nutrition (Zhao et al., 2009). However, a considerable amount of cherry tomato fruit is lost during fruit growth and after harvest. Even fruit that appears healthy when harvested may cause significant losses during storage due to the quiescent and latent infections in the field, and these latent infections become major factor for decay during transportation or storage of cherry tomato fruit (Sharma et al., 2009).

Although synthetic chemical fungicides can control certain diseases effectively (Korsten, 2006; Spadaro and Gullino, 2004), the global trend appears to be shifting towards reduced use of fungicides on produce because of the problems related to the development of pathogens' fungicide resistance and potential toxicity on human health and the environment (Sharma et al., 2009). Promising results have been achieved using antagonistic microorganisms to effectively inhibit a number of postharvest pathogens on harvested commodities (Zhao et al., 2010). However, researches have found that currently available antagonists fail to control previously established infections, such as latent and quiescent infections (Cañamás et al., 2008a; Ippolito and Nigro, 2000b). Moreover, fruits may be subjected to damage through wounds in harvesting operations and postharvest handlings (Ippolito et al., 2000b). Field applications may avoid this limitation because antagonistic microorganisms enable a preemptive colonization, which can be appropriate to protect fruit from subsequent infections.

It appears that this strategy of preharvest applications of microbial antagonists to control postharvest decay on fruits and vegetables deserves more attention and it has been quite successful in certain cases. For instance, Kovach et al. (2000) found that preharvest application with *Trichoderma harzianum* had given promising result in controlling postharvest diseases of strawberry. Moreover,

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preharvest spray of *Metschnikowia fructicola* appeared to effectively inhibit preharvest and postharvest rots of strawberry (Kurtzman and Droby, 2004). Results of Larena et al. (2005) have identified that the postharvest brown rot of peaches could be controlled effectively by field applications of *Epicoccum nigrum*. In addition, Cañamás et al. (2008b) had reported that preharvest application of *Pantoea agglomerans* at different concentrations was effective for protecting oranges against *Penicillium digitatum* during storage.

Preharvest application of antagonistic microorganisms can suppress the pathogen at the source, which may reduce harmful microorganisms on infection, and may protect the environment and human health (Ippolito et al., 2005; Tian et al., 2004). Results from our previous study have shown that cherry tomato fruit is capable of responding to the yeast *Pichia guilliermondii*, which effectively inhibits the growth of pathogenic fungi and then reduces postharvest decay (Zhao et al., 2008). Although at present, researches on preharvest applications of microbial antagonists for controlling fresh fruits' postharvest decay are common and the efficacy has been well documented, there is little information about the efficacy of preharvest spraying with *P. guilliermondii* on postharvest decay and quality of cherry tomato fruit during storage. Therefore, the objectives of this study were to examine: (1) the inhibitory effect of preharvest spraying with *P. guilliermondii* on natural decay in cherry tomato fruit during storage; (2) the influence of preharvest spraying with *P. guilliermondii* on fruit quality attributes, and (3) the changes of peroxidase (POD, EC 1.11.1.7), phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) and β-1,3-glucanase (EC 3.2.1.6) activities on cherry tomato fruit treated by preharvest spraying with *P. guilliermondii*..

MATERIALS AND METHODS

Fruit

Cherry tomato fruit [*Lycopersicon esculentum* Mill var. *cerasiforme* (Dun) Gray] of the cultivar 'Hongxing' were grown according to standard cultural practices in an organic greenhouse located in Nanjing, China. No fungicides were applied prior to harvest.

Antagonist

P. guilliermondii 2.1801, obtained from the China General Microbiological Culture Collection Center (CGMCC), was used in this study. It was maintained at 4℃ on nutrient yeast dextrose agar (NYDA) (1 L distilled water containing: 8 g nutrient broth, 5 g yeast extract, 10 g glucose and 20 g agar). Liquid cultures of the yeast were grown in 250 ml Erlenmeyer flasks. Each contained 50 ml of fresh nutrient yeast dextrose broth (NYDB: NYDA without agar), which had been inoculated with a loop of the culture. Flasks were incubated on a rotary shaker at 200 rpm for 24 h at 28℃ (stationary phase). The media were centrifuged at 2770 *g* for 10 min and washed twice with sterile distilled water in order to remove the residual growth medium. Cell pellets were then re-suspended in sterilized distilled water and brought to the desired concentration of 10^8 CFU ml^{-1} (CFU: Colony-forming units).

Preharvest treatment

This experiment, which is designed according to the method described by Meng et al. (2010), began from July, 2009 and was divided into four treatments; (A) at 15 days before harvest, a suspension of *P. guilliermondii* (concentration was 10^8 CFU ml^{-1}) in distilled water with 0.05% (w/v) Tween-80 as a surfactant was sprayed on the surface of cherry tomato fruit once by using hand-sprayer until all fruit were wet to runoff; (B) at 15 days and 1 day before harvest, a suspension of *P. guilliermondii* (concentration was 10^8 CFU ml^{-1}) in distilled water with 0.05% (w/v) Tween-80 as a surfactant was sprayed on the surface of cherry tomato fruit twice by using hand-sprayer until all fruit were wet to runoff; (C) at 15 days, 8 days and 1 day before harvest, a suspension of *P. guilliermondii* (concentration was 10^8 CFU ml^{-1}) in distilled water with 0.05% (w/v) Tween-80 as a surfactant was sprayed on the surface of cherry tomato fruit thrice by using hand-sprayer until all fruit were wet to runoff, and (CK) fruit sprayed with nothing were used as the control. Every treatment consisted of three replications and each replication contained five trees. The experiment was carried out in duplicate. Fruit were harvested in August, 2009 with a commercial level of maturity and then transferred immediately to the laboratory. All fruit samples were uniform in size and color, and free of physical injury or infection. The treated and control fruit were transferred into plastic boxes wrapped with high density polyethylene sleeve in order to retain high humidity (95%) and stored at 20℃ for 8 days. All fruit were evaluated according to following methods.

Decay assessment

The natural incidence was evaluated by means of decay index after eight days storage. Disease severity of cherry tomato fruit in each replication was assessed according to the following empirical scales: 1 = healthy fruit; 2 = one lesion smaller than 1 cm in diameter; 3 = one lesion bigger than 1 cm but the decay area smaller than 25% of fruit surface; 4 = decay area was between 25 to 40% of cherry tomato fruit surface; 5 = decay area bigger than 40% of fruit surface. Decay index was calculated by the following formula:

$$Decay\ index = \frac{1\times N1+2\times N2+3\times N3+4\times N4+5\times N5}{5\times NT}$$

Where, *N*1 to *N*5 is fruit's respective quantity of each scale and *NT* is the total number of fruit examined. Each treatment consisted of three replications and each replication contained 15 fruits. The experiment was repeated twice.

Quality attributes assessment

Fruit samples

Samples were taken from 20 fruit at time points: -15 (preharvest 15 days as the origin of experiment), 0, 4 or 8 days during storage. There were three replications for the assays in each treatment, and the experiment was repeated twice.

Quality assays

All assays were performed at ambient temperature (approximately 20ºC). Hardness was determined using Texture Analyzer (TA-XT2; Stable Micro Systems Ltd., UK) and the maximum force was recorded as fruit's hardness (N). Total soluble solids content was determined by measuring the refractive index of fruit juice with a hand-hold refractometer (WYT-4; Quanzhou Optical Instrument Co.

Ltd., Quanzhou, China) and the results were expressed as percentages. Titratable acidity content was measured by titrating 50 ml of the filtered liquid to pH 8.1 with 0.1 M NaOH and calculating the result as % citric acid according to Wright and Kader (1997). Ascorbic acid content was determined using the 2, 6-dichloro-indophenol method and the results were expressed as mg 100 g^{-1} (Özden and Bayindirli, 2002). Weight loss rate was determined before treatment (A) and after storage (B), and the (%) weight loss rate was calculated as (A - B)/A × 100%. Fruit colour was measured using a chromameter (CR-200; Minolta, Tokyo, Japan) and average readings of the luminosity (L*), chroma (C*), Hue angle (Hº) values at three pre-determined points on the fruit were recorded (McGuire, 1992).

Measurement of enzyme activity

Fruit samples

Samples were taken from 10 fruit at time points: -15 (preharvest 15 days as the origin of experiment), 0, 4 or 8 day during storage. There were three replications for enzyme assays in each treatment, and the experiment was repeated twice.

Extraction of enzymes

All procedures of enzyme extraction were performed at 4°C. For POD, 1 g of fruit tissue was ground with 3 ml of 100 mM sodium phosphate buffer (pH 6.4) containing 3% polyvinylpoly-pyrrolidone (PVPP, w/v). For PAL, fresh tissue (2 g) was ground with 5 ml of 100 mM sodium borate buffer at pH 8.7 containing 0.037% EDTA (w/v), 0.137% β-mercaptoethanol (v/v) and 3% PVPP (w/v). For β-1,3-glucanase, 10 g of sample was ground with 0.3 g of PVPP in 10 ml of citric acid-phosphate buffer (50 mM, pH 5.0). The extracts were homogenized and centrifuged at 10,000 *g* for 20 min at 4°C. Supernatant was collected for enzyme assays.

Enzyme assays

POD activity was assayed by a modified method of Kochba et al. (1977). The assay mixture contained 1 ml of enzyme extract, 1 ml of 100 mM sodium phosphate buffer (pH 6.4), 1 ml of 0.25% guaiacol (w/v) and 0.01 ml of 0.75% H_2O_2 (v/v). POD activity was measured by an increase in absorbance at 460 nm. One unit POD activity is defined as 0.01 increases in absorbance at 460 nm/min.

PAL activity was analyzed by using a modified method of Assis et al. (2001). Briefly, 0.5 ml of enzyme extract was incubated with assay medium containing 3.5 ml of 100 mM sodium borate buffer (pH 8.7), and 1 ml of 10 mM L-phenylalanine as substrate at 37°C for 1 h. The reaction was terminated by adding 0.2 ml of 6 mol L^{-1} HCl. PAL activity was measured by change in absorbance at 290 nm. One unit is defined as change of 0.01 absorbance at 290 nm/h.

β-1,3-glucanase activity was assessed by measuring the amount of reducing sugar released from laminarin by the dinitro-salicilate method (Ippolito et al., 2000a), with modification. 1 ml of enzyme extract was incubated with 0.2 ml of 0.1% laminarin (w/v) and 2.5 ml of citric acid-phosphate buffer (50 mM, pH 5.0) at 40°C for 2 h. The reaction was stopped by adding 1 ml of 3,5-dinitrosalicilate and boiling for 5 min. The final solution was diluted with 1.3 ml of distilled water and the amount of sugar reduction was measured spectrophotometrically at 540 nm. Blank control was enzyme extract mixed with laminarin but with zero incubation time. One unit is defined as the formation of 1 µmol glucose equivalents per hour.

Protein content in enzyme extracts was estimated by using the Bradford method (1976). Protein standard curve was prepared by sequential dilution of bovine serum albumin (BSA). Enzyme activity data were expressed as units per milligram protein.

Statistical analysis

Random samples were used in all experiments. Data were expressed as mean ± SD. SAS Software (Version 8.2; SAS Institute, Cary, NC, USA) was used for statistical analysis. One-way analysis of variance (ANOVA) was used to test statistical difference between treatments. Mean separations were performed by Duncan's multiple range tests. P value < 0.05 was considered as significant.

RESULTS

Effect of preharvest spraying treatments on decay index

The natural decay of most cherry tomato fruits during storage was caused by several pathogenic fungi, including *Rhizopus nigricans*, *Alternaria alternata,* and *Botrytis cinerea*, which are responsible for *Rhizopus* rot, black spot rot, and gray mold, respectively (Figure 1A). The presence of these fungi was observed in all treatments except in treatment C, which had no decayed fruit. After the cherry tomato fruits were stored at 20°C for 8 days, treatments A, B, and C were found effective in reducing the incidence of natural decay. In treatment A, the decay index was 0.4, which was significantly lower than that in CK (0.63; $P < 0.05$). The results in treatments B and C were better; the decay index of cherry tomato fruit decreased to 0.23 and 0.0, respectively, which were significant compared with that in CK ($P < 0.05$). Moreover, spraying frequency was positively correlated with the fruit's decay index, which demonstrated significant differences among the treatments ($P < 0.05$; Figure 1B).

Effect of preharvest spraying treatments on fruit firmness

With regard to the fruit's growth during preharvest and the increasing storage time, the cherry tomato fruits in all treatments suffered from a gradual softening, resulting in a significant decrease in firmness (from approximately 20.16 to 7.27 N; Figure 2). Preharvest spraying with *P. guilliermondii* and spraying frequency had no significant influence on the firmness of cherry tomato fruit ($P > 0.05$).

Effect of preharvest spraying treatments on total soluble solids and titratable acidity contents

The total soluble solids content increased rapidly with fruit maturity before harvest (Figure 3A). When stored at 20°C, the total soluble solids content in all treatments increased for four days. However, from days four to eight, total soluble solids content decreased due to nutrient consumption after harvest. Nevertheless, no significant

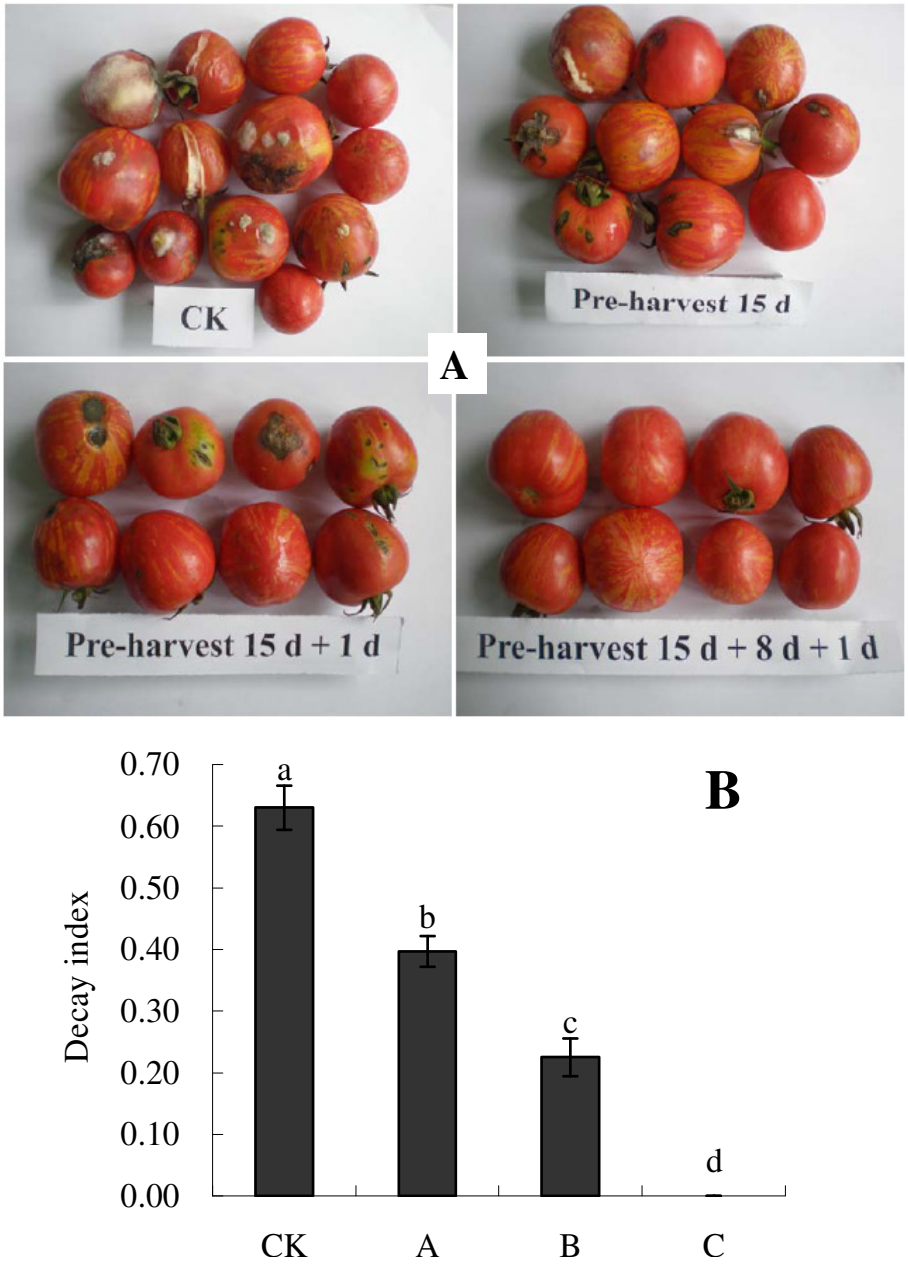

Figure 1. Development of natural decay in cherry tomato fruit after preharvest spraying with *P. guilliermondii*. (A) Symptoms of natural decay on the fruit after storage for eight days; (B) decay index of the cherry tomato fruit. Data represent the means ± S.D. Different letters are significantly different according to Duncan's multiple range test at $P < 0.05$ level. CK: Control; A: Treatment A; B: Treatment B; C: Treatment C.

differences were observed in all the treatments ($P > 0.05$).

The titratable acidity content increased with maturity and was not significantly affected by preharvest spraying with *P. guilliermondii* (Figure 3B). Significant decrease in the titratable acidity content was observed in all treatments. During the storage period, the fruits' respiration consumed parts of the titratable acidity as substrate, while the others were transformed into sugar. Moreover, preharvest spraying with *P. guilliermondii* and spraying frequency did not significantly affect the titratable acidity content during storage at 20°C ($P > 0.05$).

Effect of preharvest spraying treatments on ascorbic acid content

The trend of the change in ascorbic acid content in cherry tomato fruit was similar to those in total soluble solids and

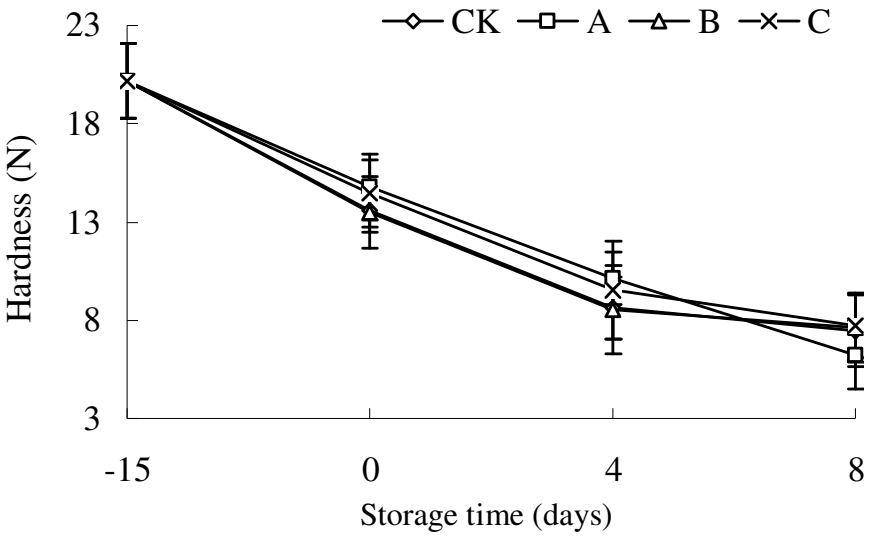

Figure 2. Effect of preharvest spraying with *P. guilliermondii* on the firmness of cherry tomato fruit. Data represent the means ± S.D. CK, control; A, treatment A; B: treatment B; C: treatment C.

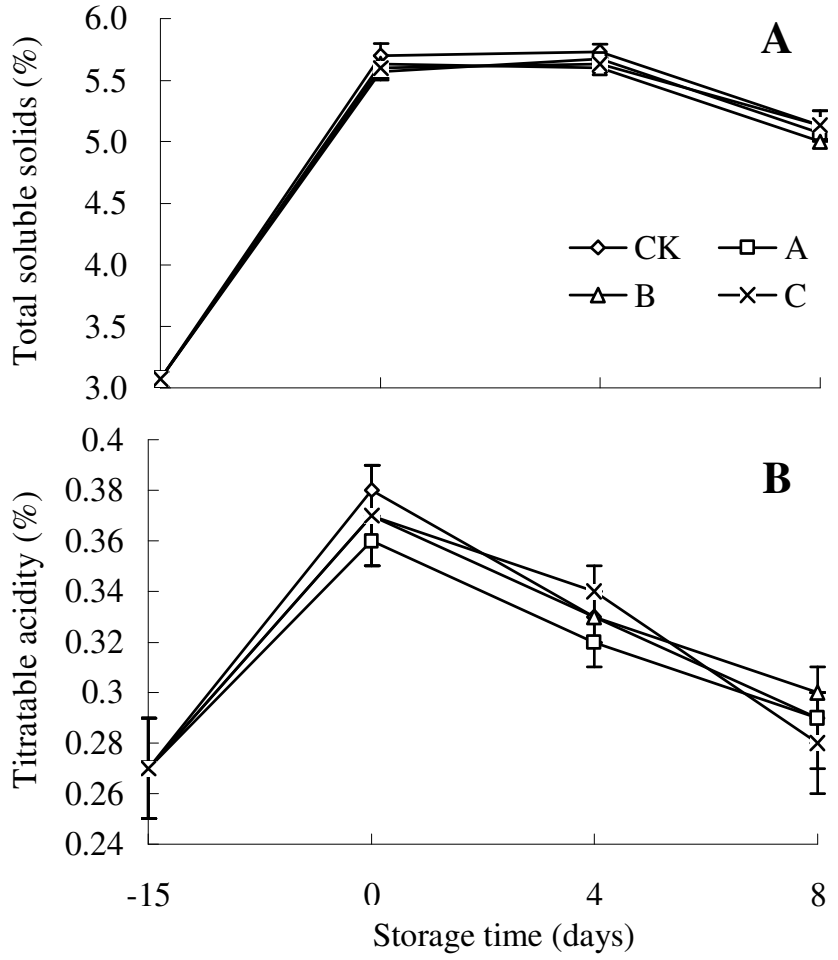

Figure 3. Effect of preharvest spraying with *P. guilliermondii* on the total soluble solids (A) and titratable acidity (B) of cherry tomato fruit. Data represent the means ± S.D. . CK, control; A, treatment A; B: treatment B; C: treatment C.

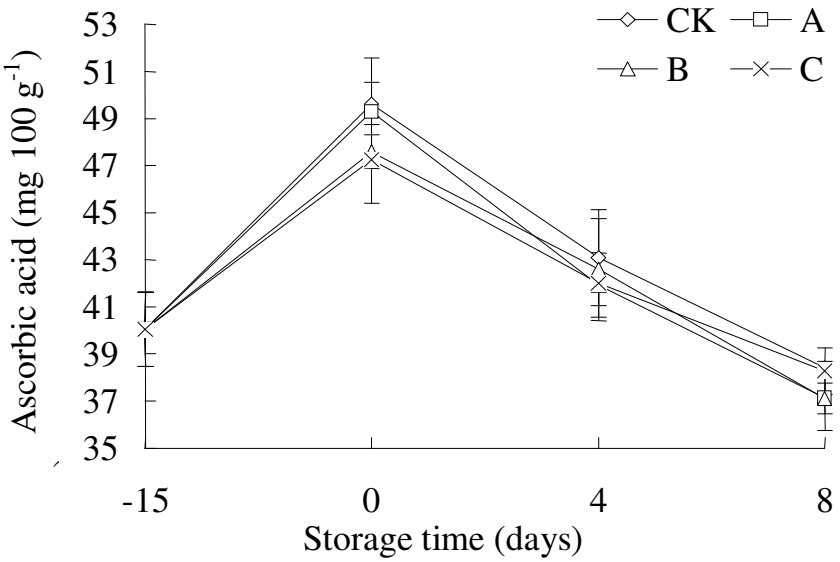

Figure 4. Effect of preharvest spraying with *P. guilliermondii* on the ascorbic acid content of cherry tomato fruit. Data represent the means ± S.D. CK, control; A, treatment A; B: treatment B; C: treatment C.

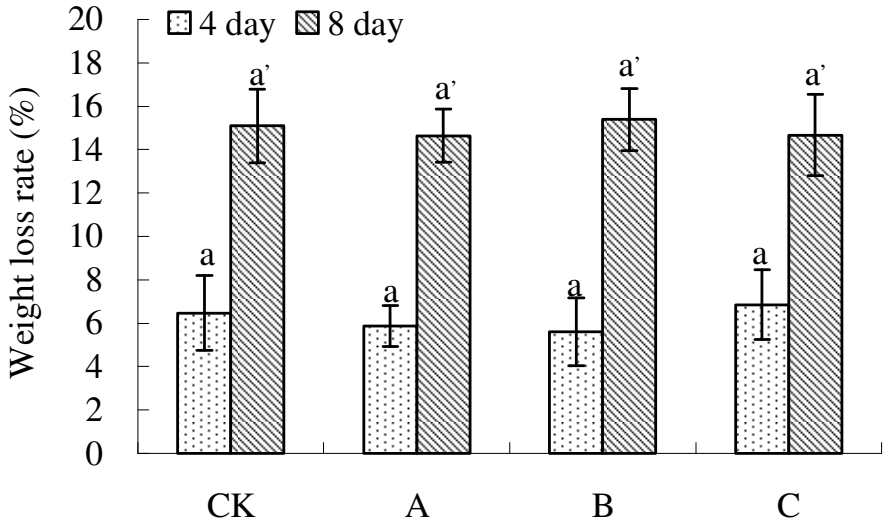

Figure 5. Effect of preharvest spraying with *P. guilliermondii* on the weight loss rate of cherry tomato fruit. . CK, control; A, treatment A; B: treatment B; C: treatment C.. Vertical bars represent standard deviations of the means. Means at the same day followed by the same letter are not significantly different according to Duncan's multiple range test at $P = 0.05$ level.

titratable acidity, which increased rapidly during the fruit growth period (Figure 4). However, because the nutrients of cherry tomato fruit were reduced gradually due to respiration, the substrate for the synthesis of ascorbic acid was insufficient; hence ascorbic acid content was unstable. After the cherry tomato fruits were stored at 20°C for eight days, the ascorbic acid content decreased significantly (approximately 22%) with no significant differences observed in all treatments ($P > 0.05$).

Effect of preharvest spraying treatments on weight loss rate

Given that weight loss occurs only after harvest, the weight of the cherry tomato fruits was determined at days four and eight during the whole storage period (Figure 5). At day four, the weight loss rate was approximately 6% in all treatments. After day eight, the weight loss rate of the control and treated fruits was approximately 15% due to

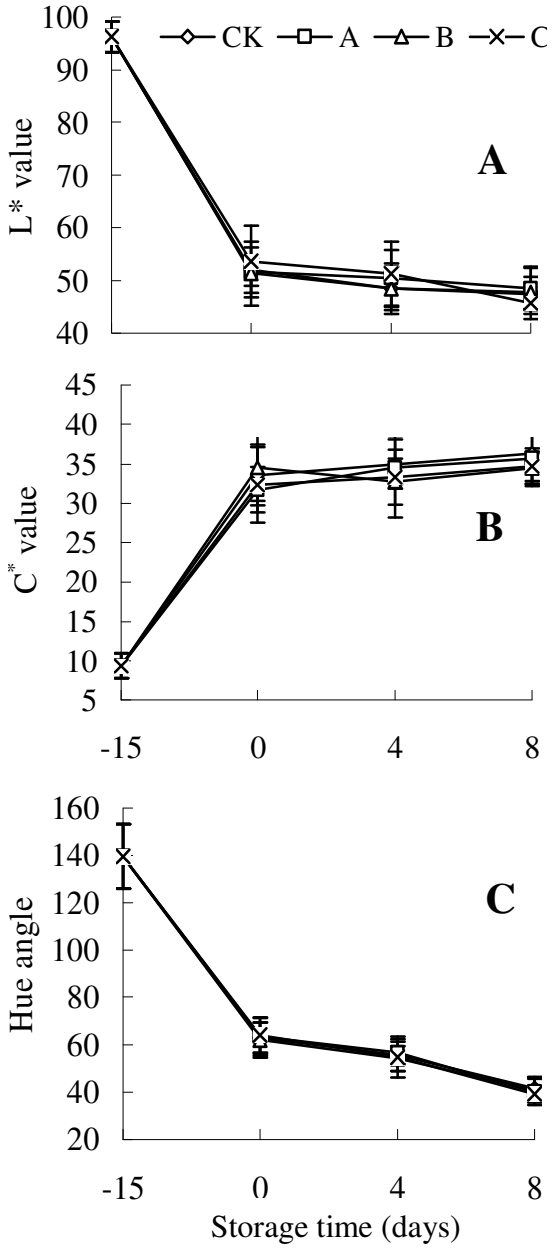

Figure 6. Effect of preharvest spraying with *P. guilliermondii* on the L* (A), C* (B) and Hº (C) values of cherry tomato fruit. Data represent the means ± S.D. CK, control; A, treatment A; B: treatment B; C: treatment C.

water loss for respiration. In addition, both preharvest spraying with *P. guilliermondii* and spraying frequency had no significant effect on weight loss rate of fruit compared with the control ($P > 0.05$).

Effect of preharvest spraying treatments on fruit colour

The colour of the cherry tomato fruits was expressed using the values of L*, C* and Hº (Figures 6A to C). The change in the trend of L* and Hº values were similar, wherein their values in all treatments decreased gradually from the initial time before harvest (-15 days) to the end of storage (day 8). Meanwhile, the C* value of all the fruits in all the treatments increased. The advance in colour of the cherry tomato fruits changed dramatically during the fruit growth period (days -15 to 0), and then gradually from the harvest time (day 0) to the end of the storage period (day 8). Furthermore, preharvest spraying with *P. guilliermondii* did not significantly affect the colour advance of the fruits in all the treatments ($P > 0.05$).

Effect of preharvest spraying treatments on enzyme activity

The effect of preharvest spraying with *P. guilliermondii* on the POD activity in cherry tomato fruit is shown in Figure 7A. In the control, the POD activity remained relatively stable from the initial time (-15 days) until the middle of storage period (four days). However, the POD activity increased from days four to eight, which is possibly caused by the extension of the lesions. In treatment A, the peak value of POD activity appeared at day four of storage time, and then declined rapidly thereafter. In treatment B, the POD activity increased to its maximum value at day 0, which was approximately 1.42 and 1.45-fold higher than those in CK and treatment A, respectively. At day 0, the POD activity decreased gradually, and then decreased rapidly starting from day four. In treatment C, the POD activity reached its maximum value at day 0, which was significantly higher than those in the other treatments ($P < 0.05$). The POD activity decreased rapidly from day 0 until it reached its lowest value at day four, which remained within that level until day eight.

As shown in Figures 7B, the PAL activity in all treatments was similar. In treatment C, the PAL activity increased after preharvest spraying with *P. guilliermondii*, which reached its maximum value at day four. Moreover, treatment C had significantly increased PAL activity throughout the whole storage period ($P < 0.05$). No significant differences in PAL activity were observed between treatments A and B from days 0 to eight ($P > 0.05$). The PAL activity reached its maximum value at day four in both treatments, which was approximately 1.67- and 1.89-fold higher than that in CK, respectively. In contrast, the PAL activity in CK increased before harvest, which remained relatively stable thereafter.

The β-1,3-glucanase activity was markedly induced in the cherry tomato fruits in all treatments, except CK (Figure 7C). In treatment C, the β-1,3-glucanase activity was significantly higher ($P < 0.05$) than that in CK after harvest (day 0 to eight). At day 0, the peak values of β-1,3-glucanase activity in treatments A and B were significantly lower than that in treatment C ($P < 0.05$) but they were still higher by 1.56- and 1.58-fold than that in

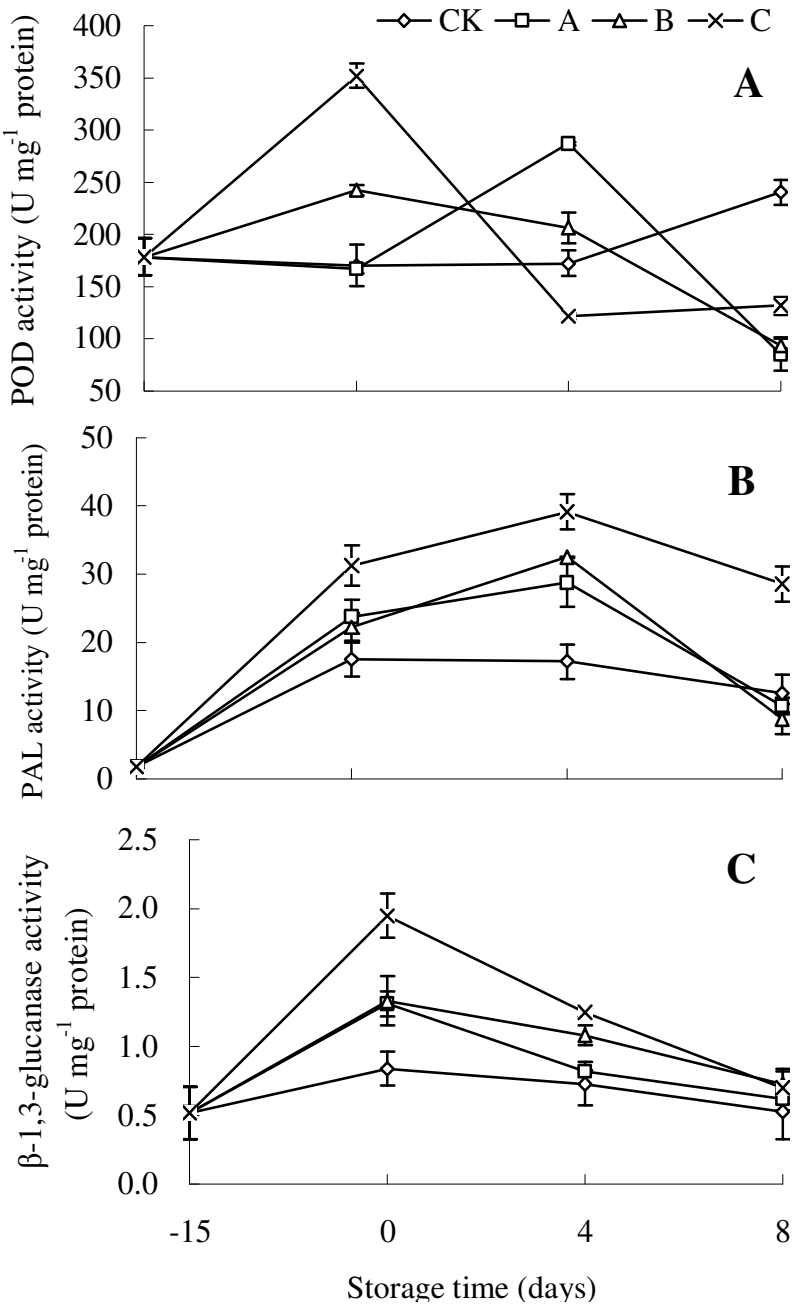

Figure 7. Effect of preharvest spraying with *P. guilliermondii* on the POD (A), PAL (B), and β-1,3-glucanase (C) activities in cherry tomato fruit during the storage period at 20°C. Data represent the means ± S.D. CK, control; A, treatment A; B: treatment B; C: treatment C.

CK. During the whole storage period, no significant change in the β-1,3-glucanase activity in CK was observed.

DISCUSSION

A substantial body of evidence has demonstrated that the infection of fruits by pathogens often occurs in the field prior to harvest (Biggs, 1995; Ippolito et al., 2000b; Sharma et al., 2009; Teixidó et al., 1998). Thus, applying antagonistic microorganisms before fruit harvest is advantageous to protect the wounds that form during picking and transport, prevent the invasion of pathogenic fungi, and prevent the incidence of fruit rot during storage. One of the important objectives of this study was to examine the efficacy of preharvest spraying with *P. guilliermondii* on the natural decay of cherry tomato fruit.

The initial time for preharvest spraying was 15 days before fruit picking, when most fruits are at the colour stages of turning (based on the USDA colour classification) and susceptible to pathogenic microorganism infections. Our results show that the incidence of natural decay in cherry tomato fruit after preharvest spraying with *P. guilliermondii* was significantly lower ($P < 0.05$) than that in the control after storage at 20°C for eight days (Figure 1). This finding is in accordance with previous studies, such as that of Tian et al. (2004). They reported that the preharvest application of 10^8 CFU ml^{-1} *Cryptococcus laurentii* efficiently controlled the postharvest decay of sweet cherry fruit, owing to the strong survival ability of *C. laurentii* on fruit surfaces under field conditions. Furthermore, our findings indicate that the inhibitory effect of preharvest spraying with *P. guilliermondii* on postharvest natural decay in cherry tomato fruit is positively correlated with its frequency of application. The result is consistent with a previous study by Larena et al. (2005), who demonstrated that the antagonist *E. nigrum* needed to be applied twice before harvest to reduce the incidence of postharvest brown rot in peaches. Therefore, given that different fruits have different growth cycles, we suggest that the frequency of preharvest application of biocontrol agents be increased appropriately.

Fruit quality is a crucial factor in evaluating the effect of strategic fresh keeping. Previous studies revealed that natural fungicides could decrease the decay index of fruit significantly with better quality attributes. For instance, Meng et al. (2008) observed that chitosan preharvest spraying and postharvest coating during storage at 20°C significantly decreased the weight loss rate of grape fruit. Castillo et al. (2010) reported that *Aloe vera*, which is effective in reducing the incidence of postharvest decay in table grapes, significantly reduced the fruit's respiration rate and weight loss rate, as well as delayed ripening parameters such as colour and firmness. We showed that preharvest spraying with *P. guilliermondii* had no significant effect on fruit firmness, total soluble solids, titratable acidity and ascorbic acid contents, as well as weight loss rate and colour value during the storage at 20°C for eight days (Figures 2 to 6; $P > 0.05$). Moreover, the results were similar regardless of the spraying frequency. The preharvest spraying with *P. guilliermondii* did not influence the postharvest quality attributes of cherry tomato fruit. Compared to natural fungicides such as chitosan and *A. vera*, the antagonistic yeast *P. guilliermondii* had no effect in delaying fruit senescence.

Researchers suggest that POD, PAL, and β-1,3-glucanase are involved in plant defence responses against fungal infections (Yao and Tian, 2005). In our previous study, we provided strong evidence that the action of reducing decay is associated with rapid increases in POD, PAL, and β-1,3-glucanase activities in cherry tomato fruit with postharvest *P. guilliermondii* treatments (Zhao et al., 2008). In this study, we assayed the changes in POD, PAL, and β-1,3-glucanase activities to evaluate the induction of host disease resistance. Compared with CK, the peak values of POD activity in treatments B and C were observed at day 0 while in treatment A it was observed at day 4 (Figure 7A). Furthermore, the peak value of POD activity in treatment C was higher than those in treatments A and B. This rapid induction of POD possibly plays a primary role in the last step of lignification. Researchers suggest that lignification enhances the mechanical resistance of fruits to pathogen penetration by increasing cell wall resistance to degrading enzymes secreted by microorganisms, thereby forming impermeable barriers to toxins (Ride, 1978). This is in accordance with previous studies such as that of Meng et al. (2010). They found that the preharvest application of *C. laurentii* enhanced the activities of POD in table grapes during the harvest and storage periods.

This study also show that preharvest spraying with *P. guilliermondii* significantly stimulated the PAL and β-1,3-glucanase activities in cherry tomato fruit. In treatment C, the peak values of PAL and β-1,3-glucanase activities were significantly higher than those in treatments A and B (Figures 7B and C). PAL is a key enzyme in the first step of phenylpropanoid pathway, which is directly involved in the synthesis of phytoalexins, lignin, and phenols that are correlated with localized resistance processes (Yao et al., 2005). β-1,3-glucanase is the most fully characterized PR protein since it can act directly by degrading a pathogen's cell wall or indirectly by releasing oligosaccharides, thereby eliciting defence reactions. Both processes are potential defence mechanisms against fungal infections (Tian et al., 2007). Considering the lowest decay level of cherry tomato fruit in treatment C (Figure 1), we deduce that the frequency of preharvest spraying with *P. guilliermondii* is therefore positively correlated with improved disease control.

Conclusions

The preharvest spraying with 10^8 CFU ml^{-1} *P. guilliermondii* is effective in controlling the postharvest natural decay of cherry tomato fruit. Moreover, the inhibitory effect is positively correlated with spraying frequency. Preharvest spraying with *P. guilliermondii* has no significant influence on the main quality attributes of cherry tomato fruit, and it induces the activities of POD, PAL, and β-1,3-glucanase, all of which are associated with plant defence responses.

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