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# Heat Treatment in Combination with Antagonistic Yeast Reduces Diseases and Elicits the Active Defense Responses in Harvested Cherry Tomato Fruit

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This study investigated the effects of heat treatment (hot air at 38 °C) and antagonistic yeast (*Pichia guilliermondii*) alone or in combination against postharvest diseases (*Botrytis cinerea*, *Alternaria alternata* and *Rhizopus nigricans*) on cherry tomato fruit, and evaluated the elicitation of active defense responses. Results showed that heat treatment at 38 °C for 24 h in combination with *P. guilliermondii* at $1 \times 10^8$ CFU $mL^{-1}$ was the most effective approach to reduce various infections on cherry tomato fruit's wounds. Moreover, the combined heat and *P. guilliermondii* treatment stimulated a rapid increase of $H_2O_2$ and higher lignin deposition in cherry tomato fruit showing that the oxidative burst and biological synthesis of lignin might play important roles in the fruit's active defense responses. In addition, the reduction of the fruit's susceptibility to pathogens by the combined treatment was positively correlated with higher activities of phenylalanine ammonia-lyase (PAL) and $\beta$-1,3-glucanase in cherry tomato fruits, both of which are associated with plant defense responses.

**KEYWORDS: Active defense responses; cherry tomato fruit; heat treatment; *Pichia guilliermondii*; postharvest diseases**

## INTRODUCTION

Tomato is an important agricultural commodity worldwide because of its contribution to human health and nutrition (*1*), and cherry tomato fruit is especially popular all over the world. However, a considerable amount of cherry tomato fruit is lost during fruit growth and after harvest due to the climate and undeveloped cold-chain transportation in China. *Botrytis cinerea*, *Alternaria alternata*, and *Rhizopus nigricans* are the three most common postharvest pathogens that are responsible for gray mold rot, black spot rot, and *Rhizopus* rot on cherry tomato fruit (*2*). Although synthetic chemical fungicides can control certain diseases effectively, problems related to the development of the pathogens' fungicide resistance and potential toxicity on human health and the environment have stimulated research of alternative measures for disease control (*3*). Promising alternatives such as postharvest heat treatment and biological control have shown potential in reducing the fruit's postharvest decay (*4*, *5*). However, heat treatment has little effect when inoculation occurs after heating (*6*). Similarly, biological control lacks eradicative activity, and its spectrum of activity is narrower than that of the synthetic fungicides (*7*). It is therefore necessary to use an integrated strategy with a desire to complement each other and even eradicate the use of chemical fungicides on postharvest commodities, rather than a single approach.

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Plants protect themselves from disease-causing organisms by activating a broad array of defense responses that ultimately inhibit the growth and spread of invading pathogens (*8*). One of the most prominent and initial defense responses is the generation of reactive oxygen intermediates (ROI) (*9*, *10*), such as superoxide anion ($O_2^-$), hydrogen peroxide ($H_2O_2$), and hydroxyl radical (•OH). They are directly toxic to pathogens and can trigger subsequent defense responses that include hypersensitive cell death, phytoalexin synthesis, lignification, and pathogenesis-related (PR) protein gene expression (*10*). These phenomena are known as the plant's active defense responses and can be initiated by a variety of biotic and abiotic elicitors.

In recent years, considerable amount of research has focused on the active resistance in harvested fruits and vegetables, which acts as an important manageable form of crop protection (*3*, *11*). Proper postharvest heat treatment can enhance the antioxidant capacity in various fruits and vegetables (*12*, *13*). Results from our previous study have shown that cherry tomato fruit is capable of responding to the yeast *Pichia guilliermondii*, which activates defense-related enzymes inducing host resistance to disease (*14*). Although disease resistance stimulated by heat treatment or antagonistic yeast has been well documented, there is little information about the elicitation of active defense responses in cherry tomato fruit when the above two methods are used in combination. Therefore, our objective was to study whether the elicitation of active defense responses, such as accumulation of $H_2O_2$, biological synthesis of lignin, and increased activity of


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several defense-related enzymes, is linked to the enhanced diseases resistance in stored cherry tomato fruit treated by a combination of postharvest heat treatment (hot air at 38 °C for 24 h) and antagonistic yeast (*P. guilliermondii* at $1 \times 10^8$ CFU $mL^{-1}$).

## MATERIALS AND METHODS

**Yeast and Pathogens.** *P. guilliermondii* 2.1801, obtained from the China General Microbiological Culture Collection Center (CGMCC), was used in this study. It was maintained at 4 °C on nutrient yeast dextrose agar (NYDA; 1 L 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 containing 50 mL of NYDB (= NYDA without added agar), which were inoculated with a loop of the culture. These flasks were incubated on a rotary shaker at 200 rpm for 24 h at 28 °C (stationary phase). The media were centrifuged at 2,770*g* for 10 min. The pellets were washed twice with sterile distilled water in order to remove the residual growth medium, then, resuspended in sterilized distilled water and diluted to the concentration of $10^8$ CFU $mL^{-1}$ (CFU: colony-forming units).

Isolates of *B. cinerea* FLB-1, *A. alternata* FLA-1, and *R. nigricans* FLR-1, originally isolated from infected cherry tomato fruit, were used in this study. They were maintained on potato dextrose agar (PDA, extract of boiled potatoes, 200 mL; dextrose, 20 g; agar, 20 g, and deionized water, 800 mL) at 4 °C. Fresh cultures were grown on PDA plates for 10 days at 26 °C before use. Spores were subsequently harvested by flooding the surfaces of cultures with sterile water. The conidial suspensions of *B. cinerea*, *A. alternata*, and *R. nigricans* were prepared in 0.05% (v/v) Tween-80, and spore concentrations were adjusted to $1 \times 10^5$ spores $mL^{-1}$ by using a hemacytometer.

**Fruit.** Cherry tomato fruit (*Solanum lycopersicum* var. *cerasiforme*) of the cultivar Jinzhu were harvested in August, 2008 at a red ripe stage (based on the USDA color classification) from Nanjing, China. Nearly 1,200 tomatoes were harvested from 120 plants that grew in the same greenhouse. Fruit were transferred to our laboratory within 2 h. All fruit were uniform in size and color with no physical injury or infection. Before treatment, fruit were surface-disinfected by immersion in 1% sodium hypochlorite for 2 min, rinsed with tap water, and air-dried at room temperature.

**Effect of Heat Treatment and *P. Guilliermondii* on Disease Incidence and Lesion Diameter of Cherry Tomato Fruit.** A wound (3 mm wide and 3 mm deep) was made at the equator of each fruit using a sterile puncture needle. Wound sites were inoculated by adding 15 $\mu$L of conidial suspension of *B. cinerea*, *A. alternata*, or *R. nigricans* at $1 \times 10^5$ spores $mL^{-1}$, and inoculated fruits were left to air-dry for 2 h before being divided into four groups: (I) CK, fruit did not receive any treatment and served as control; (II) H, fruit were left in hot air at 38 °C for 24 h (based on the result of our previous study) before being transferred to room temperature for 20 min to equilibrate the heat; (III) P, aliquots (20 $\mu$L) of *P. guilliermondii* suspension ($1 \times 10^8$ CFU $mL^{-1}$) were inoculated into wounds and dried by air at room temperature; (IV) H + P, fruits were treated with heat treatment at 38 °C for 24 h and 20 min of heat redistribution at room temperature, and aliquots (20 $\mu$L) of *P. guilliermondii* suspension ($1 \times 10^8$ CFU $mL^{-1}$) were inoculated into wounds and air-dried. All groups of fruit were kept in plastic boxes that were wrapped with a high density polyethylene sleeve in order to maintain high humidity (95%) and stored at 20 °C. Disease incidence and lesion diameter were recorded after 5 days of storage. Each group consisted of three replications and each replication contained 10 fruits. The experiment was repeated three times.

**Measurements of Active Defense Response-Related Enzymes in Cherry Tomato Fruits.** *Fruit Samples*. A wound (3 mm wide and 3 mm deep) was made at the equator of each fruit using the tip of a sterile puncture needle. Without pathogen inoculation, all wounded fruits were equally divided into CK, H, P, and H + P groups, and treated identically as described above. All fruit were transferred into plastic boxes and stored at 20 °C. In order to evaluate the elicitation of active defense responses under specific treatment conditions, samples were taken from 20 fruits at the following time points: 0, 1, 2, 3, 4, or 5 days after treatment. After removing the wound tissue (+1 mm distance from the edge of the wound), fresh tissues (+5 mm away from the edge of the wound) were collected. There were three replications for enzyme assays in each group, and the experiment was repeated twice.

*Extraction of Enzymes*. All procedures of enzyme extraction were performed at 4 °C. For superoxide dismutase (SOD, EC 1.15.1.1), 1 g of fruit tissue was ground with 3 mL of 50 mM sodium phosphate buffer (pH 7.8) containing 0.1 mM ethylene diamine tetraacetic acid (EDTA) and 3% polyvinyl polypyrrolidone (PVPP) (w/v). For catalase (CAT, EC 1.11.1.6), tissue (1 g) was ground with 3 mL of 50 mM sodium phosphate buffer (pH 7.0) containing 3% PVPP (w/v). For phenylalanine ammonia-lyase (PAL, EC 4.3.1.5), 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% $\beta$-mercaptoethanol (v/v), and 3% PVPP (w/v). For peroxidase (POD, EC 1.11.1.7) and polyphenoloxidase (PPO, EC 1.10.3.1), 1 g of fruit tissue was ground with 3 mL of 100 mM sodium phosphate buffer (pH 6.4) containing 3% PVPP (w/v). For $\beta$-1,3-glucanase (EC 3.2.1.6), 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. The supernatant was collected for enzyme assays.

*Enzyme Assays*. SOD activity was determined by using a modified method of Constantine and Stanley (*15*). Briefly, the reaction mixture (3 mL), containing 0.1 mL of enzyme extract, 50 mM sodium phosphate buffer (pH 7.8), 13 mM methionine, 75 $\mu$M nitroblue tetrazolium (NBT), 10 $\mu$M EDTA, and 10 $\mu$M riboflavin, was illuminated with a fluorescent lamp (60 $\mu$mol $m^{-2}$ $s^{-1}$) for 10 min, and the absorbance at 560 nm was recorded. An aliquot of identical solution was kept in the dark and served as the blank control. One unit of SOD activity is defined as the amount of enzyme that catalyzes 50% decrease of the SOD-inhibitable NBT reduction.

CAT activity was measured by adding 0.2 mL of the enzyme extract to 3 mL of 50 mM sodium phosphate buffer (pH 7.0) containing 0.2 mL of 0.75% $H_2O_2$ (v/v) as substrate (*16*). Decomposition of $H_2O_2$ was measured by reduction in absorbance at 240 nm (UV 1102 spectrophotometer, Shanghai, China). One unit is defined as the change of 0.01 absorbance per min.

PAL activity was analyzed by using a modified method of Assis et al. (*17*). 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 the change of 0.01 absorbance at 290 nm per h.

POD activity was assayed by a modified method of Kochba et al. (*18*). 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 of POD activity is defined as a 0.01 increase in absorbance at 460 nm per min.

PPO activity was examined by the method reported by Tian et al. (*19*). The assay mixture contained 0.1 mL of enzyme extract, 2 mL of sodium phosphate buffer (100 mM, pH 6.4), and 1 mL of catechol (65 mM). PPO activity was determined by an increase in absorbance at 398 nm. One unit of PPO activity is defined as a 0.01 increase in absorbance at 398 nm per min.

$\beta$-1,3-Glucanase activity was assessed by measuring the amount of reducing sugar released from laminarin by the dinitro-salicilate method (*20*), with modification. One milliliter 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. The blank control was the enzyme extract mixed with laminarin but with zero incubation time. One unit is defined as the formation of 1 $\mu$mol glucose equivalents per h.

Protein content in enzyme extracts was estimated by using the Bradford method (*21*). A protein standard curve was prepared by sequential dilution of bovine serum albumin (BSA). Enzyme activity data were expressed as units per milligram protein.

**Measurements of $H_2O_2$ and Lignin Contents in Cherry Tomato Fruits.** Fruit samples were collected as described above, and the experiment was repeated twice with three replications in each group. For the determination of $H_2O_2$ content, 2 g of fresh tissue was homogenized with

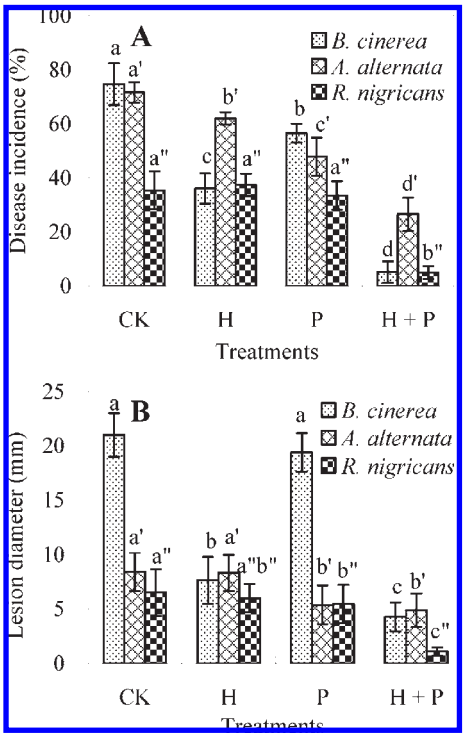


**Figure 1.** Effects of heat and *P. guilliermondii* on disease incidence (**A**) and lesion diameter (**B**) of gray mold rot, black spot rot, and *Rhizopus* rot during cherry tomato storage at 20 °C over 5 days. Data expressed as means ± SD ($n$ = 9). CK, control; H, heat; P, *P. guilliermondii*; H + P, a combination of heat and *P. guilliermondii*. Means followed by the same letter are not significantly different according to Duncan's multiple range test at $P < 0.05$ level.

5 mL of chilled 100% acetone and centrifuged at 10,000*g* at 4 °C for 20 min. The supernatant was immediately collected for analysis of $H_2O_2$ content by a method of Patterson et al. (*22*). $H_2O_2$ content was expressed as $\mu$mol $g^{-1}$ FW.

Lignin content was determined gravimetrically by using a modified method of Femenia et al. (*23*). Two grams of fresh sample was dispersed in 10 mL of 98% $H_2SO_4$ at room temperature for 12 h, diluted to 200 mL with deionized water, and heated in a boiling water bath for 6 h. Insoluble material was recovered by vacuum filtration and washed thoroughly with hot water (90 °C) until acid free before drying at 105 °C overnight. The residual weight was recorded as percentage of lignin content.

**Statistical Analysis.** Random samples were used in all experiments. Data were expressed as the 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 the statistical difference between groups. Mean separations were performed by Duncan's multiple range tests. A $P$ value $< 0.05$ was considered as significant.

## RESULTS

**Effect of Heat Treatment and *P. Guilliermondii* on Disease Incidence and Lesion Diameter of Cherry Tomato Fruits.** The effect of heat treatment and *P. guilliermondii* on disease incidence of cherry tomato fruits is summarized in **Figure 1A**. Fruit treated with heat treatment or *P. guilliermondii* showed lower occurrence of disease caused by *B. cinerea* or *A. alternata* than that of controls ($P < 0.05$). Heat treatment demonstrated a greater inhibitory effect on *B. cinerea* infection than that of *P. guilliermondii* ($P < 0.05$). The yeast-treated fruit provided higher capacity against *A. alternata* infection than the heat-treated fruit ($P < 0.05$). A combination of heat treatment and *P. guilliermondii* was proven to be the best inhibitor to *B. cinerea* and *A. alternata* infection, which reduced the disease incidence by 70% and 45%, respectively.

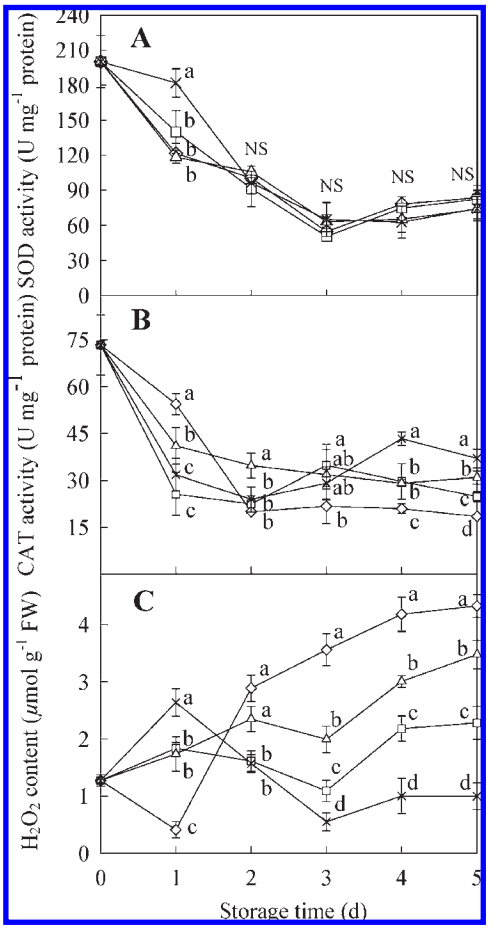


**Figure 2.** Effects of heat and *P. guilliermondii* on SOD (**A**), CAT (**B**) activities, and $H_2O_2$ content (**C**) in cherry tomatoes during storage at 20 °C. Data expressed as means ± SD ($n$ = 6). ◇, control; □, heat; Δ, *P. guilliermondii*; ×, a combination of heat and *P. guilliermondii*. Means at the same day followed by the same letter are not significantly different according to Duncan's multiple range test at the $P < 0.05$ level. NS, no significant difference.

Similarly, the inhibition to *R. nigricans* infection was enhanced by the combined treatment ($P < 0.05$).

As shown in **Figure 1B**, different treatments had various influences on the lesion diameter of cherry tomato fruit. Heat treatment alone had no inhibition ($P > 0.05$) to lesion spreading on fruit inoculated with *A. alternata* or *R. nigricans*. Similarly, *P. guilliermondii* could not effectively control the lesion spread caused by *B. cinerea*. However, there were reductions ($P < 0.05$) on lesion spreads caused by all three pathogens when cherry tomato fruits were treated with heat treatment at 38 °C for 24 h and *P. guilliermondii* at $1 \times 10^8$ CFU $mL^{-1}$ in combination.

**Effect of Heat Treatment and *P. Guilliermondii* on SOD, CAT Activities, and $H_2O_2$ Content in Cherry Tomato Fruits.** The effect of heat treatment and *P. guilliermondii* on SOD activity is summarized in **Figure 2A**. Fruit treated with heat, *P. guilliermondii*, or a combination of heat and *P. guilliermondii* demonstrated a similar pattern of reduction in SOD activity. There was a rapid decrease of SOD activity in the first 3 days of storage. Such striking reduction bottomed on day 3 and was sustained for two more days. Fruit treated with a combination of heat and *P. guilliermondii* lost less SOD activity than the control in the first 24 h ($P < 0.05$).

The effect of heat treatment and *P. guilliermondii* on CAT activity is shown in **Figure 2B**. CAT activity decreased ($P < 0.05$) in fruits treated with heat and *P. guilliermondii* alone or in

combination with heat and *P. guilliermondii* within the first 24 h of storage. A recovery of CAT activity was observed in the heat-, yeast-, and combination-treated fruit at 4−5 days ($P < 0.05$). Especially, the combination-treated fruit, exhibited approximately 2.1- and 2.0-fold higher CAT activities than the controls at 4 and 5 days, respectively.

**Figure 2C** showed that $H_2O_2$ content decreased ($P < 0.05$) in the control fruit within the first 24 h of storage; in contrast, fruit treated with heat, yeast, or a combination of heat and yeast showed a higher $H_2O_2$ content ($P < 0.05$). At the first 24 h, $H_2O_2$ content in cherry tomato fruit treated with a combination of heat and *P. guilliermondii* was approximately 6-fold higher than that of control fruit. However, it began to decrease during the rest of the storage period and dropped to the lowest level during 3−5 days. The content of $H_2O_2$ increased linearly between days 3 and 5 in the control, heat-, and yeast-treated fruit, and the heat-treated fruit showed lower $H_2O_2$ contents ($P < 0.05$) than the yeast-treated fruit.

**Effect of Heat Treatment and *P. Guilliermondii* on PAL, POD, and PPO Activities and Lignin Content in Cherry Tomato Fruits.** The effect of heat treatment and *P. guilliermondii* on PAL activity in cherry tomato fruit is summarized in **Figure 3A**. PAL activity in cherry tomato fruit varied during storage period. Compared with controls, 5.5- and 8.6-fold increases in PAL activity were observed in fruits treated with a combination of heat and *P. guilliermondii* in the first 24 h and third 24 h of storage, respectively. A similar first peak in PAL activity was also observed in fruits treated with *P. guilliermondii* alone. There was no difference in PAL activity between the heat-treated fruit and controls throughout the storage period except for days 2 and 3.

During 2−4 days, POD activity in the yeast-treated fruit maintained the highest levels compared with that of the control, heat-, and combination-treated fruit. However, it dropped sharply and showed the lowest level at day 5 (**Figure 3B**). POD activity in the combination-treated fruit began to increase after 2 days and peaked at 4 days after treatment, and the peak value was almost 2.3-fold higher than that of the control. However, POD activity in the heat-treated fruit showed lower levels ($P < 0.05$) than the controls between 2 and 4 days.

**Figure 3C** indicated that PPO activity increased gradually during 1 and 4 days and decreased sharply on the fifth day. Heat treatment and *P. guilliermondii* alone or in combination had no effect ($P > 0.05$) on PPO activity throughout the whole storage period.

The effect of heat treatment and *P. guilliermondii* on lignin content in cherry tomatoes is summarized in **Figure 3D**. Compared with controls, lignin content in cherry tomatoes treated with a combination of heat and *P. guilliermondii* increased by 1.6- and 1.7-fold on days 1 and 3, respectively ($P < 0.05$), and increased to a lower extent in fruit treated with *P. guilliermondii* alone on day 1 ($P < 0.05$). In contrast, lignin content in heat-treated fruit was lower than that of control fruit between days 1 and 4 ($P < 0.05$).

**Effect of Heat Treatment and *P. Guilliermondii* on β-1,3-Glucanase Activity in Cherry Tomato Fruits.** As shown in **Figure 4**, β-1,3-glucanase activity increased 1.6- and 1.5-fold in fruit treated with *P. guilliermondii* alone or a combination of heat and *P. guilliermondii* compared to that of the control at day 2 of storage ($P < 0.05$). In contrast, β-1,3-glucanase activity was lower in heat-treated fruit than that of controls ($P < 0.05$) between days 1 and 4 of storage.

## DISCUSSION

Preliminary in vitro tests proved that 24, 48, or 72 h heat treatment (at 38 °C) inhibited mycelial growth of *B. cinerea*, *A. alternata*, and *R. nigricans*. Furthermore, there is a positive

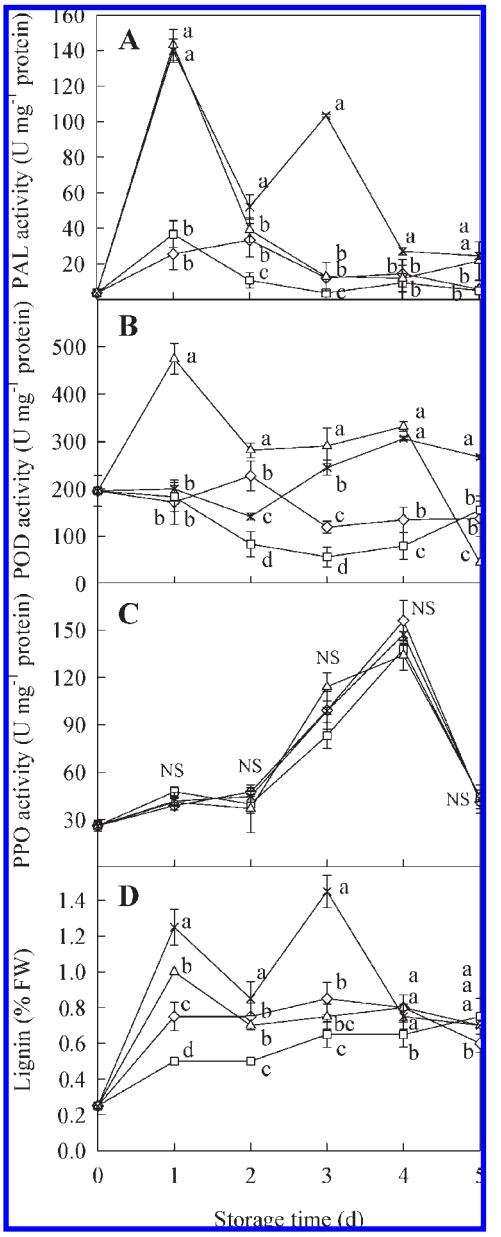


**Figure 3.** Effects of heat and *P. guilliermondii* on PAL (**A**), POD (**B**), and PPO (**C**) activities, and lignin content (**D**) in cherry tomatoes during storage at 20 °C. Data expressed as means ± SD ($n = 6$). ◇, control; □, heat; △, *P. guilliermondii*; ×, a combination of heat and *P. guilliermondii*. Means at the same day followed by the same letter are not significantly different according to Duncan's multiple range test at the $P < 0.05$ level. NS: no significant difference.

correlation between inhibitory rate and increased duration of heat treatment. Therefore, the observed reduction of pathogen attack in this study is partially due to the direct effect of heat stress on the pathogens. A major shortcoming of heat treatment is degradation of fruit quality factors, such as total soluble solids, titratable acidity, and ascorbic acid. Both 48 and 72 h heat-treated cherry tomatoes show decreases on quality factors. However, 24 h heat treatment has no influence on fruit firmness, flavor, and color (unpublished data). Therefore, heat treatment at 38 °C for 24 h was used for disease control in this study.

One of the most prominent and earliest fruit defense responses is oxidative burst, which generates ROI, including $O_2^-$, $H_2O_2$, and •OH. Generally, an appropriate intracellular balance between ROI generation and elimination exists in all cells. The SOD

and CAT, which work together with other enzymes of the ascorbate−glutathione cycle, can promote the elimination of ROI (*24*). SOD catalyzes the dismutation of $O_2^-$ into $H_2O_2$ and $O_2$, while CAT protects cells from $H_2O_2$ by catalyzing its decomposition into $O_2$ and $H_2O$ (*25*). In the present study, compared with the control, marked increase of SOD activity (**Figure 2A**) and decrease of CAT activity (**Figure 2B**) was observed in fruit treated with combined heat and *P. guilliermondii* treatment, and treatments lasting 24 h produced the highest level of $H_2O_2$ content (**Figure 2C**). This rapid accumulation of $H_2O_2$ possibly hinders microorganism penetration in plant tissues by facilitating peroxidase reactions catalyzing intra- and intermolecular cross-links between structural components of cell walls and lignin polymerization. In addition, $H_2O_2$ acts as a secondary messenger to induce expression of PR proteins and initiates the development of systemic acquired resistance (*26*). Subsequently, $H_2O_2$ content decreased sharply in fruit treated with a combination of heat and *P. guilliermondii* to protect fruit cells from oxidative damage, and between days 4 and 5 of storage, higher level of CAT activity in this experimental group may promote the elimination of $H_2O_2$ (**Figure 2B**). Because the most effective inhibitions to gray mold rot, black spot rot, and *Rhizopus* rot were observed in the combination-treated fruit (**Figure 1**), we suggest that the action of reducing decay is associated with a rapid increase of $H_2O_2$ in cherry tomato fruit.

The biological synthesis of lignin is slower than the oxidative burst in a plant's active defense response because it needs gene transcription and protein synthesis. Lignification has been implicated in enhancing the mechanical resistance to pathogen penetration by increasing cell wall resistance to degrading enzymes secreted by microorganisms, thus forming nonpermeable barriers to toxins (*27*). PAL is a key enzyme in the first step of the phenylpropanoid pathway, which is directly involved in the synthesis of phytoalexins, lignin, and phenols that are correlated with localized resistance processes (*3*). Our results provide strong evidence that the combined heat and *P. guilliermondii* treatment produced peak values of PAL activity (**Figure 3A**). This is associated with large depositions of lignin in the combination-treated fruit on days 1 and 3 of storage (**Figure 3D**). POD belongs to the PR-9 albumen family, and it plays a primary role in the last step of biological synthesis of lignin (*8*). Our findings indicate that, in cherry tomato fruit, the activities of PAL and POD were stimulated strongly by *P. guilliermondii*, and correspondingly, lignin content in the yeast-treated fruit was significantly higher than that of the control fruit within the first 24 h of storage. PPO catalyzes the formation of lignin and phenols, providing defense for plant cells in order to avoid pathogen infection. However, in this study, PPO may be not a crucial factor in the biological synthesis of lignin because no differences were observed in PPO activity between different experimental groups during the storage period (**Figure 3C**). Furthermore, activities of PAL and POD were inhibited by heat treatment in this study (**Figure 3A**,**B**), and lignin content in the heat-treated fruit was significantly lower than those in the control fruit between days 1 and 4 (**Figure 3D**). This may be due to a number of enzymes operating in the phenylpropanoid pathway being replaced by those acting in the synthesis of heat shock proteins (*28*, *29*). Therefore, considering the lowest decay of the combination-treated cherry tomato fruit (**Figure 1**), we can deduce that a combination of heat treatment with *P. guilliermondii* increases PAL activity and triggers the lignification of fruit's wound cells.

In addition to the lignification of the fruit's wound cells, the synthesis of PR protein is thought to be an important mechanism of resistance to various diseases. The current study showed that *P. guilliermondii*, alone or combined with heat treatment,

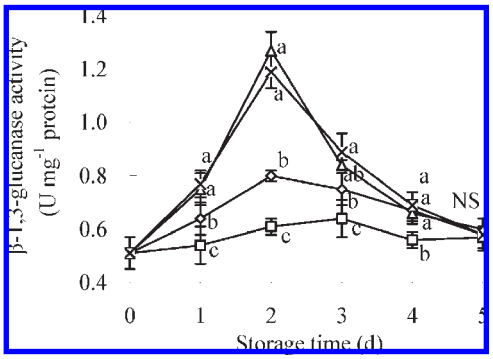


**Figure 4.** Effects of heat and *P. guilliermondii* on $\beta$-1,3-glucanase activity in cherry tomatoes during storage at 20 °C. Data expressed as means ± SD ($n$ = 6). ◇, control; □, heat; Δ, *P. guilliermondii*; ×, a combination of heat and *P. guilliermondii*. Means at the same day followed by the same letter are not significantly different according to Duncan's multiple range test at $P < 0.05$ level.

significantly stimulated the activity of $\beta$-1,3-glucanase in cherry tomato fruit (**Figure 4**). $\beta$-1,3-Glucanase is the most fully characterized PR protein, and an increasing body of evidence suggests that it can act directly by degrading a pathogen's cell wall or indirectly by releasing oligosaccharide, eliciting defense reactions. Both of these processes are potential defense mechanisms against fungal infection (*30*). Our results indicate that the significant increase of $\beta$-1,3-glucanase activity in the yeast- and combination-treated cherry tomato fruits boosts resistance against gray mold rot, black spot rot, and *Rhizopus* rot. These finds are in agreement with a previous report (*10*). Thus, $\beta$-1,3-glucanase is involved in the active defense responses of a combination-treated cherry tomato fruit.

In conclusion, a combination of postharvest heat treatment and *P. guilliermondii* is a more effective strategy to control gray mold rot, black spot rot, and *Rhizopus* rot on cherry tomato fruit than individual applications of heat or *P. guilliermondii* treatments. Furthermore, the elicitation of active defense responses in cherry tomato fruits, including a rapid increase of $H_2O_2$, higher lignin deposition, and increased PAL, $\beta$-1,3-glucanase activities, is positively correlated with improved disease control.

## LITERATURE CITED

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