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Salicylic Acid Enhances Biocontrol Efficacy of the Antagonist *Cryptococcus laurentii* in Apple Fruit

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Abstract

Biological control and induced resistance are two of the promising approaches to the control of postharvest diseases. This study was conducted to evaluate the efficacy of salicylic acid (SA) alone or in combination with an antagonistic yeast, *Cryptococcus laurentii*, in controlling the blue mold disease caused by *Penicillium expansum* on apple fruit wounds. SA alone significantly inhibited the spore germination of *P. expansum in vitro* when its concentration was increased to 1000 μg ml^{-1}, but it was not effective in controlling the disease *in vivo*. Simultaneous application of SA and *C. laurentii* to the wounds on the apple fruit surface showed that SA could improve the efficacy of *C. laurentii* against *P. expansum* in a concentration-dependent manner, being most effective at 10 μg ml^{-1} but less effective at a higher or lower concentrations. Besides reducing the blue mold incidence in the local wound sites, the combination of *C. laurentii* with SA at 10 μg ml^{-1} also had a synergistic effect on the induction of fruit resistance to the disease, which might be associated with a rapid increase in peroxidase, phenylalanineamonialyase and lipoxygenase activities. In addition, SA at 100 μg ml^{-1} or above showed an adverse effect on the growth of *C. laurentii in vitro* and *in vivo*, whereas it had no effect when its concentration was decreased to 10 μg ml^{-1} or lower. This suggested that SA could enhance the biological activity of *C. laurentii* in apple fruit by inducing resistance to pathogens based on the antagonistic activity of *C. laurentii*.

Key words: Salicylic acid; Biocontrol; *Cryptococcus laurentii*; Postharvest; Apple fruit; Induced resistance.

Introduction

Blue mold, caused by *Penicillium expansum* Link, is a major postharvest disease of apple fruits. The most effective control strategy is the postharvest treatment of apples with fungicides. However, fungicide toxicity, along with the development of fungicide resistance by pathogens, has caused the public considerable concern requiring that alternatives to synthesized fungicides be developed.

Recently, biological control with antagonistic yeasts, especially for controlling wound-invading

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pathogens, has emerged as a promising method of controlling the postharvest decay of fruits and vegetables. But to be an economically feasible alternative, the biocontrol yeasts must be further developed to enhance their performance in controlling postharvest diseases (Janisiewicz and Korsten 2002; Droby and others 2003).

At the same time, considerable attention has also been placed on the induction of resistance as an important manageable form of plant protection and control of postharvest diseases of fruits and vegetables (Wilson and others 1994; Kuć 2001; Kogel and Langen 2005). Several selected postharvest biocontrol yeasts have been shown to be capable of inducing resistance in harvested fruits (Droby and others 2002; El Ghaouth and others 2003). Furthermore, it has been reported that several physical- and chemical-based elicitors such as ultraviolet light, chitosan, harpin, methyl jasmonate, and salicylic acid (SA) can augment the biocontrol activities of selected biocontrol yeasts (de Capdeville and others 2002; Qin and others 2003; Yao and Tian 2005).

The involvement of SA as a signal molecule in local defenses and in systemic acquired resistance (SAR) has been extensively studied. Exogenous application of SA can also activate resistance in plants without pathogen-inoculation (Durrant and Dong 2004). In postharvest systems, previous data showed that SA had multifunctional effectiveness, including direct antimicrobial activity in various fruits (Terry and Joyce 2004), suppression of banana fruit ripening (Srivastava and Dwivedi 2000), and induction of disease resistance in sweet cherry fruit (Qin and others 2003).

In this study, we used SA at various concentrations alone or in combination with a biocontrol yeast *Cryptococcus laurentii* to control the postharvest blue mold of apple fruit and investigated its possible modes of action through the measurements of SA on *C. laurentii*, *P. expansum*, and fruit *in vivo* and *in vitro*.

Materials and Methods

Microorganisms and Fruit Material

Fuji apple fruits (*Malus domestica* Borkh.) were hand-picked at harvest maturity. The fruit samples were sorted to remove any with apparent injuries or infections and then stored at 4°C under high humidity before being used in biocontrol experiments. After they were immersed in a solution of 0.1% sodium hypochlorite (actual concentration of available chlorine $\geq$ 52 μg ml^{-1}) for 1 min, the samples were washed with inflowing fresh water for 10 min and then were allowed to air dry at room temperature (22°C). The yeast antagonist *C. laurentii* (Kufferath) Skinner was isolated from the surfaces of the apple fruits and identified by the VITEK 32 Automicrobic System (bioMerieux Company) and maintained at 4°C on nutrient yeast dextrose agar (NYDA) medium (containing 8 g nutrient broth, 5 g yeast extract, 10 g glucose, and 20 g agar in 1 l of distilled water). The yeast was grown with the liquid cultures in 250-ml Erlenmeyer flasks containing 50 ml of nutrient yeast dextrose broth (NYDB). After inoculation with a loop of the culture, the flasks were incubated on a rotary shaker at 28°C for 24 hours. After incubation, the cells were centrifuged at 3000 × *g* for 10 min and were washed twice with a solution of 50 ml of sterile distilled water to remove the growth medium. The cell pellets were re-suspended in sterile distilled water and were counted to 1×10^8 cells per ml by means of a hemocytometer. The pathogen *P. expansum* was isolated from an apple fruit infected by the typical blue mold and cultured on potato-dextrose agar (PDA) medium. After the spores were removed from a 1-week-old culture with a bacteriological loop, they were suspended in sterile distilled water. Spore concentration was determined with a hemocytometer.

Efficacy of *C. laurentii* and SA on Control of Blue Mold in Fruit Wounds

The apples were wounded (5-mm-diameter by 3-mm-deep wound) on the equator using the tip of a sterile dissecting needle and then treated with 30 μl of one of the following: (1) cell suspensions of *C. laurentii* at 10^8 cells ml^{-1}; (2) 1, 10, 100, or 1000 μg ml^{-1} of SA; (3) cell suspensions of *C. laurentii* at 10^8 cells ml^{-1} containing 1, 10, 100, or 1000 μg $ml^{-1}$of SA, (4) sterile distilled water as a control. Two hours later, 20 μl of 1×10^5 spores/ml suspension of *P. expansum* were challenge-inoculated onto each wound. After air drying, the apples were stored in enclosed plastic trays at 22°C with a relative humidity of about 95%. The number of infected fruits and their lesion diameters were recorded after 5 days of inoculation. There were four replicates of 24 apples per replicate (four wounds per fruit, total of 96 wounds per replicate), and the experiment described above was conducted twice.

Effect of SA on *P. expansum* Spore Germination *In Vitro*

The effect of SA on the spore germination of *P. expansum* was assessed in potato dextrose broth

(PDB). Aliquots (100 µl) of the spore suspension (5×10^6 spores per ml) of the pathogen were transferred to a 10 ml glass tube containing 2 ml PDB. Treatments were evaluated at the concentrations of 1, 10, 100, and 1000 µg ml^{-1} plus sterile distilled water as a control. After filter sterilizing, the SA solutions were added to give the final concentrations. In each replicate 150–200 spores were observed microscopically and their germination was evaluated following 12 h of incubation at 28°C on a rotary shaker (200 rpm). The treatments were replicated three times and the experiment described above was conducted twice.

Effects of SA on Populations of *C. laurentii In Vitro*

The 25 ml-Erlenmeyer flasks containing 5 ml of NYDB were prepared and the filter-sterilized SA solutions were added at the selected concentrations (0, 1, 10, 100, and 1000 µg ml^{-1}). The shake-flask cultures were started with 1×10^4 cells per ml of *C. laurentii* and incubated on a rotary shaker (200 rpm) at 28°C. The samples were collected after 24 h, and the cell suspension was counted using a hemacytometer. The treatments were replicated three times and the experiment described above was conducted twice.

Effects of SA on Population Growth of Antagonists in Wounds

The fruit samples were treated and wounded as described above. Then, the wounds were treated with 30 µl of a cell suspension of *C. laurentii* at 10^8 cells ml^{-1} containing 0, 1, 10, 100, or 1000 µg ml^{-1} of SA. The samples were taken at different times (0, 12, 24, 48, 72, and 96 h at 22°C) after the treatment. The tissue was removed with a cork borer (1 cm diameter by 1 cm deep) and ground with a mortar and pestle in 10 ml of sterile distilled water. The cells was counted using a haemacytometer. There were three replicates per treatment, and the experiment described above was conducted twice.

Effects of *C. laurentii* and SA on Induced Disease Resistance

Evaluation of induced disease resistance by *C. laurentii* and SA was performed according to the method of Droby and others (2002). The samples were gently wounded (5 mm diameter by 3 mm deep) on the equator using the tip of a sterile dissecting needle, after which a 30-µl of *C. laurentii* at 10^8 cells per ml alone, or SA at 10 µg ml alone, or the combination was piped into each wound. Wounds treated with the same amount of sterile distilled sterilized water served as a control. After 0, 24, 48, 72, and 96 h at 22°C, four fresh wounds were made at a point 5 mm from the edge of the site pre-treated with SA and *C. laurentii* (+5 mm); these wounds were inoculated with 20 µl of a spore suspension of *P. expansum* (1×10^5 spores per ml) to make sure that the pre-treatment agents and the pathogen were injected into spatially separated wounds. The fruit samples were then incubated in enclosed plastic trays at 22°C under humid conditions (approximately 95%). For each treatment at each inoculation time, the number of the infected wounds was recorded every 4 days after inoculation with the pathogen. Each treatment consisted of three replications of 12 fruit samples per treatment (four wounds per fruit, total of 48 wounds per replicate and the experiment described above was conducted twice.

Effects of *C. laurentii* and SA on Defense-related Enzyme Activities

The fruit samples were treated and wounded as described above. The wounds were then treated with 30 µl of a solution of cell suspension of *C. laurentii* at 10^8 cells ml^{-1} alone, SA at 10 µg ml^{-1} alone, *C. laurentii* at 10^8 cells ml^{-1} in combination with SA at 10 µg ml^{-1}, and sterile distilled water as a control. The enzymes were extracted at 4°C from one gram of fresh tissue from the wound site and the +5-mm site with 10 ml of cold (4°C) 50 mmol l^{-1} sodium phosphate buffer (PH 7.8) containing 1.33 mmol l^{-1} EDTA and 1% polyvinyl-polypyrrolidone (PVPP) in a mortar and pestle at different times (0, 24, 48, 72, and 96 h at 22°C) after the treatments. The homogenates were centrifuged at 4°C for 15 min at 27,000 × *g*, and the supernatant was used for assay of the enzyme activities and protein content. There were three replicates per treatment and six fruit samples per replicate; the experiment described above was conducted twice.

Following the method described by Lurie and others (1997) with some modifications, peroxidase (POD) activity was measured using guaiacol as substrate. The reaction mixtures contained 3 ml of 50 m mol/l sodium phosphate buffer (pH 6.4), 220 µl of 0.3% guaiacol, 60 µl of 0.3% H_2O_2, and 20 µl of crude enzyme extract. The reaction was initiated immediately by adding H_2O_2 at 30°C incubated in a water bath. The reaction was allowed to proceed for 5 min, and measurements of A_{470} were taken once every 30 seconds beginning 1 min after addition of H_2O_2 to the substrate. A cuvette containing all components except H_2O_2 was used as

Table 1. Effects of salicyclic acid and *Cryptococcus laurentii* on Control of Blue Mold on Apple Fruit Wounds

Treatment	Decay incidence (%)	Lesion diameter (mm)
Control	100 ± 0 a	22.8 ± 3.3 a
1000 μg ml^{-1} SA	100 ± 0 a	24.5 ± 2.8 a
100 μg ml^{-1} SA	100 ± 0 a	23.0 ± 4.4 a
10 μg ml^{-1} SA	100 ± 0 a	22.6 ± 2.6 a
1 μg ml^{-1} SA	100 ± 0 a	21.9 ± 2.6 a
C. laurentii	41.6 ± 7.6 c	12.4 ± 2.5 b
1000 μg ml^{-1} SA +*C. laurentii*	100 ± 0 a	22.6 ± 2.4 a
100 μg ml^{-1} SA +*C. laurentii*	58.3 ± 9.0 b	13.0 ± 2.3 b
10 μg ml^{-1} SA +*C. laurentii*	12.9 ± 4.9 e	12.1 ± 2.4 b
1 μg ml^{-1}SA+*C.laurentii*	25.4 ± 7.2 d	13.1 ± 2.6 b

Note: Data are means ± standard deviations of four replicates. Different lowercase letters indicate significant differences ($p < 0.05$) according to Duncan's multiple range test.

control. One unit of POD activity is defined as the amount of enzyme extract producing an increase of A_{470} by 0.01 in 1 min, and the activity is expressed as U mg^{-1} protein.

Phenylalanine amonialyase (PAL) activity was measured according to the method of Camm and Towers (1973) with some modifications. The reaction mixtures contained 1 ml supernatant with 4 ml 0.1 $mol{\cdot}l^{-1}$ borate buffer (pH 8.8) and 10 $mmol{\cdot}l^{-1}$ L-phenylalanine. After incubation of the mixtures at 30°C for 30 min, the reaction was terminated by the addition of 0.1 ml of 5N HCl. The increase in absorbance at 290 nm, due to the formation of trans-cinnamate, was measured spectrophotometrically. One unit of PAL activity is defined as the amount of enzyme extract producing an increase of A_{290} by 0.01 in 1 h; the activity is expressed as U mg^{-1} protein.

Lipoxygenase (LOX) activity was measured according to the method described by Todd and others (1990) with some modifications. The reaction mixture contained 200 μl Tween 20 and 40 μl of linoleic acid in 4.0 ml of 0.1 M phosphate, pH 7.0. Lipoxygenase activity was measured spectrophotometrically by monitoring the change in absorbance at 234 nm over a 5-min period taken once every 30 seconds. One unit of LOX activity is defined as the amount of the enzyme that causes an increase in absorption by 0.01 min^{-1} at 25°C, and the activity is expressed as U mg^{-1} protein.

The protein content was determined according to the method of Bradford (1976) using bovine serum albumin (Sigma-Aldrich) as a standard.

Statistical Analyses

The data were analyzed by analysis of variance (ANOVA) in the Statistical Program SPSS/PC ver. II.x. Statistical significance was applied at the level $p < 0.05$. When the analysis was statistically significant, Duncan's multiple range test was applied to the separate means.

Results

Effect of SA and *C. laurentii* on the Control of Blue Mold

As shown in Table 1, treatment with SA alone at all the selected concentrations wasn't effective in reducing blue mold incidence and severity *in vivo*. However, the addition of SA at the concentration of 10 μg ml^{-1} or lower to a cell suspension of *C. laurentii* produced a significant increase in the activity of *C. laurentii* against *P. expansum* in apple fruit ($p < 0.05$). At the optimal concentration (10 μg ml^{-1}), the percentage of infected fruits in the combined treatment was reduced significantly from 41.6 ± 7.6% to 12.9 ± 4.9%, as compared with that of *C. laurentii* alone. In addition, the efficacy of the combined treatment was weakened as the concentration of SA was decreased to 1 μg ml^{-1} ($p < 0.05$). What is more, when combined with SA at 1000 μg ml^{-1}, the efficacy of *C. laurentii* was entirely lost, which was similar to that of SA applied alone ($p > 0.05$). As for the combination of *C. laurentii* with SA at 100 μg ml^{-1}, the decay incidence was also much higher than that of *C. laurentii* alone ($p < 0.05$). But it should be noted that there was no significant difference of lesion diameters in the treatments of *C. laurentii* with SA at the concentration range from 0 to 100 μg ml^{-1} ($p > 0.05$).

Effect of SA on Spore Germination of *P. expansum in vitro*

The results (Figure 1) showed that at the concentrations of 1000 μg ml^{-1}, a marked inhibition in the percentage of spore germinations was observed in

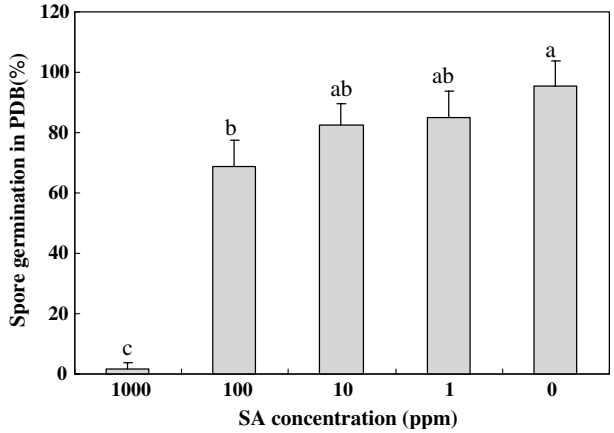

Figure 1. Spore germinations of *Penicillium expansum* in potato-dextrose broth (PDB) containing salicylic acid (SA) at the selected concentrations (1000, 100, 10, 1, 0 μg ml^{-1}). Standard deviations of three replications are given as the short bars. Different letters indicate significant differences ($p < 0.05$) according to Duncan's multiple range test.

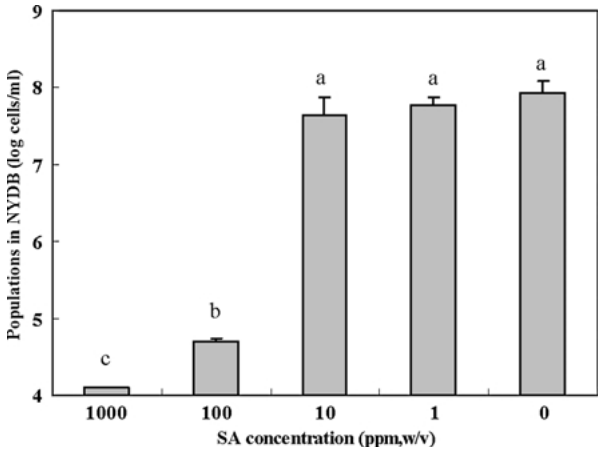

Figure 2. Populations of *Cryptococcus laurentii* in nutrient yeast dextrose broth (NYDB) containing salicylic acid (SA) at the selected concentrations (1000, 100, 10, 1, 0 μg ml^{-1}). Standard deviations of three replications are given as the short bars. Different letters indicate significant differences ($p < 0.05$) according to Duncan's multiple range test.

PDB with SA ($p < 0.05$). However, when the concentration was decreased to 100 μg ml^{-1} or lower, there was just a slight inhibition. And there was little difference in spore germination of *P. expansum* in the treatment with SA from 0 to 10 μg ml^{-1} ($p > 0.05$).

Effect of SA on *C. laurentii* Growth *In Vitro* and *In Vivo*

In NYDB (Figure 2), the growth of *C. laurentii* was not influenced by SA at the lower concentrations used ($p > 0.05$). After 24 h of incubation, the populations of *C. laurentii* were all increased nearly 10^4-fold in the presence of SA from 0 to 10 μg ml^{-1}. When increased up to 100 μg ml^{-1} or higher, SA had an obvious negative effect on the growth of *C. laurentii* ($p < 0.05$). At the highest concentration of 1000 μg ml^{-1}, SA nearly completely inhibited the growth of *C. laurentii*.

As shown in Figure 3, the ability of *C. laurentii* to grow in apple wounds in the presence of SA was similar to the results observed *in vitro*.

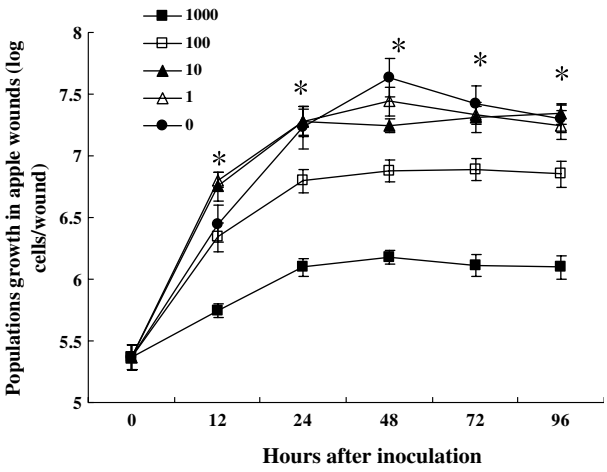

Figure 3. Population growths of *Cryptococcus laurentii* on apple wounds in presence of salicylic acid (SA) at the selected concentrations (1000, 100, 10, 1, 0 μg ml^{-1}). Standard deviations of three replications are given as the short bars. An asterisk indicates that the difference of the population in the treatments is significant ($p < 0.05$) according to Duncan's multiple range test.

Effect of SA and *C. laurentii* on the Induction of Resistance

The results (Figure 4) showed that all treatments, when applied either immediately or 24 h before challenge by *P. expansum*, had no significant effect on reduction of the decay incidence ($p > 0.05$). However, when applied 72 or 96 h prior to inoculation with *P. expansum*, the combined treatment with SA (10 μg ml^{-1}) and *C. laurentii* effectively reduced the decay incidence in comparison with the treatment with SA and *C. laurentii* alone or the control ($p < 0.05$). By 96 h, the blue mold incidence in the combined treatment was 38%, 57 %, or 50 % of that in the control, *C. laurentii*, or SA alone, respectively.

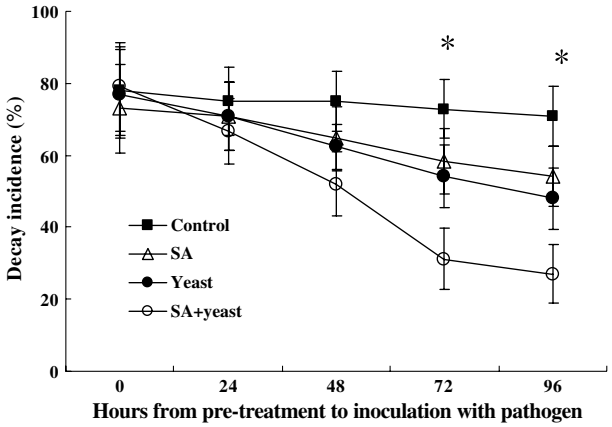

Figure 4. Effects of salicylic acid (SA) and *Cryptococcus laurentii* on inducing resistance to blue mold on apple wounds. Standard deviations of three replications are given as the short bars. An asterisk indicates that the difference of the decay incidence between the co-treatment with SA and *C. laurentii* and any other treatment is significant ($p < 0.05$) according to Duncan's multiple range test.

Effects of SA and *C. laurentii* on POD Activity

At the local wound site (Figure 5A), the combination of *C. laurentii* with SA induced an approximately eightfold increase in POD activity within 24 h of inoculation ($p < 0.05$) and then dropped by 48 h followed by a notable increase again ($p > 0.05$). However, in the tissue treated with SA alone, an induction of POD activity was also observed after 72 h of inoculation ($p < 0.05$). In the control or *C. laurentii*-treatment alone, a relatively small increase in POD activity with time was detected. As shown in Figure 5B, the treatment with SA alone also resulted in an induction of POD activity, which was increased approximately 4.6-fold within 24 h of inoculation at the +5 mm site ($p < 0.05$), although this was still lower than that of the combined treatment with *C. laurentii* ($p < 0.05$). However, in the tissue treated with *C. laurentii* alone, an induction of POD activity was observed by 72 h of inoculation ($p < 0.05$).

Effects of SA and *C. laurentii* on PAL activity

At the local wound site (Figure 6A), the combined treatment induced a peak of PAL activity within 24 h of inoculation and was fivefold higher than that measured in the control ($p < 0.05$). However, no significant increase was induced by the treatment with SA or *C. laurentii* alone ($p > 0.05$). At the +5 mm site (Figure 6B), the treatment with SA or *C. laurentii* alone, or the combination stimulated an increase in PAL activity of approximately 3.0-, 2.2-, and 5.2-fold higher than that of the control within 24 h of inoculation, respectively ($p < 0.05$).

Effects of SA and *C. laurentii* on LOX Activity

At the local wound site (Figure 7A), all treatments increased LOX activities within 24 h of inoculation, but no significant differences in them were observed within 72 h of inoculation ($p > 0.05$). In the treatment with SA alone or in combination with *C. laurentii*, LOX activity increased approximately 3-fold and 3.2-fold, respectively, compared to that observed in the control after 72 h ($p < 0.05$). At the +5-mm site (Figure 7B), LOX activity in the combined treated tissue showed a rapid and strong increase of approximately 9.6-fold within 24 h of inoculation ($p < 0.05$). However, in the tissues treated with yeast or SA as well as water alone, a moderate induction was observed only after 48 h of inoculation ($p < 0.05$).

Discussion

In this study, spore germination of *P. expansum* in PDB was significantly influenced by SA when its concentration was increased up to 100 μg ml^{-1} or higher (Figure 1), but blue mold incidence and lesion diameter caused by *P. expansum* were not influenced by SA at concentrations from 0 to 1000 μg ml^{-1} *in vivo* (Table 1). This rather different effect *in vivo* and *in vitro* was similar to that of 2-deoxy-D-glucose reported by El-Ghaouth and others (2000a). This is not surprising because results from *in vitro* studies do not always accurately represent the efficacy of that in the *in vivo* situation (Rotem and other 1978).

The antagonistic yeasts *C. laurentii* and *P. expansum* have been selected mainly for their ability to rapidly colonize and grow in the surface wounds, and subsequently to compete with the pathogen for nutrients and space, which is believed to be a major component of the mode of action of *C. laurentii* (Janisiewicz and Korsten 2002; Zhang and others 2005). In the present study, the efficacy of *C. laurentii* was reduced markedly (Table 1) when its growth was inhibited significantly in the presence of SA at 100 μg ml^{-1} or above (Figure 2, Figure 3), which was similar to the result from chitosan chloride reported by El-Ghaouth and others (2000b).

SA alone at the relatively low concentration of 10 μg ml^{-1} did not affect the populations of *C. laurentii in vitro* (Figure 2) or *in vivo* (Figure 3),

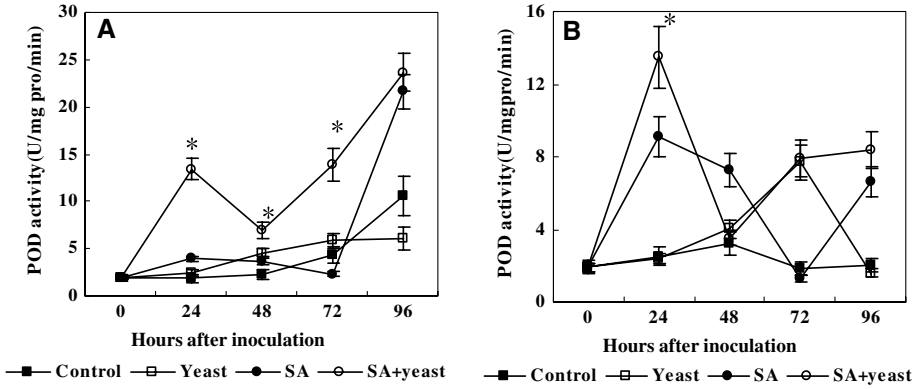

Figure 5. Effects of salicylic acid (SA) and *Cryptococcus laurentii* on the peroxidase activities in apple wounds at the local wound site (**A**) and at + 5 mm from the edge of the wound site (**B**). Standard deviations of three replications are given as the short bars. An asterisk indicates that the difference between the co-treatment with SA and *C. laurentii* and any other treatment is significant ($p < 0.05$) according to Duncan's multiple range test.

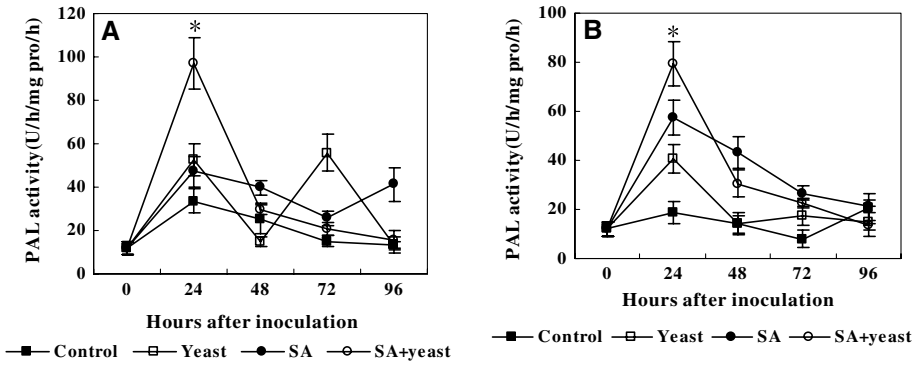

Figure 6. Effects of salicylic acid (SA) and *Cryptococcus laurentii* on the phenylalanine amonialyase activities in apple wounds at the local wound site (**A**) and at +5 mm from the edge of the wound site (**B**). Standard deviations of three replications are given as the short bars. An asterisk indicates that the difference between the co-treatment with SA and *C. laurentii* and any other treatment is significant ($p < 0.05$) according to Duncan's multiple range test.

or the spore germination of *P. expansum in vitro* (Figure 1). Interestingly, the combination of SA at this concentration (10 μg ml^{-1}) with *C. laurentii* significantly enhanced control of blue mold disease on apple fruit wounds, as compared with *C. laurentii* alone (Table 1). Moreover, this combined treatment produced a useful result in reducing the blue mold incidence when applied 72 h or more prior to the inoculation with *P. expansum* by inducing resistance in the fruit rather than by directly affecting the pathogen (Figure 4), because no direct interactions could occur when the pretreatment agents and the pathogen were inoculated into the spatially separated wounds (de Capdeville and others 2002; Droby and others 2002; El Ghaouth and others 2003).

The effectiveness of the combined treatment lay in reducing the disease incidence rather than the lesion diameters (Table 1), which was in agreement with the effect of other biologically based elicitors reported by Fajardo and others (1998). It indicated that the induced resistance could be effective, particularly during the early phase of disease progression, and that once the fruit samples were visibly infected by the pathogen, the elicitors did not reduce the fungal colonization or the severity of symptoms (Fajardo and others1998).

Additionally, the results in Table 1 also showed that the SA improved the biocontrol activity of *C. laurentii* most effectively at a concentration of 10 μg ml^{-1} but less at lower concentrations (1 μg ml^{-1}), findings in agreement with the general phenomenon that plant growth regulators are most effective when applied at optimal concentration.

The induction of disease resistance is a sensitization process that primes the plant for more rapid deployment of defenses, which has been shown to coincide with the activation of a biochemical defense

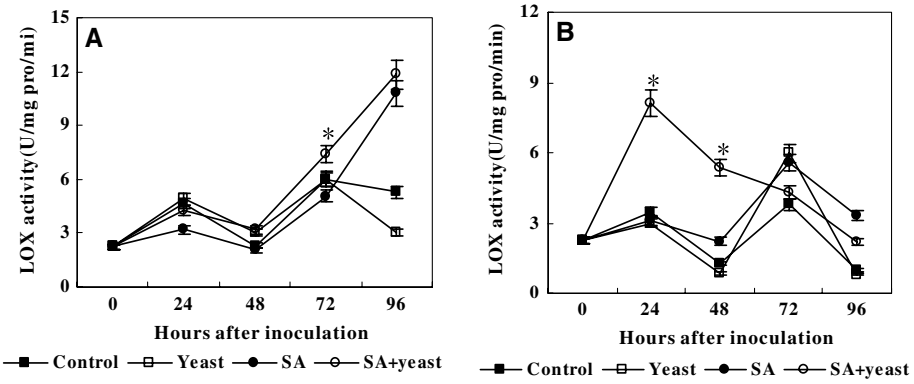

Figure 7. Effect of salicylic acid (SA) and *Cryptococcus laurentii* on the lipoxygenase activities in apple wounds at the local wound site (**A**) and at +5 mm from the edge of the wound site (**B**). Standard deviations of three replications are given as the short bars. An asterisk indicates that the difference between the co-treatment with SA and *C. laurentii* and any other treatment is significant ($p < 0.05$) according to Duncan's multiple range test.

system for disease resistance in plants, such as PAL, POD, and LOX (Kuć 2001; Conrath and others 2002). Our results showed that the combined treatment provided a rapid and strong induction of these defense-related reactions (Figure 5, 6, and 7). It may facilitate formation of appropriate defense-related reactions in the fruit to the potential attacking pathogen, because the success in the defense response depends on the speed at which the plant recognizes the attacking pathogen and the intensity with which the appropriate defense mechanism is activated (Conrath and others 2002; Ton and others 2005). However, the mechanism for the different biochemistry reactions at different sites remains to be elucidated.

In conclusion, the results presented in this study showed that the biological efficacy of *C. laurentii* against *P. expansum* on apple fruit could be significantly enhanced by adding SA, which was most effective at 10 μg ml^{-1}. The mechanism of action might be related to induced resistance to pathogens based on the antagonistic activity of *C. laurentii*. These results offer a potential for minimizing apple fruit decay in an integrated pest management strategy.

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