Indole-3-Acetic Acid Improves Postharvest Biological Control of Blue Mold Rot of Apple by *Cryptococcus laurentii*

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ABSTRACT

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Cryptococcus laurentii is a well-known postharvest biocontrol yeast; however, it cannot provide satisfactory levels of decay control when used alone. Here, we evaluated the effects of indole-3-acetic acid (IAA), a plant growth regulator, on the biocontrol efficacy of the yeast antagonist *C. laurentii* against blue mold rot caused by *Penicillium expansum* in apple fruit. Results showed that the addition of IAA at 20 µg/ml to suspensions of *C. laurentii* greatly enhanced inhibition of mold rot in apple wounds compared with that observed with *C. laurentii* alone. The addition of IAA at 20 µg/ml or lower did not influence the population

growth of *C. laurentii* in wounds, but adverse effects were seen on *C. laurentii* when the concentration of IAA was increased to 200 µg/ml or above in vitro and in vivo. *P. expansum* infection in apple wounds was not inhibited when the pathogen was inoculated into the fruit wounds within 2 h after application of IAA; however, infection was reduced when inoculated more than 12 h after IAA application. Treatment of wounds with IAA at 20 µg/ml 24 h before pathogen inoculation resulted in significant inhibition of *P. expansum* spore germination and host infection. Application of IAA at 20 µg/ml also reduced *P. expansum* infection when it was applied 48 h before pathogen inoculation in the intact fruit. Thus, IAA could reinforce the biocontrol efficacy of *C. laurentii* in inhibiting blue mold of apple fruit by induction of the natural resistance of the fruit.

Blue mold rot, caused by the wound-invading necrotrophic fungus *Penicillium expansum*, is a major postharvest disease of apple fruit (1,16). Given limited applications of synthetic fungicides, the effective and safe nonfungicide approaches to control postharvest decay have been continually explored in recent years (16). Proposed nonfungicide approaches include the following: (i) biological methods, such as using antagonistic microorganisms, natural and safe bioactive compounds, and induced resistance; and (ii) physical methods, such as cold storage, controlled or modified atmosphere storage, heat treatment, ultraviolet light irradiation, and ozone. However, none of these methods, when used alone, provide satisfactory levels of decay control compared with using synthetic fungicides. Therefore, a current principal strategy for nonfungicide control of fruit decay has been to develop an integrated management strategy that combines various biological and physical means (8,10,11,16,28).

Strains of the yeast *Cryptococcus laurentii* are widely studied for their potential in biological control. They have been found to have strong antagonistic activity against postharvest pathogens of apple fruit when used alone or in combination with certain additives, including calcium chloride, chitosan, gibberellic acid, food additives, low doses of fungicides, salicylic acid, and sodium bicarbonate (8,17,18,26,33–35).

Indole-3-acetic acid (IAA) is the most important auxin of higher plants (4), contributing to the processes of ripening and senescence of harvested produce (3,6,19) and pathogen defense mechanisms in *Arabidopsis thaliana* and tomato plants (20,21). Recent evidence indicates IAA displays in vitro antifungal activity against *Ustilago maydis* and *Saccharomyces cerevisiae* (25), and in vivo activity against *Gibberella pulicaris* and

Phytophthora infestans in potato (22,29). However, little information is available for evaluating the effects of IAA on the fungal disease of harvested fruit. We recently observed that IAA could enhance the biocontrol efficacy of *C. laurentii* against *P. expansum* and *Botrytis cinerea* in pear fruit, but the mechanism by which IAA enhanced the biocontrol efficacy was not made clear (36).

This study was conducted to evaluate (i) the efficacy of *C. laurentii* with IAA in controlling blue mold rot in apple fruit; (ii) the effects of IAA on induction of the resistance to blue mold rot in apple fruit; (iii) the antifungal activity of IAA on the spore germination of *P. expansum* in vivo and the spore survival of *P. expansum* in vitro; and (iv) the influence of IAA on the population and the survival of *C. laurentii* in vivo and in vitro.

MATERIALS AND METHODS

Fruit material, antagonist, and fungal pathogen. Fuji apple fruit (*Malus domestica* Borkh.) was harvested at commercial maturity and those having uniformity in size and ripeness and lack of any apparent injuries or infection were selected. Fruit samples were surface-disinfected with sodium hypochlorite at 0.1% (vol/vol) for 1 min, rinsed with tap water, and allowed to air dry at room temperature (20°C).

The yeast antagonist *C. laurentii* (Kufferath) Skinner strain zju-10 was originally isolated from the surface of pear fruit and identified by VITEK 32 Automicrobic System (bioMérieux Company, Marcy l'Etoile, France) and streaked on nutrient yeast dextrose agar (NYDA) medium (containing 8 g of nutrient broth, 5 g of yeast extract, 10 g of glucose, and 20 g of agar in 1 liter of distilled water) and incubated at 28°C for 48 h. The yeast cells were cultured in nutrient yeast dextrose broth (NYDB) and incubated for 24 h at 28°C on a gyratory shaker at 200 rpm. The yeast cells were harvested by centrifuging at 3,000 × g for 10 min and washed twice with sterile distilled water. The concentration of yeast cells was counted with a hemacytometer.

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The pathogen *P. expansum* strain PE-z was originally isolated from apple fruit showing typical blue rot and was cultured on potato dextrose agar (PDA) medium (containing the extract from 200 g of boiled potato, 20 g of glucose, and 20 g of agar in 1 liter of distilled water) at 28°C in the dark. Spore suspension was prepared by flooding 7-day-old sporulating cultures of *P. expansum* with sterile distilled water. The spore concentration was counted with a hemacytometer.

Control of blue mold rot by *C. laurentii* with IAA. IAA was purchased from local producers (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) and prepared by dissolving absolute alcohol and adjusting the pH to 6 with 1 mol liter⁻¹ NaOH, followed by filter sterilization (0.45 µm) for immediate use. To conduct postharvest disease assays, the surfaces of apples were wounded at four different sites with a sterile cork borer (approximately 5 mm diameter and 3 mm deep) and treated with 30 µl of a solution of the following: (i) *C. laurentii* suspension (1 × 10⁸ cells/ml); (ii) IAA at 2, 20, 200, or 2,000 µg/ml; and (iii) *C. laurentii* suspension (1 × 10⁸ cells/ml) with IAA at final concentrations of 2, 20, 200, or 2,000 µg/ml. After 2 h, a solution of 30 µl of *P. expansum* spore suspension (1 × 10⁴ spores/ml) was inoculated onto each wound. Wounds treated with sterile distilled water and with *P. expansum* spore suspension served as the inoculated positive disease control. In each experiment, wounds treated with either sterile distilled water, biocontrol yeast suspension, or IAA solutions without pathogen inoculation were used as negative controls. For all experiments, negative controls did not exhibit disease symptoms. After air drying, the apples were stored in trays covered with plastic film to maintain a high relative humidity at 20°C. The number of infected wounds and their lesion diameters were examined daily. Fruit were scored for disease incidence, based on first visual appearance of rot, and disease severity, based on lesion diameter as determined by average length of perpendicular measurements. Each experiment consisted of four replicates per treatment, consisting of 24 fruit samples per replicate, totaling 96 wound sites.

Growth of *C. laurentii* in the presence of IAA. In vitro. *C. laurentii* was cultured on NYDB amended with filter-sterilized IAA at 0, 0.1, 1, 10, 100, or 1,000 µg/ml. Shake-flask cultures were started with yeast cells (approximately 5 × 10⁴ cells/ml) and incubated on a rotary shaker (200 rpm) at 28°C. Samples were collected after 24 h, and the cell concentration was counted with a hemacytometer after diluting with sterile distilled water if necessary. There were three replicates per treatment and three flasks per replicate.

In vivo. Fruit samples were wounded at five different sites with a sterile cork borer, and each wound (apart 2 cm each) on one fruit was treated with each of the different IAA concentrations (0, 2, 20, 200, or 2,000 µg/ml) and *C. laurentii* suspension (2 × 10⁷ cells/ml, 30 µl). After air drying, the samples were stored in trays covered with plastic film to maintain a high relative humidity at 20°C. Tissues were removed at the wound site of treated fruit with a sterile cork borer (1 cm diameter by 1 cm deep) at 0, 12, 24, 48, 72, and 96 h and ground with a mortar and pestle in 10 ml of sterile water. Yeast cells were counted with a hemacytometer. There were three replicates per treatment and six fruit samples per replicate for each time point. Experiments were conducted at least twice.

Survival of *C. laurentii* or *P. expansum* in the presence of IAA. Equal amount of *C. laurentii* cells or *P. expansum* spores were incubated in solutions of IAA at various concentrations (0, 1, 10, 100, or 1,000 µg/ml) in a final volume of 1 ml for 60 s, centrifuged, and resuspended in water to remove the IAA solution prior to plating on the agar medium. Then, 100 µl of each suspension was plated on NYDA or PDA. After 36 or 72 h of incubation at 28°C, the yeast colonies or the fungal colonies were counted and calculated as the mean value of colony forming units (CFU) per plate. There were three replicates per treatment and

four plates per replicate. All treatments were arranged in a randomized complete block design and conducted at least twice.

***P. expansum* spore germination in vivo.** Two of the four wounded sites on one apple fruit were treated with 30 µl of sterile distilled water (the control), and the other two wounds were treated with 30 µl of a solution of IAA at 20 µg/ml in a randomized complete block design. After 2 or 24 h, 30 µl of the *P. expansum* spore suspension (1.5 × 10⁷ spores/ml) was inoculated into each wound at each time point. Use of the high concentration was necessary in order to observe the spores by optical microscopy. After air drying, fruit samples were stored in trays covered with plastic film to maintain a high relative humidity at 20°C. At equal 12 h postinoculation with pathogen for each time point, germinated spores of *P. expansum* were examined by an optical microscope. The number of germinated spores was divided by the total observed spores (at least 200 spores in each replicate, usually used multiple fields of view to reach 200 spores) and multiplied by 100 as the percent spore germination. There were three replicates per treatment, with each replicate consisting of nine fruit samples for each time point.

Effects of IAA on induction of apple fruit resistance in wounds. The surfaces of apples were wounded at six different sites and each of the wounds was treated with 30 µl of filter-sterilized IAA at 2, 20, 50, 100, and 200 µg/ml or with 30 µl of distilled water (the control) on one fruit in a randomized complete block design, followed by inoculation of 30 µl of the *P. expansum* spore suspension (1 × 10⁵ spores/ml) after 24 h. There were three replicates per treatment and 12 fruit samples per replicate.

In another experiment, apples were wounded at four different sites and two of the wounds were treated with 30 µl of filter-sterilized IAA at 20 µg/ml or with 30 µl of distilled water (the control) on one fruit in a randomized complete block design, followed by the inoculation of 30 µl of the *P. expansum* spore suspension at 1 × 10⁶ spores/ml or at 1 × 10² spores/ml onto each wound after 2, 12, and 24 h. There were 12 fruit samples per replicate and three replicates per treatment for each time point.

Then, all of the above samples were stored in trays covered with plastic film to maintain a high relative humidity at 20°C. The number of infected fruit and their lesion diameters were examined daily and recorded after equal time of incubation for each timing treatment. The percent inhibition (%) for each treatment was calculated by comparison with the water control: Inhibition (%) = [(average lesion diameter of infected wounds in the control • average lesion diameter of infected wounds in the treatment)/average lesion diameter of infected wounds in the control] × 100.

The correlation of the efficacy of the induced resistance in inhibiting the average lesion diameter by IAA from 2 to 200 µg/ml and the incubation time and of the efficacy of the induced resistance by IAA from 2 to 200 µg/ml and the efficacy of *C. laurentii* with IAA at the corresponding concentration in inhibiting the average lesion diameter were analyzed by the Pearson correlation test (2-tailed) in Statistical Program (SPSS/PC version II.x).

Effects of IAA on induction of resistance in intact apple fruit. The intact fruit samples were dipped in IAA (20 µg/ml) solution for 20 min or in the distilled water for 20 min as the control. After 0, 24, 48, 72, 96, and 120 h, one of six sets of the treated fruit was wounded (5 mm diameter and 3 mm deep) at four different sites on the fruit surface and inoculated with 30 µl of *P. expansum* spore suspension (1 × 10⁴ spores/ml). Then the fruit samples were stored in the enclosed plastic trays to maintain high humid conditions at 20°C. The disease incidence was recorded on every fourth day after inoculation. The percentage of the rotted wounds (% of control) was calculated as follows: the number of the average of rotted fruit obtained from each treatment was divided by the values of the control (pretreated with water and then inoculation with *P. expansum* for each time interval) and then multiplied by 100. There were three replicates

per treatment and 15 fruit samples (total of 60 wounds) per replicate for each time interval.

All treatments above were arranged in a randomized complete block design and conducted at least twice. Data are from one individual experiment and are representative of two independent experiments with similar results. The analysis of variance from Statistical Program (SPSS/PC version II.x) was performed on the data and the Duncan's multiple range test was used for means separation.

RESULTS

Inhibition of blue mold rot by *C. laurentii* with IAA. As shown in Figure 1, IAA at concentrations from 20 to 2,000 µg/ml did not directly inhibit blue mold infection in apple wounds ($P < 0.05$). In contrast, application of the yeast *C. laurentii* inhibited

Fig. 1. Effects of indole-3-acetic acid on blue mold rot caused by *Penicillium expansum* (10^5 spores/ml) in apple and pear fruit. Values of lesion diameter represent the mean of four independent replicates and bars represent the standard errors. No significant differences ($P = 0.05$) were observed between treatments according to Duncan's multiple range test for each time point.

Fig. 2. Development of blue mold rot **A**, incidence and **B**, lesion diameter on apple fruit treated with *Cryptococcus laurentii* at 1×10^8 cells/ml (□) and indole-3-acetic acid (IAA) at 2,000 µg/ml (▲), 200 µg/ml (△), 20 µg/ml (●), or 2 µg/ml (○) followed by inoculation with *Penicillium expansum* at 1×10^4 spores/ml within 2 h (20°C). The treatment with distilled water and inoculated with *P. expansum* served as the inoculated control (■). Values of rot incidence and lesion diameter represent the mean of four independent replicates and bars represent the standard errors.

both blue mold incidence and lesion diameter, especially during the early stages of an 8-day incubation period (Fig. 2). The incidence of disease and its average lesion diameter in the treatment by *C. laurentii* were 58.6 and 79.3% of those in the inoculated control on day 5 ($P < 0.0001$). However, the efficacy of *C. laurentii* decreased rapidly with incubation time. On day 8, there was no difference in rot incidence between treatments with *C. laurentii* and that of the inoculated control ($P > 0.05$), although the mean value for lesion diameter in wounds treated with *C. laurentii* was lower than that in wounds of the inoculated control (77.1% of the water control, $P < 0.01$).

The addition of IAA at concentrations between 2 and 20 µg/ml to *C. laurentii* suspensions resulted in remarkably increased efficacy in inhibiting the blue mold disease compared with the treatment of *C. laurentii* alone between 5 and 8 days after inoculation (Fig. 2). Rot incidence in wounds treated with *C. laurentii* and IAA at 20 µg/ml was only 6.7% of the *C. laurentii* alone on day 5 ($P < 0.0001$). On day 7, rot incidence and the lesion diameter in wounds treated with *C. laurentii* and IAA at 20 µg/ml were lower than with *C. laurentii* and IAA at 2 µg/ml ($P < 0.001$ for rot incidence and $P < 0.05$ for lesion diameter). Treatments of *C. laurentii* with IAA at 200 µg/ml also enhanced the inhibition of blue mold incidence on day 5 and on day 6 ($P < 0.01$) compared with *C. laurentii* alone; however, this treatment was less effective than treatments with *C. laurentii* and IAA at 2 and 20 µg/ml ($P < 0.01$). In contrast to lower concentrations of IAA, a combination of *C. laurentii* with IAA at 2,000 µg/ml resulted in adverse effects on reduction of the *P. expansum* infection compared with the treatment with *C. laurentii* alone; the higher IAA concentration treatment resulted in disease incidence similar to the untreated disease control (Fig. 2).

Effect of IAA on the biocontrol yeast *C. laurentii* and the pathogen *P. expansum* in vitro. In general, IAA exhibited adverse effects on both *C. laurentii* cells and *P. expansum* spores under in vitro conditions (Table 1). When *C. laurentii* was incubated in NYDB with the addition of IAA at high concentrations of 100 and 1,000 µg/ml for 24 h, *C. laurentii* population levels did not increase above detectable levels of 5×10^4 CFU/ml ($P < 0.05$). Even at the lowest IAA concentration of 0.1 µg/ml, growth of *C. laurentii* was significantly inhibited compared with the untreated control ($P < 0.05$).

On the surface of the agar culture medium NYDA (Table 1), IAA between concentrations of 1 to 1,000 µg/ml also exhibited adverse effects on the growth of *C. laurentii* ($P < 0.05$). However, for the pathogen *P. expansum*, significant growth inhibition was shown only at the high concentration of 1,000 µg/ml IAA ($P < 0.05$), whereas no differences were observed with IAA treatments between 0 and 100 µg/ml on PDA ($P > 0.05$).

Growth of the yeast antagonist in apple wounds. *C. laurentii* proliferated rapidly in apple wounds, especially during the first 24 h (Fig. 3). Growth was not influenced by the addition of IAA at concentrations of 20 µg/ml or lower during 96 h of incubation ($P > 0.05$). However, IAA concentrations at 200 µg/ml or above significantly inhibited the growth of yeast in apple wounds, especially during the first 48 h of incubation ($P < 0.05$). Furthermore, at 24 h, *C. laurentii* populations incubated with IAA at the highest concentration of 2,000 µg/ml were reduced more than sevenfold compared with populations of the untreated control ($P < 0.05$). However, little difference was detected in the *C. laurentii* populations for all treatments after 72 h inoculation ($P > 0.05$).

Pathogen spore germination in apples wounds. When the time interval between IAA application and *P. expansum* spore inoculation onto fresh wounds was 2 h, nearly 100% of spores germinated within 12 h after inoculation regardless of the presence or absence of IAA at 20 µg/ml (Fig. 4). In contrast, germination was inhibited in wounds inoculated with *P. expansum* spores 24 h after treatment with either IAA or the water control by 1.2 or 19.4%, respectively.

Effects of IAA concentration, timing of application, pathogen spore density, and incubation after pathogen inoculation on blue mold rot development on apple fruit. Because IAA at 20 µg/ml could strongly enhance the biological control of blue mold rot by *C. laurentii* and induce the fruit resistance against the blue mold (Fig. 5), this concentration of IAA was chosen for the following experiment. The results showed that the time between IAA application and pathogen inoculation had a significant effect on disease development. Treatments of IAA at 20 µg/ml had no effect on blue mold rot in apple wounds when applied within 2 h prior to pathogen spore inoculation onto apple wounds (Fig. 1).

The timing between application of IAA and inoculation of the pathogen at two different spore densities were tested. At a time interval of 24 h, greater inhibition of rot, as determined by lesion diameter, was observed at day 3 to day 5 compared with time intervals of 2 and 12 h. Between the time intervals of 2, 12, and 24 h, differences in the amount of inhibition remained significant at all three observation days when fruit wounds were inoculated with the low pathogen density of 10^2 spores/ml (at least $P < 0.05$, Table 2). Regardless of pathogen inoculum density, effective reduction in lesion diameter was significantly different when

comparing time intervals of 12 h with 2 h between application of IAA and inoculation with *P. expansum*, but the level of effectiveness at 12 h was much lower compared with the 24 h time interval ($P < 0.05$). In addition, a significant difference in inhibition was observed between inoculum densities of 10^2 and 10^6 spores/ml, but only on day 4 and only for timing of application of 24 h.

Regression analysis (Fig. 6) showed the relationship of average lesion diameter for IAA treatments at different concentrations (y) and the incubation time after pathogen inoculation (x), which was described by the following equation: at 2 µg/ml, $y = -22.131x + 104.08$, $r^2 = 0.994$ (Pearson correlation coefficient = -0.975 , $P = 0.000$); at 20 µg/ml, $y = -24.3x + 127.54$, $r^2 = 0.9883$ (Pearson correlation coefficient = -0.984 , $P = 0.000$); and at 200 µg/ml, $y = -2.0631x + 11.655$, $r^2 = 0.9853$ (Pearson correlation coefficient = -0.421 , $P = 0.152$). These results indicated that the efficacy of IAA in inhibiting lesion size was closely correlated with the concentrations of IAA and incubation time.

The slope and x -axis position of regression lines for IAA at 200 µg/ml was nearly linear, while for IAA at 2 or 20 µg/ml were similar in slope and the x -axis position for IAA at 20 µg/ml was delayed by the equivalent of a full day (Fig. 6).

In addition, there was a significant positive Pearson correlation coefficient (0.938, $P = 0.000$) of the efficacy of the induced resis-

Fig. 3. Effects of indole-3-acetic acid (IAA) on population growth of *Cryptococcus laurentii* in apple fruit wounds at 20°C. Each value is the mean of three independent determinations and bars represent the standard errors.

TABLE 1. Effects of indole-3-acetic acid (IAA) on *Cryptococcus laurentii* and *Penicillium expansum* in vitro at 28°C^w

IAA (µg/ml)	<i>C. laurentii</i> in NYDA ^x (log cells/ml)		<i>C. laurentii</i> on NYDA ^y (CFU/ml)		<i>P. expansum</i> on PDA ^z (CFU/ml)	
	Reduction (% of the control)		Reduction (% of the control)		Reduction (% of the control)	
0	8.94 ± 0.13 a		1,950 ± 143 a		2,440 ± 95 a	
0.1	8.70 ± 0.04 b	2.7	...		...	
1	8.58 ± 0.04 b	4.0	1,590 ± 69 b	18.5	2,550 ± 179 a	0.0
10	7.48 ± 0.15 c	16.3	1,550 ± 48 b	20.5	2,690 ± 155 a	0.0
100	Undetectable*		1,100 ± 61 c	43.6	2,620 ± 84 a	0.0
1,000	Undetectable*		83 ± 45 d	95.7	770 ± 26 b	68.4

^w Each value is the mean of three separate determinations and the standard error. Different letters indicate significant differences ($P = 0.05$) according to one-way analysis of Duncan's multiple range test.

^x *C. laurentii* was cultured in nutrient yeast dextrose agar (NYDA) added to filter-sterilized IAA. Yeast cells were collected after 24 h. * The amount of yeast cells was lower than the detectable threshold (5×10^4 cells/ml) determined by a hemacytometer.

^y After the *C. laurentii* cells were incubated in IAA at various concentrations for 1 min, 100 µl of each suspension was plated on NYDA. After 36 h at 28°C, the colonies per plate were counted and converted to CFU/ml.

^z After the *P. expansum* spores were incubated in IAA at various concentrations for 1 min, 100 µl of each suspension was plated on potato dextrose agar (PDA). After 72 h at 28°C, the colonies per plate were counted and converted to CFU/ml.

Fig. 4. In vivo detection of spore germination of *Penicillium expansum* from apples treated with indole-3-acetic acid (IAA). Each value is the mean of three independent determinations of the percentage of spore germination and the standard error. Values followed by a different letter within a column show significant inhibition of spore germination ($P < 0.00001$) according to Duncan's multiple range test.

Fig. 5. Effects of indole-3-acetic acid (IAA) concentration on reduction of blue mold rot in apple fruit wounds at 20°C. Apple fruit were wounded and treated with IAA at 2, 20, 50, 100, and 200 µg/ml 24 h prior to inoculation with *Penicillium expansum*. Data were recorded at a further 4 days after inoculation. The percent inhibition (%) of the lesion diameter of infected wounds was calculated by comparison with the control as described in the text. Each value is the mean of three separate determinations and bars represent the standard errors. Different letters indicate significant differences ($P = 0.01$) according to Duncan's multiple range test.

tance by IAA at 2, 20, and 200 µg/ml and efficacy of *C. laurentii* with IAA at the comparable concentration and the comparable incubation time (IAA alone on day 3 to day 6; *C. laurentii* combined with IAA on day 5 to day 8).

Application of IAA at a concentration of 20 µg/ml also significantly reduced rot incidence on intact apple fruit, especially as time between application and pathogen inoculation was 48 h, whereas its effective was remarkably decreased when the time interval was reduced to 24 h, or increased to 120 h (Fig. 7).

However, it should be noted that the induced resistance by IAA was most effective in inhibiting the early stages of infection (Table 2; Fig. 6) but decreased as incubation time after pathogen inoculation went by.

DISCUSSION

The results from this study demonstrated that the combination of IAA at 20 µg/ml with the biocontrol yeast *C. laurentii* was a much more effective means to control blue mold rot in apple fruit wounds than the application of *C. laurentii* alone. Similar control effects of postharvest blue and gray mold rot using a combination of *C. laurentii* and IAA was observed in pear fruit (36). Since IAA has no residue problem when used as plant regulator on all food commodities after harvest (31), this combination of *C. laurentii* with IAA might be considered to be a safe and effective means for controlling *P. expansum* infection in harvested fruit.

Fig. 6. Effects of indole-3-acetic acid (IAA) at different concentrations on induction of diseases resistance against blue mold rot in apple fruit wounds over time at 20°C. Apple fruit were wounded and treated with IAA at 2, 20, and 200 µg/ml 24 h prior to inoculation with *Penicillium expansum*. The percent inhibition (%) of the lesion diameter of infected wounds was calculated by comparison with the control as described in the text. Each value is the mean of three separate determinations.

TABLE 2. Effects of timing between indole-3-acetic acid (IAA) application and pathogen spore density on blue mold rot in apple fruit wounds at 20°C^z

Timing application	Inoculum density	Inhibition of blue mold after inoculation (%)		
		D3*	D4**	D5**
24 h	10 ⁶ spores/ml	83.1 ± 4.6 a	28.2 ± 5.4 b	22.3 ± 3.2 ab
	10 ² spores/ml	94.8 ± 5.2 a	79.2 ± 8.4 a	39.7 ± 6.9 a
12 h	10 ⁶ spores/ml	10.4 ± 3.6 b	6.3 ± 3.7 c	4.3 ± 3.0 b
	10 ² spores/ml	12.6 ± 3.1 b	10.6 ± 2.1 c	15.6 ± 5.5 bc
2 h	10 ⁶ spores/ml	0 ± 0 c	0 ± 0 d	0 ± 0 d
	10 ² spores/ml	0 ± 0 c	0 ± 0 d	0 ± 0 d

^z Apple fruit were wounded and treated with 30 µl of filter-sterilized IAA at 20 µg/ml or with 30 µl of distilled water as the control at 2, 12, or 24 h before inoculation. The treatment with distilled water and inoculated with *Penicillium expansum* served as the control at each time point. The inhibition data (percent reduction in lesion diameter) were calculated compared with the control, which was described in the text. The rot symptoms were observed 3 days (D3), 4 days (D4), and 5 days (D5) after inoculation, respectively. Each value is the mean of three independent determinations and the standard error. Values followed by a different letter show significant inhibition (* = 0.000001 level; ** = 0.05 level) according to Duncan's multiple range test.

Results from in vitro growth experiments showed that IAA at concentrations between 0.1 and 1,000 $\mu\text{g/ml}$ could inhibit the yeast *C. laurentii* on NYDA, whereas it produced the inhibition of the fungus *P. expansum* only when the IAA concentration was at 1,000 $\mu\text{g/ml}$ on PDA. Inhibitory effects of IAA were also observed with the yeast *S. cerevisiae*, and the fungal pathogen *U. maydis* and the oomycete *P. infestans* under different in vitro conditions (22,25). However, in apple fruit wounds, inhibition by IAA on the growth of *C. laurentii* was observed only when the concentration of IAA was increased to 200 $\mu\text{g/ml}$ or above, whereas IAA at concentrations ranging from 2 to 2,000 $\mu\text{g/ml}$ had no direct antifungal activity against blue mold infection.

Previously, we also observed that IAA had no direct antifungal activity against *P. expansum* and *B. cinerea* in pear fruit (36). These data suggested in vitro results did not accurately represent in vivo situations (27) and IAA had little direct fungicidal activity in vivo. However, the results from the present study revealed that IAA could exhibit a strong inhibition of the blue mold rot when the time interval between the IAA treatment and the *P. expansum* inoculation was increased to 24 h in the wounds or to 48 h in the intact fruit. Similar results were obtained with *P. infestans* on the detached potato leaves in which a reduction in the late blight infection was observed when the leaves were incubated in a solution of IAA for 24 h and then inoculated with *P. infestans* (22). Accordingly, we suggested that IAA could induce host-mediated resistance against fungal pathogens, but requires a sufficient induction time to be effective. It has been demonstrated that fruit resistance induced by various elicitors, such as β -aminobutyric acid, chitosan, harpin, postharvest biocontrol yeasts (*Candida oleophila* and *C. saitoana*), salicylic acid, UV irradiation, and sodium silicate, usually develops in one or a few days (5,9,12,13,23,34).

Induced plant resistance triggered by various elicitors that do not act directly against pathogens is an attractive means of disease control because of their potential to be far less toxic than fungicides (32). However, it is worth noting that success of inducing plant resistance as a disease control method relies on rapid development and at adequate intensity upon challenge by a

pathogen (7,30), because necrotrophic pathogens can start the infection process immediately after their spores land on wound sites (15). Consequently, the pathogen is capable of killing host cells or causing extensive tissue damage at the very early stages of infection (24). Our data showed that the spores of *P. expansum* germinated quickly within 12 h in apple fruit wounds, which was consistent to that reported by Amiri et al. (2) who also found that emergence of the germ tube of *P. expansum* was observed in 12 to 24 h of inoculation on apple wounds followed by the formation of the conidiophore and sporulation. Hence, we deduce here that the time lag between IAA treatment and induced resistance might be the key reason for the failure of IAA alone to reduce the blue mold infection directly; the time needed for the IAA-induced resistance in apple fruit (24 to 48 h) was much longer than for the spore germination of *P. expansum* in vivo (12 h).

In our tests, the biocontrol yeast *C. laurentii* proliferated rapidly within the first 12 to 24 h of incubation in apple fruit wounds, which was in agreement with that reported by Roberts (26). This suggested that the biocontrol yeast could rapidly colonize apple wounds and preemptively utilize available nutrients needed for pathogen colonization (14,16,26). Furthermore, we speculate that in combination with IAA, the biocontrol yeast *C. laurentii* would be the first and primary line of defense against the mold infection, while IAA could be considered a second line of defense that reinforces the biocontrol efficacy of *C. laurentii*, resulting in an integrated, more effective biological means to reduce the postharvest decay than either method alone.

Our data showed that *C. laurentii* alone or with IAA had most effective results in inhibiting the early stages of infection (within 5 days), whereas their efficacy decreased as the time of incubation went by and could not provide complete protection from the mold infection, although *C. laurentii* with IAA could delay the mold rot development by another 1 to 2 days under the designed experiment conditions compared with the *C. laurentii* alone. These results imply that treatments involving *C. laurentii* and IAA primarily produced fungistatic, but not fungicidal, effects on the mold growth. Therefore, the effectiveness and consistency of this combined control means of using *C. laurentii* and IAA need to be further improved for practical purposes.

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Fig. 7. Effects of indole-3-acetic acid (IAA) on incidence of blue mold rot in intact apple fruit at 20°C. The intact fruit samples were dipped in IAA (20 $\mu\text{g/ml}$) solution for 20 min, or in the distilled water for 20 min as the control. After 0, 24, 48, 72, 96, and 120 h, one of six sets of the treated fruit was wounded and inoculated with 30 μl of *Penicillium expansum* spore suspension (1×10^4 spores/ml). The disease incidence was recorded on every fourth day after inoculation. The percentage of the rotted wounds (% of control) was calculated as described in the text. Each value is the mean of three separate determinations and bars represent the standard error. Different letters indicate significant differences ($P = 0.05$) according to Duncan's multiple range test. Bars represent standard errors of three replicate.

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