Postharvest Biological Control of Rhizopus Rot of Nectarine Fruits by *Pichia membranefaciens*

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ABSTRACT

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A new yeast antagonist, *Pichia membranefaciens*, isolated from wounds of peach fruit, was evaluated for its biocontrol capability against *Rhizopus stolonifer* on nectarine fruits at different temperatures and with other treatments. *P. membranefaciens* at 5×10^8 CFU/ml of washed-cell suspension completely inhibited Rhizopus rot in nectarine wounds artificially inoculated with 5×10^4 spores per ml at 25, 15, and 3°C. A culture filtrate of the yeast antagonist failed to provide any protection against Rhizopus rot in nectarine fruits. The yeast mixed with iprodione at 100 µg a.i./ml gave better control of *R. stolonifer* than either yeast or iprodione alone. A solution of 20 g CaCl₂ per liter enhanced the efficacy of *P. membranefaciens* (10^7 to 10^8 CFU/ml) as an aqueous suspension. Rapid colonization of the yeast in wounds was observed during the first 48 h at 25 and 15°C. *P. membranefaciens* at 5×10^8 CFU/ml was effective when applied 0 to 72 h before the pathogen, while at 1×10^8 CFU/ml, its efficacy was best when applied 24 to 48 h prior to inoculation with *R. stolonifer*. However, its efficacy was significantly reduced when the yeast was applied simultaneously with the pathogen, with disease incidence of 60% and lesion diameter of 37 mm.

Additional keywords: calcium chloride, *Prunus*

Rhizopus rot, caused by *Rhizopus stolonifer* (Ehrenb.: Fr) Vuill., is one of the most destructive postharvest diseases of stone fruits in China. The spores of *R. stolonifer* are very common in the atmosphere and this infection of fruits occurs mainly at wound sites during harvest or packing. Chemical treatment is a very important method for controlling this disease (20,25). However, the application of fungicides may lead to the development of fungicide-resistant strains of postharvest pathogens (14,29). In addition, public concern over the presence of chemical residues in food has resulted in cancellation of some of the most effective fungicides (1,15). Therefore, research has been focused on the development of management alternatives that are both effective and economically feasible (13,14,33). Microbial biocontrol agents have shown promise (13,32) as potential alternatives to fungicides (6,16,27).

Although the biocontrol of postharvest diseases has been widely reported (27,32), *R. stolonifer* has received little attention in the past (19,21,31). Isolates of *Kloeckera apiculata*, *Candida guilliermondii* (21),

and *Enterobacter cloacae* (31) partially controlled postharvest Rhizopus rot of peach fruits, whereas *Cryptococcus laurentii* and *Rhodotorula glutinis* also limited Rhizopus rot in apple, table grapes, and strawberries (19). Yeasts appear to be particularly promising biocontrol agents in the postharvest environment, since production of antibiotic probably is not involved in their activity (11).

A new yeast antagonist, *Pichia membranefaciens* Hansen, isolated from peach fruits, was used in this study. Our objectives were to determine its effectiveness against *R. stolonifer* infection of nectarine fruits as affected by: (i) antagonist concentration; (ii) calcium chloride treatment; (iii) time of application; and (vi) combination with the fungicide iprodione.

MATERIALS AND METHODS

Pathogen inoculum. *Rhizopus stolonifer*, isolated from peach fruits, was maintained on potato dextrose agar (PDA). A sporangiospore suspension of the pathogen was prepared from 10-day-old culture dishes incubated at 25°C by flooding with 10 ml of sterile distilled water containing 0.5 ml of Tween 80 per liter. The spore concentration was determined with a hemacytometer and adjusted to 5×10^4 spores per ml with sterile distilled water.

Antagonists. *P. membranefaciens* was isolated from wounds of peach fruits with the method described by Wilson et al. (33). The yeast (IMI number 382465) was identified by CABI Bioscience Identification

Services (International Mycological Institute, UK). Yeast cells cultured in 250-ml Erlenmeyer flasks with 50 ml of nutrient yeast dextrose broth (NYDB: 8 g of nutrient broth, 5 g of yeast extract, and 10 g of dextrose in 1 liter water) on a rotary shaker at 200 rpm for 24 h at 28°C. The cells were harvested by centrifugation at 6,000 rpm (about 2,500 g) for 10 min, washed twice with 0.05 M phosphate buffer at pH 7.0, and resuspended in distilled water. The concentration of cells in the suspension was counted with a hemacytometer and adjusted to 5×10^8 CFU/ml with sterile distilled water. Culture filtrates were prepared by centrifuging cultures of the antagonist through a 0.2-µm polycarbonate membrane filter. The unwashed cells were in the 24-h culture filtrate and adjusted to 5×10^8 CFU/ml with additional culture filtrate. The treatments were as follows: (A) 5×10^8 CFU of unwashed culture filtrate per ml; (B) culture filtrate; and (C) 5×10^8 CFU of washed cell suspension per ml in sterile distilled water; (D) sterile distilled water.

Fruits. Nectarine (*Prunus persica* var. *nectarina*) fruits were harvested at a fruit firmness of 6.74 kg/cm² from Jinzhou in Liaoning province. The fruit was transported to Beijing by a refrigerator car (2 to 4°C) soon after harvest, then placed in refrigerated storage at 0°C. At the beginning of the biocontrol experiment, fruit firmness was 5.70 to 5.50 kg/cm² after 40 to 50 days storage at 0°C. Fruits were disinfected with 2% sodium hypochlorite for 2 min, washed with tap water, and air dried prior to wounding.

Efficacy of *P. membranefaciens* for controlling of Rhizopus rot. A uniform 4 mm deep and 3 mm wide wound was made at the equator of each fruit (put on its side) using the tip of a sterile dissecting needle. A 30-µl drop of above-mentioned suspensions of A, B, C, or D was pipetted onto each wound site. Four hours later, each wound was inoculated with 15 µl of a suspension of 5×10^4 spores of *R. stolonifer* per ml. Fruits were air dried, then put into 400 × 300 × 100 mm plastic trays wrapped with high density polyethylene sleeve to retain high humidity (about 95%). There were three replicate trials of 10 fruits per treatment with a complete randomization of trays in each test. The test was conducted three times. The fruits were stored at 25, 15, and 3°C, and infection incidence and lesion diameter were observed after 4, 6, and 30 days. Fruits stored at 3°C for 30

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days were transferred to 25°C for 3 to 6 days in order to determine disease development during simulated shelf life. Two diameters were measured at right angles across lesions on the surface of infected fruit. Data presented are the means of lesion diameters. The lesion diameter was recorded as 75 mm when the lesion exceeded half of the fruit perimeter.

Effects of Ca^{2+} and antagonist concentrations on *Rhizopus* rot. To evaluate biological control activity in the presence and absence of Ca^{2+} , cell suspensions were made in 2% (wt/vol) and 0% (distilled water) CaCl_2 solutions. Wounds were made as described above, then inoculated with 30 μl of 5×10^8 , 5×10^7 , 5×10^6 , 5×10^5 , and 0 CFU/ml suspensions of *P. membranefaciens*. After 4 h, 15 μl of 5×10^4 spores per ml of *R. stolonifer* were added to each wound. Treated fruits were stored at 25°C in wrapped trays as described above. Disease incidence and severity were recorded 4 days after challenge inoculation. There were three replicates of 10 fruits (one wound per fruit) per treatment. The test was conducted three times.

Colonization of wounds by *P. membranefaciens*. Three wounds per fruit were made equidistant around the fruit "equator," then 30 μl of washed-cell suspension of *P. membranefaciens* at 5×10^8 CFU/ml was pipetted onto each wound. Fruit samples were taken to determine the antagonist population 1 h after inoculation (time 0) and after 7, 14, 21, and 28 days at 3°C. The population in wounds of fruits stored at 25 and 15°C were sampled each day for 7 days. Yeast cells were recovered as described by Janisiewicz et al. (18). Fruits were wounded and 30 μl of 5×10^8 CFU/ml suspension was applied to each wound. The antagonist was recovered by removing the wound tissue, grinding it in a mortar with 0.05 M phosphate buffer, and plating 0.1 ml of a 10-fold dilution on NYDA. Plates were incubated at 26°C for 48 h, and colony counts were expressed as

the $\log_{10}$ transformed number of cells per wound. An analysis of variance of the $\log_{10}$ transformed data was carried out for each sampling day. There were three replicates of one fruit (with three wounds) for each treatment, and the experiment was conducted twice.

Preinoculation with yeast to control *Rhizopus* rot. A 30- μl drop containing a suspension of *P. membranefaciens* at 5×10^8 or 1×10^8 CFU/ml was pipetted onto the wound simultaneously with the *R. stolonifer* spores (5×10^4 spores per ml) or at 4, 24, 48, and 72 h before the pathogen. After inoculation, the fruits were incubated at 25°C, and decay development was determined after 4 days. Each treatment consisted of three replicates of 10 fruits in each experiment, and the experiment was conducted three times.

Combination of yeast and iprodione on incidence of *Rhizopus* rot. The efficacy of *P. membranefaciens* was compared with iprodione. The treatments were as follows: iprodione at 1,000 μg a.i./ml; yeast at 5×10^6 CFU/ml plus iprodione at 100 μg a.i./ml; yeast at 5×10^6 CFU/ml; iprodione at 100 μg a.i./ml; and sterile distilled water. Each wound was inoculated with 30 μl at each treatment. After 4 h, 15 μl of *R. stolonifer* at 5×10^4 spores per ml was inoculated onto each wound. Fruits were kept at 25°C, and the incidences of *Rhizopus* rot were observed 4 days later. Each fruit was wounded once, with three replicates of 10 fruits each per treatment, and the experiment was conducted three times.

***P. membranefaciens* in vitro effects on *R. stolonifer*.** The effects of *P. membranefaciens* on spore germination and germ tube elongation of *R. stolonifer* were assessed in potato dextrose broth (PDB) by the method of Piano et al. (26). An aliquot (100 μl) of *R. stolonifer* at 5×10^6 spores/ml was transferred into a glass tube (180 $\times$ 16 mm) containing 5 ml of PDB. A 100- μl quantity of 5×10^8 , 5×10^7 , 5×10^6

CFU/ml living yeast cell suspensions, culture filtrate, or autoclaved 5×10^8 CFU/ml cell suspension was added to each tube, respectively. At least 200 spores of *R. stolonifer* per replicate were observed microscopically to determine germination rate and germ tube length after 15 h incubation at 25°C on a rotary shaker (50 rpm). Treatments were replicated three times, and the test was conducted two times.

Statistical analysis. All statistical analyses were performed with SAS (SAS Institute, Cary, NC). To test for the effect of the treatment, the data were analyzed by one-way analysis of variance (ANOVA). Means were separated with the Student-Newman-Keuls.

RESULTS

Efficacy of *P. membranefaciens* for controlling of *Rhizopus* rot. The highest level of control of *Rhizopus* rot was achieved with 5×10^8 CFU of washed yeast cells per ml, which completely inhibited lesion development of *R. stolonifer* in nectarine fruit at all temperatures (Fig. 1). The infection incidence and lesion diameter of fruit treated by cell-culture filtrate suspension were 30% and 16 mm after 4 days at 25°C, 20% and 6 mm after 6 days at 15°C, respectively, which were significantly lower than those in washed cell suspension ($P < 0.01$). There was a significant difference between cell-culture filtrate suspension and washed cell suspension at 25 and 15°C ($P < 0.01$). The culture filtrate did not inhibit the development of *Rhizopus* rot at 25 and 15°C with infection incidence of 100%, but the infection incidence (20%) was significantly reduced compared with the control (80%) in 3°C storage at 25°C simulated shelf study ($P < 0.01$). No infection incidence occurred for all treated fruits after 30 days storage at 3°C.

Effects of Ca^{2+} and antagonist concentrations on *Rhizopus* rot. Significant

Fig. 1. Inhibition of *Rhizopus* rot of nectarine fruits as affected by various treatments of the antagonist. Each value is the mean from 30 inoculation sites. A = 5×10^8 CFU of *Pichia membranefaciens* per ml in 24 h culture filtrate; B = culture filtrate; C = 5×10^8 CFU suspension of washed cells of *Pichia membranefaciens* per ml; D = sterile distilled water. Vertical bars represent standard error. Lesion diameters (right) and infected wounds (left) were measured after 4 days incubation at 25°C, 6 days incubation at 15°C, and 30 days storage at 3°C followed by 6 days at 25°C.

effects of Ca^{2+} on the inhibition of *Rhizopus* rot were observed. Infection incidence and lesion diameter of fruits treated with 2% CaCl_2 were significantly lower than for those treated with water at all yeast concentrations except at 5×10^8 CFU/ml where both treatments completely controlled *Rhizopus* rot ($P < 0.01$) (Fig. 2). In addition, there were significant negative relationships between infection severity and yeast concentrations at both 0 and 2% CaCl_2 . At the lowest concentration (5×10^5 CFU/ml), the infection incidence and lesion diameter were significantly lower than in the control ($P < 0.01$).

Colonization of wounds by *P. membranefaciens*. The populations of *P. membranefaciens* in drop-inoculated wounds in fruits increased rapidly. At the beginning of the experiment (time 0), the yeast population was 3.2×10^6 CFU per wound, and increased to 1.8×10^7 CFU at 25°C, 2.5×10^7 CFU at 15°C, and 6.8×10^6 CFU at 3°C per wound within the first 2 days (Fig. 3), then stabilized thereafter (1.8×10^7 to 8.5×10^7 CFU per wound at 25 and 15°C, 6.8×10^6 to 2.9×10^7 CFU per wound at 3°C). Temperature and incubation time also affected population of the antagonist in wounds. The highest populations of the antagonist in wounds were 8.3×10^7 CFU, 7.7×10^7 CFU, and 3.0×10^7 CFU per wound after incubation for 4 days at 25°C, 6 days at 15°C, and 28 days at 3°C, respectively, which were 26.3, 24.4, and 9.5 times greater than at the beginning of the experiment.

Preinoculation with yeast to control *Rhizopus* rot. Complete control of *Rhizopus* rot was achieved with application of *P. membranefaciens* at 1×10^8 CFU/ml both at 24 and 48 h before inoculation with the pathogen. The biocontrol efficacy was reduced when the antagonist was applied at the same time as the pathogen or 72 h before the pathogen. The infected wounds and lesion diameter were 60% and 37 mm at 0 h, 70% and 5 mm after 72 h, respectively (Table 1). Preinoculation with *P. membranefaciens* at $5 \times$

10^8 CFU/ml provided complete control of *Rhizopus* rot, irrespective of the time interval prior to the inoculation with *R. stolonifer*.

Effects of combination of yeast and iprodione on incidence of *Rhizopus* rot. Fruit infection and lesion diameters of all treatments were significantly reduced compared with the untreated control ($P < 0.01$) (Table 2). Iprodione at 1,000 μg a.i./ml completely controlled the disease. At a concentration of 100 μg a.i./ml, control was less active than at the higher rate, with 85% infection and 60 mm lesion di-

ameter. The concentration at 5×10^6 CFU/ml partially inhibited the pathogen, with 45% infection and 38 mm lesion diameter. The combination of *P. membranefaciens* and iprodione (5×10^6 CFU/ml plus 100 μg a.i./ml) resulted in significantly lower infection incidence and lesion diameter than those treated singly with 5×10^6 CFU/ml or iprodione at 100 μg a.i./ml ($P < 0.01$).

***P. membranefaciens* in vitro effects on *R. stolonifer*.** Spore germination of *R. stolonifer* in PDB was totally inhibited with *P. membranefaciens* at 5×10^8

Fig. 3. Population dynamics of *Pichia membranefaciens* in wounds of nectarine fruits incubated at 25 to 15 (A) and 3°C (B). The fruits were wounded and 30 μl of 5×10^8 CFU/ml was applied to each wound. The antagonist was recovered by removing the wound tissue, grinding it in a mortar with 0.05 M phosphate buffer, and plating 0.1 ml of a 10-fold dilution on NYDA. There were three replicates of one fruit for each treatment, and the experiment was conducted twice. Data were pooled from two experiments. Bars represent standard error of the means.

Fig. 2. Relationship between development of *Rhizopus stolonifer* on nectarine fruits and cell concentration of *Pichia membranefaciens* suspended in 2% and 0% CaCl_2 . Pathogen concentration was 5×10^4 spores per ml. Lesion diameter (right) and percent infected wounds (left) were determined after 4 days of storage at 25°C. There were three replicates of 10 fruits (one wound per fruit) per treatment. The test was conducted three times, and data were pooled from three experiments. Means of lesion diameter and infected wounds of the control were 75 mm and 100%, respectively.

CFU/ml (Table 3). Cell suspensions of 5×10^7 and 5×10^6 CFU/ml significantly reduced germination rate and germ tube length ($P < 0.01$). Yeast cells killed by autoclaving did not affect spore germination and germ tube growth, whereas spores grown in culture filtrate had comparable germination and longer germ tube length than those in the control.

DISCUSSION

Rhizopus rot of nectarine was effectively controlled by the application of washed cells of *P. membranefaciens*. Wound areas treated with *P. membranefaciens* showed no darkening or necrosis associated with application of a concentration of 5×10^8 CFU/ml to wounds. Some reports have demonstrated that a direct relationship exists between the population density of an antagonist and the efficacy of postharvest biological control treatment (15,16). Our results also showed that the antagonistic activity of *P. membranefaciens* was dependent on the concentration of the antagonist. Concentrations from 5×10^5 to 5×10^7 CFU/ml did not provide a satisfactory level of control, while 5×10^8 CFU/ml completely limited rot development. The same level of control was found with 1×10^8 CFU/ml applied at 24 to 48 h and in 5×10^8 CFU/ml at 0 to 72 h before inoculation with the pathogen. These results indicated that when saturation of wound sites with *P. membranefaciens* was achieved, there was no difference between different applied concentrations, because the nutrients and/or the moisture present at the wound site limited yeast growth if the applied yeast reached saturation density (24). Our results also agreed with Piano et al. (26) using *Metschnikowia pulcherrima* to control gray mold of apple. When yeast was applied at a higher concentration (10^8 to 10^9 CFU/ml), the biocontrol activity was not apparently related to the time of pathogen application.

Wounds treated with culture filtrate of *P. membranefaciens* developed rapidly expanding lesions, with the exception of the 3°C storage/25°C simulated shelf study, where some inhibition occurred after inoculation with *R. stolonifer*. In addition, culture filtrate co-cultured with pathogen spores did not affect germination or germ tube elongation. This may indicate that *P. membranefaciens* produced no apparent antibiotic substance. This is quite different from some postharvest bacterial antagonists (17,27).

Unwashed cells of *P. membranefaciens* in culture filtrate showed decreased biocontrol effectiveness compared with the washed cells, which supported the premise that competition for nutrients may play a major role in the biocontrol capability of *P. membranefaciens* against *R. stolonifer*. The importance of nutrient competition has been previously demonstrated with other antagonistic yeasts (11,17,21,28). How-

ever, the in vitro experiments did not show the same result as in vivo experiments where *P. membranefaciens* limited spore germination and germ tube elongation of *R. stolonifer*, even under the nutrient rich conditions of PDB, which is in agreement with the result obtained by Piano et al. (26).

Rapid colonization of *P. membranefaciens* in nectarine wounds was observed at 25, 15, and 3°C at an early stage and then stabilized for the remaining time, as previously observed for other antagonistic yeasts (24,26,28). The initial recovery after 1 h was 3.2×10^6 CFU per wound, representing only 21% recovery of 1.5×10^7 CFU per wound (30 µl of 5×10^8 CFU/ml). However, the yeast population increased rapidly after 2 days incubation. The rapid growth of the yeast in wounds over a wide range of temperatures and its ability to control Rhizopus rot indicated that *P. membranefaciens* is well adapted to the wound environment in fruit and has considerable potential as a biocontrol

agent. The ability of yeast to multiply rapidly in wounds may benefit its utilization of available nutrients, thus may facilitate biocontrol by nutrient competition.

Calcium plays an important role in controlling physiological disorders and diseases of fruits and vegetables. Application of supplemental calcium to apples is a routine practice in orchards that are prone to calcium-related nutritional disorders such as cork spot and bitter pit (2). Increased calcium content of fruits and vegetables can prolong their storage life (8). This is due mainly to the role of calcium in ameliorating physiological disorders and thus indirectly reducing pathogen activity (10). The effectiveness of calcium chloride against brown rot has been ascribed to the enhancement of host resistance by calcium (9). The role of calcium in resistance may be in interfering with the activity of pectolytic enzymes (9,10). Besides, calcium can directly inhibit the pathogens. Biggs (2) found calcium salts directly suppress the bitter rot pathogens

Table 1. Effect of wounding and applying *Pichia membranefaciens* (P.m.) prior to the inoculation of nectarine fruits with *Rhizopus stolonifer* for control of Rhizopus rot

Time between application of P. m. and inoculation (h)	Infected wounds (%)		Lesion development (mm)	
	5×10^8 CFU/ml	1×10^8 CFU/ml	5×10^8 CFU/ml	1×10^8 CFU/ml
0	0 b ^z	60 c	0 b	37 b
4	0 b	5 d	0 b	2 d
24	0 b	0 e	0 b	0 e
48	0 b	0 e	0 b	0 e
72	0 b	70 b	0 b	5 c
Control	100 a	100 a	75 a	75 a

^z Data were measured 4 days after inoculation with the pathogen at 25°C. Values of each column followed by the same letter are not statistically different according to Student-Newman-Keuls ($P = 0.01$).

Table 2. Effect of *Pichia membranefaciens* (P.m.) and chemical treatment with iprodione against *Rhizopus stolonifer* on nectarine

Treatments ^y	Infected wounds (%)	Lesion diameter (mm)
Iprodione, 1,000 µg/ml	0 e ^z	0 e
P.m., 5×10^6 CFU/ml + iprodione, 100 µg/ml	20 d	12.5 d
P.m., 5×10^6 CFU/ml	45 c	38 c
Iprodione, 100 µg/ml	85 b	60 b
Sterile distilled water	100 a	75 a

^y Lesion diameter and infected wounds were recorded after 4 days storage at 25°C. Each treatment is the mean from 10 wounds, and data were pooled from three trials.

^z Values of each column followed by the same letter are not significantly different according to Student-Newman-Keuls ($P = 0.01$).

Table 3. Effect of different concentrations of *Pichia membranefaciens*, autoclaved cells, and cell-free culture filtrates on spore germination and germ tube elongation of *Rhizopus stolonifer*

Treatment ^y	Spore germination (%)	Germ tube length (µm)
5×10^6 CFU/ml	30 b ^z	22.8 d
5×10^7 CFU/ml	13 c	15.4 d
5×10^8 CFU/ml	0 d	0.0 e
5×10^8 CFU/ml (autoclaved)	96 a	205.5 c
Culture filtrate	100 a	249.0 a
Control	97 a	218.4 b

^y Germination and germ tube length were measured microscopically after 15 h incubation at 25°C in potato dextrose broth. Data were pooled from two trials.

^z Values of each column followed by the same letter are not significantly different according to Student-Newman-Keuls ($P = 0.01$).

by reducing germ tube growth and mycelial growth in vitro, and by reducing severity of infection of host tissue pretreated with calcium. Calcium salts have also been shown to reduce mycelial growth in vitro and reduce disease incidence and severity of peach fruits and shoots by *Monilinia fructicola* and *Leucostoma persoonii* (3,4,5). Biocontrol effectiveness of antagonists could be enhanced in the presence of calcium chloride (12,21,22). MacLaughlin et al. (21) found that control of gray mold and blue mold of apples was enhanced when strain 138 of *Kloeckera apiculata* was applied as an aqueous suspension (10^8 CFU/ml) in 2% CaCl_2 . Our study showed that calcium chloride significantly improved biocontrol obtained with *P. membranefaciens*. The ability of antagonists to reduce decay in the presence of Ca^{2+} may be partially due to nutrient competition and/or site exclusion (22), or directly inhibit spore germination and growth of pathogen through an effect on the activity of cell-wall macerating enzymes (34). The effect of calcium in this study implied that *P. membranefaciens* may be used in the future on a commercial scale for the control of postharvest diseases in fruit. However, the mode of action of calcium-*P. membranefaciens* synergism should be further studied.

The use of iprodione at a concentration of 100 μg a.i./ml in combination with *P. membranefaciens* at 5×10^6 CFU/ml enhanced the biocontrol efficacy compared to the treatments used separately, similar to the results of Mari et al. (23). This approach could lead to a reduction of the chemical residue on fruit as proposed previously (3,6,7,23,30). Chand-Goyal and Spotts (7) reported that better control of blue mold and brown rot were achieved by combining *Cryptococcus infirmo-minutus* or *C. laurentii* with low dose of thiabendazole (15 μg /ml) and iprodione (20 μg /ml). Spotts et al. (30) found incidence of brown rot of sweet cherry was reduced from 41.5% in the control to 0.4% by combining preharvest iprodione and postharvest *C. infirmo-minutus* treatments with modified atmosphere packaging.

P. membranefaciens, which is present in soil, fruit, wine, beer, and grape must, is generally considered to be nonpathogenic (Identification report-H37/80/YC7, CABI Bioscience Identification Services) and would be safe to use for biological control proposes. Moreover, we found that the use of *P. membranefaciens* for biocontrol is compatible with several common postharvest practices including iprodione, Ca^{2+} treatment, and cold storage, thus permitting an integrated control approach under commercial conditions.

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