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Postharvest biological control of gray mold decay of strawberry with *Rhodotorula glutinis*

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Abstract

Rhodotorula glutinis was evaluated for its activity in reducing postharvest gray mold decay of strawberry caused by *Botrytis cinerea* in vitro and in vivo. In the test on PDA plates, *R. glutinis* significantly inhibit the growth of *B. cinerea*. Spore germination of pathogens in PDB was greatly controlled in the presence of living cell suspensions. Rapid colonization of the yeast in wounds was observed during the first 3 days at 20 °C, and then the populations stabilized for the remaining storage period. On strawberry wounds kept at 4 °C, the increase in population density of *R. glutinis* was lower than those kept at 20 °C, but continued over 8 days after application of the antagonist until it reached a high level. Number of inoculated strawberry fruit treated with 1×10^8 CFU/ml washed cell suspension of *R. glutinis* was 10% after 2 days at 20 °C, compared to 100%, respectively, in the control. Washed cell suspensions of yeast controlled gray mold better than yeast in culture broth. Treatment of wounds with autoclaved cell cultures or cell-free culture filtrate did not prevent decay. The concentrations of antagonist had significant effects on biocontrol effectiveness: the higher the concentrations of the antagonist, the lower the disease incidence regardless of whether the fruit was stored at 20 °C for 2 days or 4 °C for 7 days. At concentrations of *R. glutinis* 1×10^9 CFU/ml, the incidence of gray mold was reduced by 94.7 or 95%, respectively, compared with control, after storage at 20 °C for 2 days or 4 °C for 7 days, respectively. *R. glutinis* significantly reduced the natural development of decay of fruit following storage at 20 °C for 3 days or 4 °C for 5 days followed by 20 °C for 3 days.

Keywords: Strawberry; Gray mold; Postharvest decay; Biocontrol; *Rhodotorula glutinis*

1. Introduction

Strawberry fruit have a very short postharvest life, due in part to gray mold caused by *Botrytis cinerea* Pers.:Fr. (Wszelaki and Mitcham, 2003). Infection may occur in the flower, remain quiescent until fruits mature, and then develop abundantly, causing fruit decay accompanied by profuse sporulation of the pathogen (Kovach et al., 2000). *B. cinerea* also causes significant losses during shipping and marketing (Ceponis et al., 1987), making it one of the most economically important pathogens of strawberry (Mertely et al., 2002; Romanazzi et al., 2001). Control of *B. cinerea* is normally carried out by the application of fungicides. Although fungicides use reduces yield losses due to *Botrytis* in strawberries, there are studies that show that commercially available fungicides can reduce pollination and cause fruit malformation (Kovach et al., 2000). Fungicide efficacy is frequently decreased by the development of resistant strains of pathogens. In addition, public concern and regulatory restrictions about the presence of fungicide residues on crops have emphasized the need to find alternative methods for disease control (Smilanick, 1994).

Microbial biocontrol agents have shown great potential as an alternative to synthetic fungicides for the control of postharvest decay of fruits and vegetables (Wisniewski and Wilson, 1992). Several biological control agents are effective in reducing decay caused by gray mold on strawberry (Peng and Sutton, 1991; Swadling and Jeffries, 1996; Lima et al., 1997; Guinebretiere et al., 2000).

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However, few of these have been tested under postharvest storage conditions.

The objective of the present study was to study the postharvest control of gray mold of strawberry by *Rhodotorula glutinis* and determine: (1) biocontrol in vitro; (2) the population dynamics of *R. glutinis* in wounds; (3) efficacy of *R. glutinis* in controlling of gray mold decay of strawberry; (4) effect of concentration of *R. glutinis* on biocontrol efficacy at ambient temperatures and cold storage condition; (5) efficacy of *R. glutinis* in controlling of natural decay development of strawberry.

2. Materials and methods

2.1. Pathogen inoculum

Botrytis cinerea Pers.: Fr. was isolated from infected strawberry fruits. The culture was maintained on potato-dextrose agar medium (PDA: extract of boiled potatoes, 200 ml; dextrose, 20 g; agar, 20 g and distilled water, 800 ml) at 4 °C; fresh cultures were grown on PDA plates at 25 °C before use. Spore suspensions were prepared by removing the spores from the sporulating edges of a 10-day-old culture with a bacteriological loop, and suspending them in 5 ml of sterile distilled water. Suspensions were filtered through four layers of cheesecloth to remove fungal mycelium and spore concentrations were determined with a hemocytometer with the concentration being adjusted as required for different experiments by adding sterile distilled water.

2.2. Antagonist

The yeast antagonist *R. glutinis* (Fresenins) Harrison was isolated from the surfaces of strawberries harvested in unsprayed orchards and identified by VITEK 32 Automicrobic system (bioMérieux Company, Marcy l'Etoile, France). *R. glutinis* isolates were 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. Liquid cultures of the yeast were grown in 250-ml Erlenmeyer flasks containing 50 ml of NYD Broth (NYDB) which had been inoculated with a loop of the culture. Flasks were incubated on a rotary shaker at 28 °C for 20 h. Following incubation, cells were centrifuged at 5000*g* for 10 min and washed twice with sterile distilled water in order to remove the growth medium. Cell pellets were re-suspended in sterile distilled water and adjusted to an initial concentration of 2×10^9 to 5×10^9 CFU/ml (CFU, colony-forming units) before being adjusted to the concentrations required for different experiments.

Culture filtrates were prepared by filtering the supernatant of centrifuged cultures of the antagonist through a 0.2 μm polycarbonate membrane filter. Autoclaved cultures were prepared by autoclaving a sample containing yeast in culture broth for 20 min at 121 °C. Unwashed cells from 20-h cultures were adjusted to 1×10^8 CFU/ml by adding additional culture filtrate. Treatments were as follows: A, 1×10^8 CFU/ml unwashed cell culture mixture; B, culture filtrate; C, autoclaved culture; D, 1×10^8 CFU/ml washed cell suspension; and E, sterile distilled water as control.

2.3. Fruits

Strawberries (*Fragaria ananassa* Duch.) cultivars '*chunxing*' were harvested from randomized blocks and rapidly transferred to the laboratory. Berries were sorted on the basis of size, color (75% full red color), and absence of physical damage, and were randomly divided into lots of ten fruit.

2.4. In vitro antagonism

To evaluate the interactions between the antagonist and the pathogens in culture, we cut 5-mm-diameter disks from potato-dextrose agar (PDA) plates, then, a 300-μl quantity of 1×10^8 CFU/ml washed cell suspension of *R. glutinis* or sterile distilled water as a control was, respectively, added into each wound site of PDA plates. After these, 100 μl of 1×10^4 spores/ml suspension of *B. cinerea* was inoculated onto each wound. The plates were incubated at 28 °C for 5 days after which the colony diameter of *B. cinerea* were recorded. There were three replicate trials of three plates in each test. The test was repeated three times.

The effect of *R. glutinis* on spore germination and germ tube elongation of pathogen was tested in potato-dextrose broth (PDB). A 100-μl quantity of 1×10^8 CFU/ml washed cell suspension of *R. glutinis* or sterile distilled water as a control was added into 10 ml glass tube containing 5 ml PDB, respectively. At the same time, aliquots (100 μl) of spore suspensions (1×10^7 spores/ml) of *B. cinerea* were added into each tube. After 20 h incubation at 25 °C on a rotary shaker (50 rpm), at least 100 spores per replicate were observed microscopically to determine germination rate and germ tube length. All treatments consisted of three replicates, and experiments were repeated three times (Droby et al., 1997).

2.5. Population studies of R. glutinis in fruit wounds

Strawberries were rinsed in fresh water and air dried. A uniform 3 mm deep by 3 mm wide wound was made at the equator of each fruit (put on its side) using the tip of a sterile dissecting needle. Twenty μl of 1×10^8 CFU/ml washed cell suspension of *R. glutinis* was applied to wounds on fruit. Fruits were incubated at 20 °C (90% relative humidity) or at 4 °C (98% relative humidity). *R. glutinis* was recovered from the wounds after incubation at 20 °C for 0 (just prior to storage), 1, 2, 3, and 4 days, and at 4 °C, for 0 (just prior to storage), 2, 4, 6, and 8 days, respectively. Wounded tissue was removed with an ethanol-flamed,