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Postharvest Biology and Technology 35 (2005) 245–251
Postharvest Biology and Technology
www.elsevier.com/locate/postharvbio

Control of *Botrytis cinerea* strains resistant to iprodione in apple with rhodotorulic acid and yeasts

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Received 31 January 2004; accepted 18 September 2004

Abstract

A *Rhodotorula* strain (*Rhodotorula glutinis* ySL 30) in combination with a siderophore was evaluated for postharvest control of a *Botrytis cinerea* strain resistant to iprodione on apple. The biocontrol yeast was less effective for the control of iprodione-resistant *B. cinerea* than the iprodione-sensitive *B. cinerea*. A combination of *R. glutinis* and rhodotorulic acid, a siderophore produced by yeasts belonging to *Rhodotorula* genus, was evaluated as a way to improve the control of the resistant strain. In experiments "in vitro", rhodotorulic acid retarded the spore germination of the fungus, and in biocontrol experiments on apple wounds, the disease was more effectively controlled by the antagonistic agent in combination with the siderophore than by the antagonistic agent alone. *R. glutinis* reduced decay severity by 54% and *R. glutinis* in combination with siderophore reduced decay severity by 72%, in comparison with the non-treated control.

Keywords: Biocontrol agents; *Botrytis cinerea*; Iprodione; Rhodotorulic acid; Siderophore; Yeasts; *Rhodotorula glutinis*; Postharvest fruit protection

1. Introduction

Botrytis cinerea causes gray mould, which is one of the important postharvest diseases of fruits and vegetables worldwide (Wilson and Wisniewski, 1994). Gray mould is difficult to control satisfactorily with fungicides because the fungus is genetically variable and has developed strains resistant to many of the chemicals introduced in the last 20 years (Staples and Mayer, 1995). Chemical control of diseases caused by *B. cinerea* has largely depended upon the use of the benzimidazole and dicarboximide fungicides. Benzimidazole resistance is now widespread because of frequent and indiscriminant use. The dicarboximides, such as iprodione were developed in response to resistance problems with benzimidazoles. However, today it is common to detect dicarboximide-resistant isolates of *B. cinerea* from a variety of host plants (Steel, 1996). In addition, there are also pressures from consumers to

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doi:10.1016/j.postharvbio.2004.09.005

reduce pesticides in the food chain (Hileman, 1993). For these reasons, there is an increased interest in alternatives to fungicides for disease control.

Biological control can be used as an alternative to fungicides. It employs saprophytic microorganisms to protect fruits and vegetables from infection by pathogens. However, the control of postharvest diseases with antagonistic microorganisms is not often as consistent as control with synthetic fungicides. The period between harvesting and placing fruit in storage, from less than a day to a few days, requires rapid antagonist action. Once fruit is placed in cold storage, metabolic rates of the host and associated microflora will decline depending on the temperature regime selected. It may be necessary either to improve the activity of antagonistic microorganisms or to combine antagonistic microorganisms with other measures for diseases control (Wilson et al., 1994). In this way, antagonistic microorganisms have been used in combination with fruit curing methods (Cook et al., 1999), or combined with metabolites such as siderophores (Calvente et al., 1999b; Lemanceau et al., 1993).

Because the period before the storage may be critical, our efforts were directed to search for rapid colonizers of the wound site or for mechanisms that help wound colonization. Rhodotorulic acid, a siderophore produced by yeasts belonging to the genus *Rhodotorula*, has shown ability to inhibit spore germination of various plant pathogens including *B. cinerea* (Calvente et al., 2001). The aim of this work was to evaluate the possibility of combining rhodotorulic acid with saprophytic yeasts to control iprodione-resistant *B. cinerea* strains.

2. Materials and methods

2.1. Microorganisms

Six *B. cinerea* strains recovered from apple, strawberry and grape fruits were used in this study. Isolates were maintained on potato dextrose agar (PDA) at 4 °C. For conidial production, *B. cinerea* were grown on PDA at 20–25 °C. When the mycelium appeared, cultures were kept at 15 °C for inducing sporulation. After a week, spores were harvested and suspended in 10 mL of sterile-distilled water containing 0.05% (v/v) Tween 80. The concentration of spore suspension was determined with a Neubauer chamber and adjusted with sterile-distilled water to 1×10^5 spores/mL.

Antagonistic yeasts, *Rhodotorula glutinis* ySL 18 and ySL 30, *Rhodotorula rubra* ySL 16 and ySL 25, *Cryptococcus albidus* ySL 28, *Cryptococcns laurentii* ySL 11, ySL 24, ySL 26 and ySL 27, and *Candida guillermondii* ySL 23 isolated from red delicious apples (Calvente et al., 1999a) were used in the experiments and maintained on a semi synthetic minimal medium: glucose 5 g/1, $(NH_4)_2SO_4$ 0.5 g/1, yeast extract 0.05 g/1, KH_2PO_4 0.2 g/1, $Mg_2Cl{\cdot}6H_2O$ 0.2 g/1, $FeSO_4$ 0.005 g/1, agar–agar 20 g/1, pH 4.5.

2.2. Fungicide sensitivity

Sensitivity of conidial germination of *B. cinerea* isolates to iprodione was determined in potato dextrose broth (PDB) amended with iprodione. The chemical, iprodione "Rovral 50 WP" (Rhône-Poulenc Agrochimie) was dissolved in a acetone–water mixture. Concentrated stock solution was prepared and progressively diluted in sterile water to provide appropriate concentrations for the assay and to reduce the amount of solvent to non-toxic levels.

To test fungicide sensitivity, 100 μl of the conidial suspension, 100 μl of the fungicide suspension (in appropriate concentration) and 200 μl of PDB were mixed. Concentration levels of fungicide of 200, 250, 300, 350, 400, 450, 500, 750, 1000, 1500 and 2000 mg L^{-1} were assayed. In the controls, sterile water was used instead of the suspension of fungicide. In addition, to determine if acetone had an effect in the bioassay, an acetone solution was tested at the final concentration employed. Mixtures prepared as indicated above were placed in capped sterile Eppendorf tubes and incubated without shaking for 24 h at 28 °C, following the procedure of Lorito et al. (1994). Approximately 50 μl of the samples were placed on microscope slides and 100 spores per slide were evaluated. Three replicates for each concentration of fungicide were made and the minimum dose of fungicide required for inhibiting germination of conidia (MID) was determined as the concentration of fungicide at which 100% inhibition of conidial germination occurred. Experiments were repeated twice.