Food Chemistry 120 (2010) 490–495

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effect of chitin on the antagonistic activity of *Rhodotorula glutinis* against *Botrytis cinerea* in strawberries and the possible mechanisms involved

Lingling Ge [a], Hongyin Zhang [b,*], Keping Chen [a], Longchuan Ma [b], Zhanli Xu [b]

[a] Institute of Life Sciences, Jiangsu University, Zhenjiang, 212013 Jiangsu, People's Republic of China
[b] College of Food and Biological Engineering, Jiangsu University, Zhenjiang, 212013 Jiangsu, People's Republic of China

ARTICLE INFO

Article history:
Received 9 June 2009
Received in revised form 17 August 2009
Accepted 13 October 2009

Keywords:
Chitin
Rhodotorula glutinis
Antagonistic activity
Botrytis cinerea
Strawberries
Possible mechanisms

ABSTRACT

The influence of chitin on the efficacy of *Rhodotorula glutinis* in controlling postharvest grey mould of strawberries and the possible mechanisms involved were investigated. The results showed that the antagonistic activity of *R. glutinis* harvested either from the culture media of NYDB amended with chitin at 0.5% or from the culture media in which chitin was the sole carbon source (NYCB) was improved greatly compared with that without chitin. The application of *R. glutinis* cultivated in the culture media of the chitin-supplement (0.5%) induced higher β-1,3-glucanase activity and reduced more MDA content of strawberries compared with that *R. glutinis* cultivated in the NYDB. Moreover, the control efficacy of the cell-free filtrate of the chitin-supplement culture media (0.5%) and NYCB were higher than that of cell-free culture filtrates of NYDB in 2 days incubation, with the associated high level of chitinase activity.

1. Introduction

Postharvest fungal decay may result in serious economic losses to strawberries. Grey mould decay caused by *Botrytis cinerea* is one of the most destructive postharvest diseases of strawberries (Zhang et al., 2007). Traditionally, *B. cinerea* is controlled mainly by treatment with synthetic fungicides. However, there is increasing concern about the potential harmful effects on the environment and human health that synthetic fungicides may have. Furthermore, the development of fungicide resistance in pathogens has prompted an urgent need for alternative control with good efficacy, low residues, and little or no toxicity to non-target organisms (Janisiewicz & Korsten, 2002; Wisniewski & Wilson, 1992).

Biological control with antagonistic yeasts has emerged as a promising way to reduce synthetic fungicide usage (Janisiewicz & Korsten, 2002). Strains of *Rhodotorula glutinis* are widely known postharvest biocontrol yeasts, which have been shown with high antagonistic activity in strawberries (Zhang et al., 2007) and other fruits (Tian, Qin, & Xu, 2004; Zheng, Zhang, & Sun, 2005). However, like other non-fungicides means, presently all the biocontrol yeasts cannot reduce postharvest diseases as effectively as synthetic fungicides. So for biological control to be accepted as an economically viable option, consistency and the effect of antagonistic yeasts in controlling postharvest disease must be enhanced (Droby, Wsiniewski, Ei-Ghaouth, & Wilson, 2003).

Several attempts have been proposed to enhance the efficacy of postharvest biocontrol yeasts. Beside the use of mixed antagonists and substances (organic and inorganic additives) (Droby, 2006; Droby et al., 2003; Ippolito & Nigro, 2000; Janisiewicz & Korsten, 2002), the physiological manipulation may also be a useful method (Janisiewicz & Korsten, 2002). For instance, Teixido, Vinas, Usall, and Magan (1998) found that the biocontrol activity of *Candida sake* to blue mould of apples could be enhanced by cultivation of the yeast in a low water activity modified liquid media. Li and Tian (2006) reported that induced accumulation of internal trehalose could improve *Cryptococcus laurentii* viability and biocontrol efficacy on *Penicillium expansum* in apples under stresses of low temperature and controlled atmosphere. Recently, the fact that amending with chitin in the culture media may greatly improve the antagonistic activity of *C. laurentii* was also reported (Yu, Wang, Yin, Wang, & Zheng, 2008). However, the physiology and genetic characteristics of most postharvest antagonistic yeasts are poorly known and the antagonistic mechanism by which the yeast antagonists inhibit the fungal diseases is not fully understood yet. Therefore, enhancing the efficacy of antagonistic yeasts through the physiological manipulation is a field in its infancy (Janisiewicz & Korsten, 2002).

Chitin is the second most abundant biopolymer renewable source in nature after cellulose, which has a versatile application potential in the agriculture-food industry (Prashanth & Tharanathan,

* Corresponding author. Tel.: +86 511 88780174; fax: +86 511 88780201.
E-mail address: zhanghongyin126@126.com (H. Zhang).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.10.042

2007), such as a biopesticide which has been approved by US Environmental Protection Agency (EPA) and as a food additive which has been approved by US Food and Drug Administration (FDA). San-Lang et al. (2002) reported that crude fungicides, obtained from the culture broth of two strains of *Bacillus*, grown in a medium containing chitin, displayed antifungal activity on pathogenic *Fusarium oxysporum*. Subsequently, Vivekananthan, Ravi, Ramanathan, and Samiyappan (2004) reported that the antagonistic efficacy of biocontrol bacteria *Pseudomonas fluorescens* and *Bacillus subtilis* increased after being cultivated in a growth media amended with chitin. Recently, chitinase production by *Trichoderma harzianum* was found to be induced using wheat bran-based solid medium containing 1% colloidal chitin, and the chitinase lead to the lysis of the phytopathogenic fungus *Colletotrichum gloeosporioides* (Sandhya, Binod, Nampoothiri, Szakacs, & Pandey, 2005). However, to the best of our knowledge, there is little information concerning the influence of chitin on the efficacy of the antagonistic yeast *R. glutinis* in controlling postharvest grey mould decay caused by *B. cinerea* in strawberries.

The objective of this study was to determine the influence of chitin on the efficacy of the *R. glutinis* in controlling postharvest grey mould decay in strawberries, the chitinase activity excreted by *R. glutinis* and to evaluate whether the activities of the chitinase, β-1,3-glucanase and malondialdehyde (MDA) content of strawberries would be affected by the application of *R. glutinis* incubated in the media of NYDB or NYDB amended with chitin or NYCB (chitin as the sole carbon source instead of dextrose in the media of nutrient yeast dextrose broth).

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and solvents used were of analytical grade or purer. Chitin, desalted snail gut enzyme and laminarin were obtained from Sigma (St. Louis, MO). Nutrient broth, yeast extract, glucose, agar and *N*-acetyl-D-glucosamine (GlcNAc) were obtained from Sangon (Shanghai, China). Sodiumazide, sodium acetate, potassium phosphate, potassium tetraborate, ρ-dimethylaminobenzaldehyde (DMAB), thiobarbituric acid (TBA), trichloroacetic acid, EDTA, polyvinyl pyrrolidone (PVPP), acetic acid and 3,5-dinitro-salicylate were obtained from Sinopharm Chemical Reagent Company (Beijing, China).

2.2. Fruits

Strawberries (*Fragaria ananassa* Duch.) cultivars 'fengxiang' were harvested at commercial maturity from Zhenjiang of the Jiangsu Province early in the morning and then were used immediately after rapid transferring to the laboratory. Berries were sorted on the basis of size, colour (75% full red colour) and berries with apparent injuries or infections were removed.

2.3. 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 nutrient yeast dextrose broth (NYDB) which had been inoculated with a loop of the culture. Flasks were incubated on a QYC-200 rotary shaker (FuMa, China) at 28 °C for 20 h. Following incubation, cells were centrifuged at 7000×*g* for 10 min by using a TGL-16M centrifuge (XiangYi, China) and washed twice with sterile distilled water in order to remove the growth medium. Cell pellets were re-suspended in sterile distilled water, counted by means of a JSQA hemocytometer (HongTai, China) and adjusted to an initial concentration of 5×10^8 cells ml^{-1}. Then, 1 ml of the above-mentioned suspensions were added and cultivated in nutrient yeast dextrose broth (NYDB) or NYDB amended with chitin powder at 0.5% (NYDB + chitin) or NYCB (chitin as the sole carbon source instead of dextrose in the media of nutrient yeast dextrose broth) on a rotary shaker at 200 rpm at 28 °C for 24 h. Then the yeast cells were harvested by centrifuging at 7000×*g* for 10 min and were washed twice with sterile distilled water. The yeast cell was counted using a hemocytometer. Cell pellets were re-suspended in sterile distilled water and adjusted to the concentrations required for different experiments. The growth media was filtered through a Millipore membrane (0.45 μm) and the cell-free culture filtrate was used for biocontrol and chitinase activity assay.

2.4. Fungal pathogen

The pathogen *B. cinerea* was isolated from infected strawberry fruits. The culture was maintained on potato dextrose agar (PDA: extract of boiled potatoes, 200 ml; dextrose, 20 g; agar, 20 g and deionised water, 800 ml) at 4 °C, and fresh cultures were grown on PDA plates before use. Spore suspensions were prepared by removing the spores from the sporulating edges of a 7 days old culture with a bacteriological loop, and suspending them in sterile distilled water. Spore concentrations were determined with a haemocyometer, and adjusted as required with sterile distilled water.

2.5. Efficacy of R. glutinis harvested from different media in controlling of grey mould decay of strawberries

The surface of strawberries was wounded with a sterile cork borer (approximately 3-mm-diameter and 3-mm-deep) and treated with 30 μl of (1) the cell suspensions of *R. glutinis* (1×10^8 cells ml^{-1}) which were harvested from the media of NYDB, (2) the cell suspensions of *R. glutinis* cells (1×10^8 cells ml^{-1}) which were harvested from the media of NYDB amended with chitin, (3) the cell suspensions of *R. glutinis* (1×10^8 cells ml^{-1}) which were harvested from the media of NYCB and (4) sterile distilled water as the control. Two hours later, 30 μl of *B. cinerea* suspensions (1×10^5 spores ml^{-1}) were inoculated onto each wound. After air-drying, the strawberries were stored in enclosed plastic trays to maintain a high relative humidity (above 95%) and incubated at 20 °C. The number of the infected fruit wounds was examined daily. There were three replicates per treatment and 18 fruits each replicate. All treatments were arranged in a randomised complete block design, and the experiment was conducted twice (Zhang et al., 2007).

2.6. Efficacy of cell-free culture filtrate of R. glutinis incubated in different media in controlling grey mould decay of strawberries

Freshly harvested fruits were treated as described above to evaluate the efficacy of *R. glutinis* harvested from different media in the control of the grey mould decay of strawberries. The wounds of fruits were treated with 30 μl of (1) the solution of the cell-free culture filtrate of *R. glutinis* harvested from the media of NYDB, (2) the solution of the cell-free culture filtrate of *R. glutinis* harvested from the media of NYDB amended with chitin, (3) the solution of the cell-free culture filtrate of *R. glutinis* harvested from the media of NYCB and (4) sterile distilled water as the control. Two hours later, 30 μl of *B. cinerea* suspensions (1×10^5 spores ml^{-1}) were