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Burdock fructooligosaccharide enhances biocontrol of *Rhodotorula mucilaginosa* to postharvest decay of peaches

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ABSTRACT

The influence of adding burdock fructooligosaccharide (BFO) in the culture media on the efficacy of *Rhodotorula mucilaginosa* in controlling postharvest decay of peaches and its possible mode of action were investigated. The antagonistic activity of *R. mucilaginosa* to *Rhizopus* decay and blue mold decay of peaches was greatly enhanced through cultivation in the nutrient yeast dextrose agar (NYDA) medium amended with BFO at the concentration of 0.32%, compared with that cultivated in NYDB without BFO. *R. mucilaginosa* at 1×10^8 cells/mL cultivation in the NYDB media did not reduce the natural decay incidence of peaches, compared with the control after 30 d at 4 °C followed by 7 d at 20 °C. However, *R. mucilaginosa* cultivation in the NYDB media amended with BFO at the concentration of 0.32% reduced the natural decay incidence of peaches. The population of *R. mucilaginosa* harvested from NYDB amended with BFO at 0.32% increased rapidly in peach wounds compared to that harvested from NYDB without BFO no matter peaches were stored at 20 °C or 4 °C. The activities of chitinase and β-1,3-glucanase of cell-free culture filtrate of *R. mucilaginosa* harvested from NYDB amended with BFO at 0.32% were higher than that at other concentrations and the control.

1. Introduction

Economical losses which caused by postharvest diseases of fruits are serious in the world, particularly in developing countries for being short of freezing equipments. Blue mold decay caused by *Penicillium expansum* Link and *Rhizopus* decay caused by *Rhizopus stolonifer* (Ehrenb.: Fr) Vuill. are two of the most destructive postharvest diseases of peaches (Karabulut & Baykal, 2004). Currently, these diseases are primarily controlled by application of synthetic fungicides (Holmes & Eckert, 1999). However, due to the development of fungicides-resistant isolate of pathogens, concern for public safety, environmental pollution and so on, an urgent search for alternative control measures with good efficacy, non-fungicide, and little or no toxicity to non-target organisms is becoming important (Calvo, Calvente, Orellano, Benuzzi, & Tosetti, 2007; Janisiewicz & Korsten, 2002; Sharma, Singh, & Singh, 2009).

Biological control using microbial antagonists is regarded as the most potential alternative reducing synthetic fungicides usage (Droby, Wsiniewski, Ei-Ghaouth, & Wilson, 2003; Zhang et al., 2007, 2008). Some of the antagonistic yeasts have been registered and are commercially available (Ippolito, EI Ghaouth, Wilson, & Wisniewski, 2000). However, for biological control to be accepted as an economically viable option, consistency, convenience of application and efficacy of antagonistic yeasts (including *R. mucilaginosa*) in controlling postharvest diseases must be improved (Droby et al., 2003; Janisiewicz & Korsten, 2002).

Many attempts have been proposed to improve the performance of postharvest biocontrol yeasts. For these years, the physiological manipulation have emerged as a promising method to enhance the biocontrol efficacy of antagonistic yeast (Janisiewicz & Korsten, 2002; Li & Tian, 2006). For example, Yu, Wang, Yin, Wang, and Zheng (2008) found that amending with chitin in the culture media may greatly increase the antagonistic activity of *C. laurentii*. Ge, Zhang, Chen, Ma, and Xu (2010) found that the antagonistic activity of *Rhodotorula glutinis* against *B. cinerea* in strawberries could be enhanced by adding chitin in the culture.

BFO (burdock fructooligosaccharide) is a fructose oligomer first isolated from the root tissue of *Arctium lappa* by Hao, Chen, Zhong, Chen, and Li (2005). It is composed of a linear chain of twelve β-2,1-linked fructofuranose residues with one terminal α-1,2-linked glucopyranose. It is cheap, non-toxic and safe to animals. BFO could increase the quantity and species of volatile organic compounds of *Lycopersicon esculentum* and its resistance to *Botrytis cinerea* (He, Tian, Chen, Hao, & Li, 2006). It could also enhance the contents of endogenous salicylic acid (SA), the resistance against *Colletotrichum orbiculare*, and the activities of defensive enzymes of cucumber

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seedlings (Zhang, Wang, Liu, & Chen, 2009). Sun, Zhang, Guo, Yu, and Chen (2013) reported that BFO treatment controlled postharvest diseases in grape, apple, banana, kiwi, citrus, strawberry and pear.

Rhodotorula mucilaginosa is a strain of antagonistic yeast which was isolated from the soil sample of an unsprayed orchard by our research team. Our research team found that *R. mucilaginosa* showed biocontrol efficacy against blue mold and gray mold of apples caused by *P. expansum* and *B. cinerea* respectively (Li, Zhang, Liu, & Zheng, 2011). However, to our knowledge there is no information concerning enhancement the biocontrol efficacy of antagonistic yeast to postharvest decay of fruits by amending BFO in the culture media.

The primary object of this study was to evaluate the influence of adding BFO in the culture media on the efficacy of *R. mucilaginosa* against postharvest *Rhizopus* decay, blue mold decay and natural decay of peaches and explore the possible mechanisms involved.

2. Materials and methods

2.1. Fruits

Peaches (*Prunus persica* Batsch) cultivars 'dajiubao' were harvested at commercial maturity from an orchard in Zhenjiang of Jiangsu province early in the morning, and selected for uniformity of size, ripeness and absence of apparent injury or infection. Fruits were selected randomly and disinfected with 0.1% sodium hypochlorite for 1 min, washed with tap water, and allowed to air dry at room temperature (20 °C).

2.2. BFO preparation

BFO with purity more than 90% was bought from "Ruoxiya" company (Yantai, China).

2.3. Antagonist and growth conditions

The yeast antagonist *R. mucilaginosa* was isolated from the surfaces of peach blossom picked in unsprayed orchards. Classical methods based on colony and cell morphologies were used for a preliminary characterization of the yeast (Kurtzman & Fell, 1998). Subsequently, sequence analysis of the 5.8S internal transcribed spacer (ITS) ribosomal DNA (rDNA) region was used to identify yeast colony, following previously published methods (Li et al., 2010). *R. mucilaginosa* isolates were maintained at 4 °C on 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 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-16 M centrifuge (XiangYi, China) and washed twice with sterile distilled water in order to remove the growth medium. Yeast cell pellets were resuspended in sterile distilled water, counted by means of a hemocytometer and adjusted to an initial concentration of 5×10^8 cells/mL. Then, 1 mL of the above-mentioned yeast suspensions were added and cultivated in NYDB or NYDB amended with BFO at different concentrations (0.04%, 0.08%, 0.16%, 0.32%, 0.64%) (NYDB+ BFO) on a rotary shaker at 200 rpm at 28 °C for 24 h. The yeast cells were harvested by centrifuging at 7000 × *g* for 10 min and were washed twice with sterile distilled water. Cell pellets were resuspended in sterile distilled water, counted using a hemocytometer and adjusted to a concentration of 1×10^8 cells/mL. The growth media was filtered through a Millipore membrane (0.45 μm) and the supernatant was used for chitinase and β-1,3-glucanase activity assay.

2.4. Pathogen inoculum

The pathogens *Penicillium expansum* Link and *Rhizopus stolonifer* (Ehrenb.: Fr) Vuill. were isolated from infected peach fruits respectively. These cultures were maintained on potato dextrose agar (PDA: extracted from boiled potatoes, 200 mL; dextrose, 20 g; agar, 20 g and deionized 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 d old culture with a bacteriological loop, and suspending them in sterile distilled water. Spore concentrations were determined with a haemocytometer, and adjusted as required with sterile distilled water.

2.5. Effects of BFO on biocontrol efficacy of R. mucilaginosa to Rhizopus decay and blue mold decay of peaches

Two uniform wounds (5 mm diameter and approximately 3 mm deep) were made at the equator of each peach fruit using the tip of a sterile dissecting needle and treated with 30 μL (1) the cell suspensions of *R. mucilaginosa* (1×10^8 cells/mL) which were harvested either from the media of NYDB or NYDB+ BFO at different concentrations (0.04%, 0.08%, 0.16%, 0.32%, 0.64%) in which the yeast was incubated for 24 h, (2) sterile distilled water as the control. Two hours later, 30 μL of *R. stolonifer* suspensions (1×10^4 spores/mL) or *P. expansum* suspensions (5×10^4 cells/mL) were inoculated into each wound. After air-drying, the samples were stored in trays covered with plastic film to maintain a high relative humidity (about 95%) and incubated at 20 °C. The number of the infected fruits was examined after 3 d inoculation at 20 °C. There were three replicates per treatment and 20 fruits each replicate. All treatments were arranged in a randomized complete block design, and the experiment was conducted twice.

2.6. Effects of BFO on biocontrol efficacy of R. mucilaginosa to natural decay development and quality parameters of peaches

Intact fruits were inoculated by dipping them in the cell suspensions of (1) *R. mucilaginosa* (1×10^8 cells/mL) which were harvested from the media of NYDB, (2) *R. mucilaginosa* (1×10^8 cells/mL) which were harvested from the media of NYDB+ BFO at the concentrations 0.32% and (3) sterile distilled water as the control respectively for 30 s, and subsequently air-dried. The treated fruits were weighed and then sealed in polyethylene-lined plastic boxes to retain high humidity. Fruits were stored at 4 °C for 30 d followed by 20 °C for 7 d in order to determine disease development under normal shelf-life conditions. Infection rate was recorded afterwards. There were three replicate trials of 20 fruits with a complete randomization in each test and experiments were repeated twice.

Quality parameters were measured after storage, on three replicates of five fruits each, and performed at ambient temperature (about 20 °C). The experiment was repeated twice. The testing methods are described below.

Weight loss. The mass of peach was measured by an MP2000-2 balance (±0.001 g) (Shanghai Balance Instruments, China) before treatment (A) and after storage (B), respectively, and the weight loss was calculated as (A − B)/A.

Fruit firmness. Firmness values of each individual peach were measured at three points of the equatorial region by using the TA-XT2i Texture Analyser (Microstable Instruments, UK) with a 5 mm diameter flat probe. The probe descended toward the sample at

1.0 mm s^{-1} and the maximum force (N) was defined as firmness (Zhang et al., 2008).

Total soluble solids. Total soluble solids (TSS) were determined by measuring the refractive index of the same juice with a hand refractometer and the results expressed as percentages (g per 100 g fruit weight) (Larrigaudière, Pons, Torres, & Usall, 2002).

Ascorbic acid. 2,6-dichloroindophenol titrimetric method was used to determine the ascorbic acid content of pressed fruit juice. Results were expressed as milligrams of ascorbic acid per 100 g sample (Özden & Bayindirli, 2002).

2.7. Effects of BFO on population growth of R. mucilaginosa in peach wounds

Fruit samples were wounded as described above. Then, the wounds were treated with 30 μL of 1×10^8 cells/mL *R. mucilaginosa* solution which were harvested either from NYDB or NYDB+ 0.32% BFO. The samples were taken at 0, 1, 2, 3, and 4 d at 20 °C and 0, 3, 6, 9, 12 d at 4 °C after the treatment. The tissue was removed with a sterile cork borer (9-mm-diameter and 10-mm-deep) and ground with a 150-mL Erlenmeyer flasks, by glass rod directly pounding to pieces in 10 mL of sterile 0.85% sodium chloride solution. Serial 10-fold suitable dilutions were made and 0.1 mL of suitable dilution was spread in NYDA. The plates were incubated at 28 °C for 2 d, and the colonies were counted. Population densities of *R. mucilaginosa* were expressed as log10 CFU (colony-forming units) per wound. There were two replicates per treatment and 3 fruits per replicate and the experiments were repeated twice.

2.8. Assay for chitinase and β-1,3-glucanase activity of the cell-free culture filtrate of R. mucilaginosa incubated in different growth media

Chitinase activity was assayed by measuring the amount of reducing N-acetyl glucosamine released from the substrate by the method reported by Tang (2003) with some modifications. Crude enzyme sample (supernatant extract) of 1 mL was routinely added to 1 mL of 0.1 mol/L, pH 7.0 phosphate buffer (containing 1% colloidal chitin) and incubated at 37 °C for 1 h. The reaction was stopped by adding 2 mL of 3,5-dinitro-salicylate and boiling for 10 min on a water bath. After it came to the room temperature, the mixer was centrifugated at 1500 × *g* for 8 min. The amount of reducing N-acetyl glucosamine was measured spectrophotometrically at 530 nm using a UV-1601 spectrophotometer. One unit of the chitinase was defined as the formation of 100 microgram N-acetyl glucosamine equivalents per hour and the specific activity was expressed as the U per mL. There were three replicates in each experiment, and the experiment was conducted twice.

β-1,3-glucanase was assayed by measuring the amount of reducing sugar released from the substrate by the method reported by Ippolito et al. (2000), with some modifications. Crude enzyme sample of 250 μL was routinely added to 250 μL of 0.2% laminarin (w/v) in 50 mM, pH 5.0, potassium acetate buffer and incubated at 37 °C for 1 h. For the control, the same mixture was similarly diluted at zero incubation time. The reaction was stopped by adding 1.5 mL of 3,5-dinitro-salicylate and boiling for 5 min on a water bath. And the amount of reducing sugars was measured spectrophotometrically at 500 nm using a UV-1601 spectrophotometer (Shimadzu, Japan). One unit of the β-1,3-glucanase was defined as the formation of 1 mg glucose equivalents per hour and the specific activity was expressed as the U per mL. There were three replicates in each experiment, and the experiment was conducted twice.

2.9. Statistical analysis

The data were analyzed by the analysis of variance (ANOVA) in the statistical program SPSS/PC version II.x, (SPSS Inc. Chicago, Illinois, USA) and the Duncan's multiple range test was used for means separation. The statistical significance was applied at the level $P < 0.05$.

3. Results

3.1. Effects of BFO on biocontrol efficacy of R. mucilaginosa to Rhizopus decay and blue mold decay of peaches

All treatments with application of *R. mucilaginosa* at 1×10^8 cells/mL could reduce the disease incidence of *Rhizopus* decay and blue mold decay of peaches, compared with the control after 3 d storage at 20 °C (Fig. 1, $P < 0.05$). The antagonistic activity of *R. mucilaginosa* was shown to be greatly enhanced through cultivation in the NYDB media amended with BFO at the concentration of 0.16%, 0.32%, and 0.64% compared with that cultivated in NYDB without BFO ($P < 0.05$), and BFO at the concentration of 0.32% showed the best enhancement efficacy to *R. mucilaginosa* in controlling *Rhizopus* decay and blue mold decay of peaches compared with the other concentrations.

3.2. Effects of BFO on biocontrol efficacy of R. mucilaginosa to natural decay development and quality parameters of peaches

R. mucilaginosa at 1×10^8 cells/mL cultivated in the NYDB media did not reduce the natural decay incidence of peaches, compared with the control after 30 d at 4 °C followed by 7 d at 20 °C (Table 1). However, treatments with application of *R. mucilaginosa* cultivation in the NYDB media amended with BFO at the concentration of 0.32% significantly reduced the natural decay incidence of peaches compared with the control.

After 4 °C for 30 d followed by 20 °C for 7 d, the application of *R. mucilaginosa* cultivated in the NYDB and BFO-supplement media (0.32%) could prevent peaches from weight losing. As shown in Table 1, the peaches treated with *R. mucilaginosa* cultivated in the NYDB, and *R. mucilaginosa* cultivated in the NYDB media amended with BFO at the concentration of 0.32% had lower weight lose, and

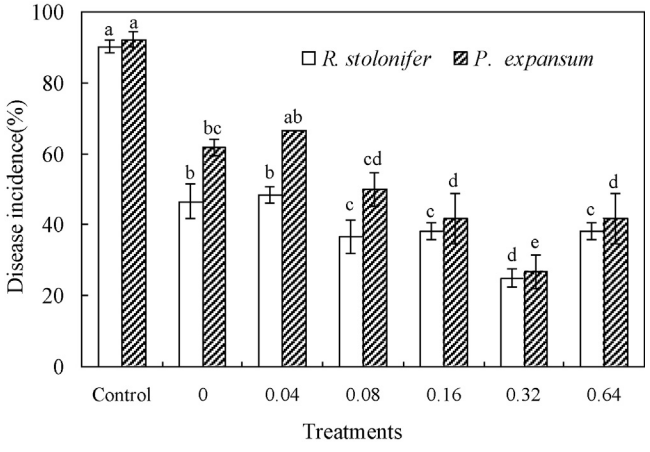

Fig. 1. Effect of BFO on biocontrol of *R. mucilaginosa* to *Rhizopus* decay and blue mold of peaches. Fruit treatments are as follows: control = sterile distilled water, 0 = *R. mucilaginosa* which were harvested from the media of NYDB, (0.04, 0.08, 0.16, 0.32, 0.64) = *R. mucilaginosa* which were harvested from the media of NYDB+ BFO at different concentrations (0.04%, 0.08%, 0.16%, 0.32%, 0.64%). Each value is the mean of two experiments. Bars represent standard deviations. Different letter indicates significant differences ($P = 0.05$) according to the Duncan's multiple range test and the data from each time point are separated.

Table 1
Effects of BFO on biocontrol efficacy of *R. mucilaginosa* to natural decay development and quality parameters of peaches.

Treatments	Disease incidence (%)	Weight lose (%)	Firmness (N)	Soluble solids (%)	Ascorbic acid (mg/100 g)
Control	73.45 ± 4.27a	4.1 ± 0.04a	2.14 ± 0.22b	9.27 ± 0.10a	10.17 ± 1.26a
NYDB[a]	69.36 ± 6.17a	3.27 ± 0.18b	2.41 ± 0.10a	9.91 ± 0.07a	10.38 ± 1.43a
0.32	55.09 ± 5.64b	2.27 ± 0.07c	2.51 ± 0.18a	9.52 ± 0.18a	10.52 ± 1.06a

[a] NYDB = *R. mucilaginosa* which were harvested from the media of NYDB, 0.32% = *R. mucilaginosa* which were harvested from the media of NYDB+ BFO at the concentration of 0.32%. Disease incidence and quality parameters were measured after 4 °C for 30 d followed by 20 °C for 7 d. Means are averaged values of three trials ± the standard error. Values within columns followed by different letters at the same storage condition are significantly different at $P = 0.05$ according to Duncan's multiple range test.

higher firmness than that of the control peaches ($P < 0.05$), but had no significant effect on soluble solids and ascorbic acid, compared to that of the control fruit. In short, application of *R. mucilaginosa*, no matter it was cultivated in the NYDB media or the NYDB media amended with BFO at the concentration of 0.32% did not produce impaired quality parameters including weight lose, firmness, soluble solids and ascorbic acid to peaches, after storage at 4 °C for 30 d followed by 20 °C for 7 d.

3.3. Effects of BFO on population growth of *R. mucilaginosa* in peach wounds

The population of *R. mucilaginosa*, no matter it was cultivated in the NYDB or NYDB media amended with 0.32% BFO, grew rapidly at 20 °C in the first day in peach fruit wounds (Fig. 2(A)). For *R. mucilaginosa* cultivated in the NYDB amended with BFO at 0.32%, it's population decreased slowly after 1 d, but still stabilized at a high level thereafter. However, the population of *R. mucilaginosa* cultivated in NYDB in peach wounds decreased fastly after 3 d, which was significantly lower than that of *R. mucilaginosa* cultivated in the NYDB amended with BFO at 0.32% ($P < 0.05$).

Similarly, in peach wounds kept at 4 °C, rapid colonization of *R. mucilaginosa*, no matter it was cultivated in the NYDB or NYDB media amended with 0.32% BFO, was observed in peach wounds during the first 3 d, and then the population slowly increased to the maximum at 6 d (Fig. 2(B)). Afterward, the population decreased occasionally but still stabilized at a high level. The population of *R. mucilaginosa* cultivated in NYDB amended with BFO at 0.32% in peach wounds was significantly higher than that of *R. mucilaginosa* cultivated in NYDB at 3 and 9 d.

3.4. Assay for chitinase activity of the cell-free culture filtrate of *R. mucilaginosa* incubated in different growth media

After 24 h incubation, the chitinase activities of the cell-free culture filtrates of *R. mucilaginosa* grown in NYDB plus BFO at 0.04, 0.32 and 0.64% were all higher than that grown in NYDB. Especially at the optimal BFO concentration 0.32 and 0.64%, the chitinase activity of the cell-free culture filtrates was higher than that of the cell-free culture filtrates grown in NYDB plus BFO at other concentrations (Fig. 3(A), $P < 0.05$). However there was no significant promotion of the chitnase activity of the cell-free culture filtrates of *R. mucilaginosa* grown in NYDB media amended with 0.08 or 0.16% BFO compared with that grown in NYDB media.

3.5. Assay for β-1,3-glucanase activity of the cell-free culture filtrate of *R. mucilaginosa* incubated in different growth media

After 24 h incubation, the β-1,3-glucanase activities of the cell-free culture filtrates of *R. mucilaginosa* grown in NYDB plus BFO at the concentration of 0.32% was higher than that grown in NYDB and in NYDB plus BFO at other concentrations (Fig. 3(B), $P < 0.05$). However, the β-1,3-glucanase activity of the cell-free culture filtrates of *R. mucilaginosa* grown in NYDB media amended with 0.04, 0.08%, 0.16% or 0.64% BFO was not higher than that of *R. mucilaginosa* grown in NYDB.

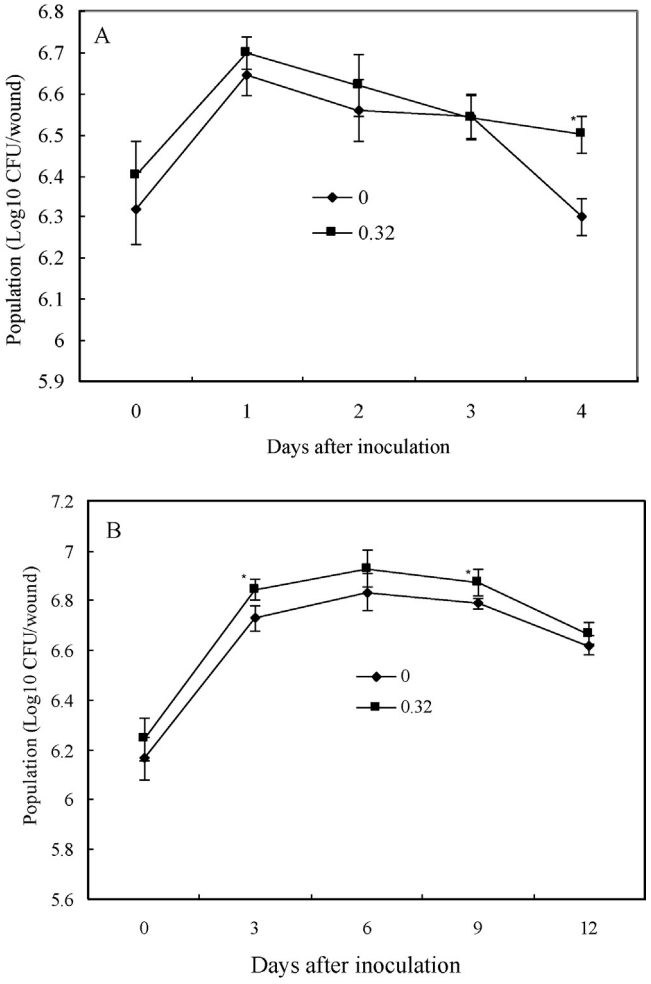

Fig. 2. Effects of BFO on population growth of *R. mucilaginosa* in peach wounds. 0 = *R. mucilaginosa* which were harvested from the media of NYDB, 0.32% = *R. mucilaginosa* which were harvested from the media of NYDB+ BFO at the concentration of 0.32%. Fruits were incubated at 20 °C (A) or at 4 °C (B). Bars represent standard errors. An asterisk (*) indicates significant differences ($P = 0.05$) according to the Independent samples *t* test.

4. Discussion

Data in this present study showed that the biological control activity of *R. mucilaginosa* against the *Rhizopus* decay and blue mold decay of peaches was greatly enhanced when the yeast was cultivated in the media of NYDB amended with BFO at the concentrations more than 0.08% compared with the case that *R. mucilaginosa* obtained from NYDB without BFO. What's more, the

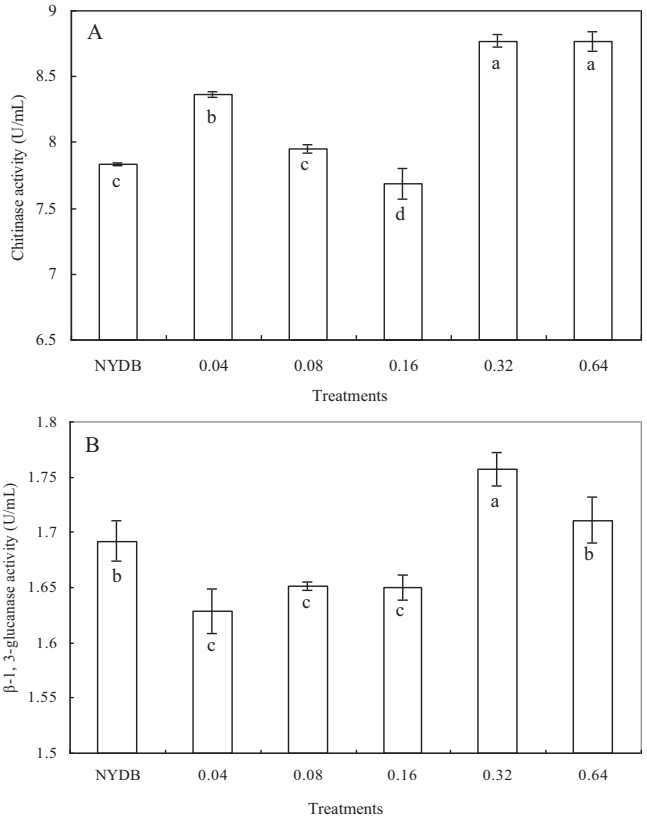

Fig. 3. Assay for chitinase activity (A) and β-1,3-glucanase (B) activity of the cell-free culture filtrate of *R. mucilaginosa* incubated in different growth media. *NYDB* = *R. mucilaginosa* which were harvested from the media of NYDB, (0.04%, 0.08%, 0.16%, 0.32%, 0.64%) = *R. mucilaginosa* which were harvested from the media of NYDB+ BFO at different concentrations (0.04%, 0.08%, 0.16%, 0.32%, 0.64%). Each value is the mean of two experiments. Bars represent standard deviations. Different letter indicates significant differences ($P = 0.05$) according to the Duncan's multiple range test and the data from each time point are separated.

concentration of BFO significantly influenced the biocontrol effectiveness of *R. mucilaginosa* to postharvest decay of peaches. BFO at the concentration of 0.32% showed the best enhancement efficacy to *R. mucilaginosa* in controlling *Rhizopus* decay and blue mold decay of peaches. The results above indicated that the utilization of BFO might be an effective, safe and economic approach to enhance the biocontrol efficacy of *R. mucilaginosa* to postharvest decay of fruits. To our knowledge, this is the first report of adding BFO to growth media to enhance the biocontrol efficacy of antagonistic yeast to postharvest decay of fruits.

Furthermore, in the experiment of effects of BFO on biocontrol efficacy of *R. mucilaginosa* to natural decay development, the natural decay incidence of peaches was significantly reduced by *R. mucilaginosa* cultivated in the NYDB media amended with 0.32% BFO compared with the control and that without BFO following storage at 4 °C for 30 d followed by 20 °C for 7 d. Blue mold decay (caused by *P. expansum*) and *Rhizopus* decay caused by *R. stolonifer* are two of the most destructive postharvest diseases of peaches (Karabulut & Baykal, 2004). The significant control efficacy of the treatment of *R. mucilaginosa* cultivated in the NYDB media amended with 0.32% BFO to postharvest natural infections of peaches suggests that it has a control efficacy at a wide range of pathogens including *P. expansum* and *R. stolonifer*. These results also imply that adding 0.32% BFO to the NYDB media can enhance the biocontrol efficacy of *R. mucilaginosa* to postharvest decay of peaches.

In the experiment of effects of BFO on quality parameters of peaches, the data show that application of *R. mucilaginosa*, no matter it was cultivated in the NYDB media or the NYDB media amended with BFO at the concentration of 0.32% did not produce impaired quality parameters including weight lose, firmness, soluble solids and ascorbic acid to peaches. What's more, the peaches treated with *R. mucilaginosa* cultivated in the NYDB, and *R. mucilaginosa* cultivated in the NYDB media amended with BFO at the concentration of 0.32% had lower weight lose, and higher firmness than that of the control peaches. The reasons for this are unknown and should be further studied. These suggest that *R. mucilaginosa* cultivated in the media of NYDB amended with 0.32% BFO can help maintain the quality of peach fruits, which may have potential for commercialization.

Furthermore, growth curves of the antagonist demonstrated that *R. mucilaginosa* could colonize and grow faster when the yeast was cultivated in the media of NYDB amended with 0.32% BFO than that without BFO in peach wounds whether at 20 °C or 4 °C. We assume that enhancing growth of *R. mucilaginosa* in fruits might be a major reason leading to the increased biocontrol efficacy against the *Rhizopus* decay and blue mold decay of peaches by adding 0.32% BFO to the NYDB media.

Recently, great interest has been shown in the action of exocellular lytic enzymes such as chitinases and glucanases secreted by yeasts and acting as depolymerases on fungal cell walls (Castoria, De Curtis, Lima, & De Cicco, 1997; Meng, Yang, Kennedy, & Tian, 2010). Chitinases and β-1,3-glucanases catalyze the hydrolysis of chitin and β-1,3-glucan, respectively, both polymers major components of the fungi cell walls. These enzymes appear to have antifungal activity (Dahiya, Tewari, & Hoondal, 2006) and to be actively involved in the phenomenon of mycoparasitism (Chiu & Tzean, 1995). Wisniewski et al. (1991) reported that isolate 87 of *Pichia guillermondii*, an effective biocontrol agent against *B. cinerea* in apple fruit, produced *in vitro* higher levels of β-1, 3-glucanase activity than isolate 117 of *Debaryomyces hansenii*, which was ineffective against the pathogen. To further understand the mechanisms of enhancement the biocontrol efficacy of *R. mucilaginosa* to postharvest decay of peaches by adding 0.32% BFO to the NYDB media, chitinase activity and β-1,3-glucanase activity were detected in different cell-free culture filtrate of *R. mucilaginosa* cultivated in different media. We found that at the BFO concentration 0.32%, the chitinase activity and β-1,3-glucanase activity of the cell-free culture filtrates of *R. mucilaginosa* were both higher than that of the cell-free culture filtrates grown in NYDB media and NYDB plus BFO at other concentrations. Besides, in the experiment of effects of BFO on biocontrol efficacy of *R. mucilaginosa* to *Rhizopus* decay, blue mold decay and natural decay development, *R. mucilaginosa* cultivated in the media of NYDB amended with 0.32% BFO showed the best control efficacy to *Rhizopus* decay, blue mold decay and natural decay of peaches. These results suggested that the enhancement of chitinase activity and β-1,3-glucanase activity of *R. mucilaginosa* may be another mode of action of enhancement the biocontrol efficacy of *R. mucilaginosa* to postharvest diseases of peaches.

In conclusion, our results showed that the antagonistic activity of *R. mucilaginosa* could be enhanced by BFO induced incubation, which may offer great practical potential in reducing the postharvest diseases of peach fruits. The mode of action may be involved in its ability to enhance growth of *R. mucilaginosa* in fruits, to enhance chitinase activity and β-1,3-glucanase activity of antagonistic yeast. Future studies should also assess the possible induction of defense enzymes of peaches such as PPO and POD by BFO induced incubation antagonistic yeast. Moreover, BFO may have an effect on the ultrastructural and cytochemical characteristics of *R. mucilaginosa* thus enhancing it's antagonistic activity. All these should be further studied.

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