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Evaluation of yeasts from Tibetan fermented products as agents for biocontrol of blue mold of Nashi pear fruits*

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Abstract: A total of 20 strains of yeast isolated from Tibetan fermented products were screened for antagonism against blue mold of pear caused by *Penicillium expansum*. Six isolates that inhibited incidence of postharvest decay by 35% or more were selected for further screening. Among them, the most effective was *Rhodotorula mucilaginosa*. The results showed that washed cell suspensions of *R. mucilaginosa* yielded better antagonistic efficacy than unwashed cell-culture mixtures, cell-free culture filtrates, and autoclaved cell cultures. Biocontrol activity improved with increasing concentrations of incubated cells. The best concentration was 1×10^8 cells/ml, at which the incidence of decay was only 16.7% after 6 d of incubation. The germination of conidia of *P. expansum in vitro* was significantly inhibited by both washed cell-suspensions and unwashed cell-culture mixtures. Rapid colonization by yeast at different concentrations showed a relationship between yeast-cell concentration and biocontrol activity. Although the titratable acidity of pear fruits increased after treatment, *R. mucilaginosa* did not affect the total soluble solids or ascorbic acid content. This is the first study to report that the yeast *R. mucilaginosa* from Tibet Autonomous Region of China may have potential as an antagonist to control the postharvest decay of pear fruits.

Key words: Biocontrol, Postharvest, *Rhodotorula mucilaginosa*, Tibetan yeast isolates, Pear
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1 Introduction

The pear (*Pyrus* spp.) is one of the most important and popular fruits in China (Liu *et al*., 2013; Yue *et al*., 2014). During postharvest storage, blue mold is a major disease of pear (Sanderson and Spotts, 1995; Jurick *et al*., 2010). Currently, the application of synthetic fungicides is the main method adopted to control the disease (Lennox *et al*., 2004; Sugar and Basile, 2011). However, with the application of postharvest fungicides, the resistance of the pathogen gradually increases, and fungicidal residues that raise food safety issues for consumers are also of great concern. Therefore, there is an urgent need to explore other methods to replace synthetic fungicides for the control of postharvest diseases of pear (Wilson and Wisniewski, 1989; Wisniewski and Wilson, 1992). The use of microbial antagonists, yeasts in particular, to control postharvest decay is regarded as a promising alternative (Wilson *et al.*, 1991; Spadaro and Gullino, 2004; Sharma *et al.*, 2009).

Among numerous antagonists, antagonistic yeasts show great promise (Zhang *et al.*, 2007; Xu *et al.*, 2008; Wang *et al.*, 2009; 2011; Lutz *et al.*, 2011; Janisiewicz *et al.*, 2014; Lu *et al.*, 2014). Because most yeasts do not produce antibiotics, they are considered to be safer than other kinds of microbial antagonists (Wisniewski *et al.*, 1991). Furthermore, the colonization of fruit wounds by yeasts is rapid

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(Castoria *et al.*, 2005). One of the main mechanisms by which antagonistic yeasts may control postharvest decay is by competing for space and nutrients (Droby *et al.*, 2009; Jamalizadeh, 2011). Finally, many biocontrol yeasts show the ability to be compatible with fungicides and other chemicals (An *et al.*, 2012; Yu *et al.*, 2012; D'Aquino *et al.*, 2013). Antagonistic yeasts have been isolated from soil, fruit surfaces, and fruit wounds (Janisiewicz and Korsten, 2002). Due to the unique geographical environment of Tibet Autonomous Region of China, there has been little research on the use of Tibetan yeasts for the control of postharvest diseases of fruit. Zhao *et al.* (2012) reported that a *Streptomyces* sp. was selected to control gummy stem blight. In this study, the yeast *Rhodotorula mucilaginosa* was selected from Tibetan fermented products, following the screening of twenty yeast isolates for their effectiveness in biocontrol. The aim of this study was to investigate and evaluate the efficacy of *R. mucilaginosa* from Tibet in inhibiting postharvest blue mold decay in pear caused by *Penicillium expansum*. Although some studies have been conducted on *R. mucilaginosa* as a biocontrol agent (Li *et al.*, 2011; Lutz *et al.*, 2011; Robiglio *et al.*, 2011), this is the first report of *R. mucilaginosa* from Tibet being used as an agent for controlling the blue mold of pear.

2 Materials and methods

2.1 Fruits

Nashi pear fruits (*Pyrus pyrifolia* Nak., cultivar "Shuijing"), of uniform ripeness and size and without any apparent damage or infection, were harvested at commercial maturity in Hangzhou, Zhejiang Province, China. After being disinfected with a solution of 0.1% (v/v) sodium hypochlorite for 1 min, the fruits were rinsed with tap water and air-dried at room temperature (20 °C) prior to experiments.

2.2 Yeasts

The Tibetan yeasts for screening were provided by the Key Laboratory of Dairy Biotechnology and Engineering, Ministry of Education (Inner Mongolia Autonomous Region, China). They were isolated from yak yoghourt, identified by large-subunit (26S) ribosomal DNA (rDNA) D1/D2 domain sequence analysis, stored in ampoule tubes in lyophilized form at −80 °C, and activated on nutrient yeast dextrose agar (NYDA: 8 g nutrient broth, 5 g yeast extract, 10 g glucose, and 20 g agar in 1 L of distilled water) for two generations at 28 °C in the dark. Before the experiments, yeasts were inoculated by immersing a loop in 50 ml NYD broth (NYDA without agar) and incubated at 28 °C on a rotary shaker at 200 r/min for 24 h. To remove the medium, the cells were centrifuged at 3500*g* for 10 min and rinsed twice in sterile distilled water. Then, the pellets were re-suspended in sterile distilled water and adjusted to the required concentrations by counting cells with a hemocytometer.

2.3 Pathogen

P. expansum was originally isolated from symptomatic pear fruits. The pathogen was cultured in a test tube with potato dextrose agar (PDA; consisting of 200 ml boiled potato extract, 20 g glucose, and 20 g agar in 1 L of distilled water) at 28 °C for 7 d. Conidia were removed by rubbing the surface of the medium with a bacteriological loop and suspending the loop in sterile distilled water. The final spore concentration was adjusted to 1×10^4 spores/ml by use of a hemocytometer.

2.4 Preliminary screening of Tibetan yeasts to control blue mold of pears

Preliminary screening of Tibetan yeasts for antagonistic activity was based on their ability to inhibit the infection of pear fruit by *P. expansum*. The biocontrol activities of 20 yeast isolates from Tibetan fermented products were evaluated *in vivo* in the pear fruits at 20 °C, according to the method described by Wilson *et al.* (1993). The fruits were wounded with a sterile cork-borer (5 mm diameter×3 mm deep) and six wounds were bored on each pear. An aliquot (50 μl) of the yeast cell suspensions in sterile distilled water at a concentration of 1×10^9 cells/ml was inoculated into each wound, while the same volume of sterile distilled water was applied as a control. Each wound was dried at room temperature for about 3 h, and then injected with 30 μl of *P. expansum* spore suspension (1×10^4 spores/ml). Then the samples were kept in enclosed plastic trays so as to maintain a high relative humidity at 20 °C in dark. Each yeast treatment consisted of three replicates, each consisting of 12 fruits. The whole experiment was performed twice.

The decay incidence and lesion diameter on the treated pears were observed on the third day. Inhibition rates were calculated from the results using the following formulas: DI=(N_C−N_T)/N_C×100% and LI=(d_C−d_T)/d_C×100%, where DI is decay inhibition (%), N_C is the number of decay wounds for the control, and N_T is the number of decay wounds for the treatment; LI is lesion diameter inhibition (%), d_C is lesion diameter of decay wounds for the control, and d_T is lesion diameter of decay wounds for the treatment. Treatments that showed a decay incidence inhibition rate higher than 35% were selected and re-evaluated in further experiments.

2.5 Further screening of selected Tibetan yeasts to control blue mold of pears

Six most promising Tibetan yeasts (JZ1-1, DX1-2, NQ11-2, NQ4-2, DX2-2, and RKZ1-5) that showed a decay incidence inhibition rate higher than 35% were selected and re-evaluated. Pear fruits were wounded as described above, then 50 μl of the selected Tibetan yeast suspensions were incubated into each wound at 1×10^8 cells/ml. After drying, the wounds were treated and kept as described previously. Each yeast treatment consisted of three replicates, each comprising 12 fruits. The experiment was conducted twice and results were reported as the percentage of decay incidence and the average lesion diameter: I_d=N/N_{total}×100% and d=(d_{total}/N), where I_d is decay incidence (%), N is the number of decayed wounds, N_{total} is the number of total wounds, d is lesion diameter (mm), and d_{total} is total lesion diameter. The yeasts that gave the lowest decay incidence compared with the control were selected for further research.

2.6 Antagonistic efficacy of the selected strain of *R. mucilaginosa*

The antagonistic efficacy of the selected strain of *R. mucilaginosa* (RKZ1-5) to *P. expansum* was evaluated on pears. The procedure was similar to that described in Section 2.4. Six wounds were made on each pear with a sterile cork-borer. *R. mucilaginosa* suspension (50 μl) in sterile distilled water at 1×10^5, 1×10^6, 1×10^7, 1×10^8, or 1×10^9 cells/ml were pipetted into each wound, respectively. The same volume of sterile distilled water was pipetted into wounds to be used as controls. After 3 h, 30 μl of pathogen spores at 1×10^4 cells/ml were inoculated into the wounds. The pear samples were incubated as described in Section 2.4. The biocontrol activity was assessed 6 d after treatment. Each yeast treatment consisted of three replicates, each consisting of 12 fruits. The experiment was conducted twice.

2.7 Antagonistic action of different preparations of *R. mucilaginosa* to *P. expansum* on pears

The pear fruits were wounded using the procedure described in Section 2.4. Based on the results of the experiments (Section 2.6), the most effective cell concentration of *R. mucilaginosa* antagonistic to *P. expansum* was 1×10^8 cells/ml and this was used in further tests based on the following preparations: (1) 1×10^8 cells/ml unwashed cell culture mixture (i.e. the yeast cells in the nutrient yeast broth medium); (2) 1×10^8 cells/ml washed cell suspension (prepared as described in Section 2.4); (3) cell-free culture filtrate (made by filtering the supernatant of the centrifuged yeast culture medium through 0.22 μm sterile Millipore filtration); (4) autoclaved cell culture (the *R. mucilaginosa* culture was sterilized by moist heat sterilization at 121 °C for 20 min); (5) Sterile distilled water was used as a control. After air-drying, the treated pear samples were stored as described in Section 2.4. Decay incidence and lesion diameter were recorded after 6 d. Each yeast treatment consisted of three replicates, each consisting of 12 fruits. The whole experiment was conducted twice.

2.8 Antagonistic action of different preparations of *R. mucilaginosa* to spore germination of *P. expansum in vitro*

The antagonistic action of *R. mucilaginosa in vitro* was also evaluated by recording pathogen spore germination in potato dextrose broth (PDB) in the presence of the antagonistic yeast. The following yeast preparations were added to the PDB: 100 μl of (1) 1×10^8 cells/ml unwashed yeast culture mixture; (2) 1×10^8 cells/ml washed yeast suspension; (3) a cell-free culture filtrate; (4) autoclaved cell culture; (5) sterile distilled water as a control. They were pipetted into 15 ml glass tubes each containing 5 ml PDB. In parallel, the same volume of *P. expansum* suspension at 1×10^7 spores/ml was added to each tube. All tubes were incubated in a rotary shaker (QYC 2102, Shanghai FUMA, China) at 150 r/min at 28 °C. After 18 h, the germination rate of a volume containing about 200 spores of pathogen was measured

under a light microscope, as described by Droby *et al*. (1998). Each treatment consisted of three replicates and the whole experiment was performed twice.

2.9 Effects of *R. mucilaginosa* on the quality of wounded pears

To rapidly evaluate the effect of the antagonistic *R. mucilaginosa* yeast on the quality of pear fruits, freshly harvested fruits were wounded as described in Section 2.4 and two wounds were made parallel to each other (about 6 cm apart). Then, 50 μl of *R. mucilaginosa* at 1×10^8 cells/ml was added to one wound, and sterile distilled water to the other as a control. Quality parameters were measured after 6 d of storage at 20 °C in dark. The treatment consisted of three replicates, each consisting of three fruits. The whole experiment was performed twice. Quality measurements were conducted as follows: (1) The total soluble solid (TSS) content of pear juice was determined using a hand refractometer and the results expressed as g TSS/100 g fruit weight (Porat *et al.*, 1999). (2) The ascorbic acid content of each pear was determined by the 2,6-dichloroindophenol titrimetric method and results were expressed as mg ascorbic acid/100 g sample (Pérez *et al.*, 1999). (3) Titratable acidity was measured by titrating pear juice obtained from 10 g of pear tissue and diluted with 100 ml of distilled water to pH 8.1 with 0.1 mol/ml NaOH. Titratable acidity is expressed as the percentage of malic acid (Wszelaki and Mitcham, 2000).

2.10 Population dynamics of *R. mucilaginosa* in pear wounds

The pear fruits were prepared and wounded as described in Section 2.4 and three wounds were made on each pear. Then, the wounds were inoculated with 50 μl of a washed *R. mucilaginosa* cell suspension at 1×10^6, 1×10^7, and 1×10^8 cells/ml. After these treatments, the fruits were stored as described above. Tissue was taken from these pears using a sterile cork-borer (10 mm diameter×5 mm deep) at different time points (0, 12, 24, 36, 48, 72, and 96 h). The samples were ground in 10 ml of sterile water using a sterile mortar and pestle. A hemocytometer was used to count the yeast cells (Yu and Zheng, 2006). The treatment consisted of three replicates, each consisting of three pears. The whole experiment was performed twice, independently.

2.11 Statistical analysis

The data presented are means from one individual experiment, representative of two independent experiments with similar results. The data were analyzed by one-way analysis of variance (ANOVA) using the statistical software SPSS 20.0 (SPSS Inc., Chicago, Illinois, USA). When the analysis result was significant at the level of $P<0.05$, separation of means was performed using Duncan's multiple range tests. The data from each experiment were analyzed separately.

3 Results

3.1 Preliminary screening of Tibetan yeasts to control blue mold of pears

The antagonistic efficacy of all 20 strains of yeast isolated from Tibetan local fermented products to *P. expansum* on pear fruits was expressed by the inhibition rate of decay incidence and lesion diameter (Table 1). In terms of decay incidence, most of the yeast isolates had a clear biocontrol activity, while four strains (NQ5-1, DX5-2, JZ1-2, and DX1-1) promoted disease development and one strain (LM1-1) had no significant effect. Among the fifteen yeasts with a positive effect, six strains (NQ 4-2, DX1-2, NQ11-2, JZ1-1, DX2-2, and RKZ1-5) inhibited fruit infection by more than 35% and one of them (RKZ1-5) almost completely inhibited the disease (Table 1). Almost all the yeast isolates inhibited the development of decay and *R. mucilaginosa* (RKZ1-5) was the most effective. The yeast isolates showing an inhibition of infection more than 35% were chosen for further screening.

3.2 Further screening of selected Tibetan yeasts to control blue mold of pears

Results from further evaluation of six Tibetan yeasts, selected as reported in Section 3.1, are shown in Table 2. At the common concentration of the antagonist of 1×10^8 cells/ml, the yeast isolate RKZ1-5 exhibited a significantly higher biocontrol activity in terms of decay incidence than the other five yeast strains. As regards lesion diameters, almost all six yeasts displayed protection, but RKZ1-5 was still the best. Based on these results, the yeast *R. mucilaginosa* was selected for further study.

Table 1 Inhibition activity of screened Tibetan yeast isolates on blue mold of pears *in vivo* after 6 d of incubation

Tibetan yeasts from fermented products	Decay incidence inhibition rate (%)	Lesion diameter inhibition rate (%)
Control	0^{ef}	0^{j}
Issatchenkia orientalis (JZ10-1)	23.38 ± 43.29^{cde}	52.73 ± 20.93^{cdefg}
Issatchenkia orientalis (NQ5-1)	-36.61 ± 3.28^{g}	26.75 ± 0.08^{hi}
Issatchenkia orientalis (DX5-2)	-19.94 ± 19.94^{fg}	38.60 ± 3.96^{efgh}
Pichia fermentans (JZ1-1)	44.44 ± 11.98^{bc}	68.79 ± 8.47^{bcd}
Pichia membranifaciens (JZ5-8)	19.84 ± 21.60^{cde}	50.94 ± 11.15^{defg}
Rhodotorula mucilaginosa (RKZ1-5)	100^{a}	100^{a}
Geotrichum sp. (JZ2-1)	0^{def}	13.72 ± 1.33^{ij}
Pseudozyma sp. (DX4-2)	11.11 ± 11.11^{cdef}	36.12 ± 7.65^{fgh}
Yarrowia lipolytica (JZ7-2)	18.52 ± 16.97^{cde}	54.29 ± 11.69^{cdef}
Candida zeylanoides (DX2-2)	59.26 ± 12.83^{b}	78.68 ± 5.18^{b}
Torulaspora delbrueckii (JZ3-1)	22.22 ± 22.22^{cde}	50.88 ± 8.99^{defg}
Torulaspora delbrueckii (NQ4-2)	37.04 ± 16.97^{bcd}	65.34 ± 5.69^{bcd}
Torulaspora delbrueckii (DX6-4)	18.52 ± 6.42^{cde}	55.20 ± 1.52^{cde}
Pichia fermentans (NQ11-2)	43.98 ± 37.69^{bc}	71.30 ± 20.31^{bc}
Pichia fermentans (DX1-2)	38.89 ± 34.70^{bc}	65.14 ± 6.17^{bd}
Saccharomyces cerevisiae (JZ1-2)	-4.17 ± 7.22^{efg}	13.33 ± 6.40^{ij}
Saccharomyces cerevisiae (DX1-1)	-4.17 ± 7.22^{efg}	5.52 ± 14.97^{j}
Saccharomyces cerevisiae (LM1-1)	0^{def}	26.23 ± 5.97^{hi}
Kluyveromyces marxianus (NQ10-2)	7.41 ± 12.83^{cdef}	34.52 ± 12.01^{gh}
Kluyveromyces marxianus (DX3-3)	8.46 ± 7.50^{cdef}	28.46 ± 11.10^{hi}

JZ and RKZ were obtained from the Xigaze area of Tibet; NQ and LM from Nagqu County and DX from the Damxung County, Tibet. The uppercase letters combined with numbers stand for the strain number. The concentration of washed Tibetan yeast suspension incubated into wounds on pear fruits was 1×10^{9} cells/ml. The pathogen conidial suspension was added into wounds at 1×10^{4} spores/ml. Results are presented as mean±standard deviation (SD) (n=3). In the two columns of inhibition rates, mean values followed by different letters are statistically different at $P<0.05$ according to Duncan's multiple range test

Table 2 Antagonistic activity of six selected Tibetan yeasts on blue mold of pears *in vivo*

Selected Tibetan yeasts	Decay incidence (%)	Lesion diameter (mm)
Control	100^{a}	20.80 ± 1.98^{a}
Pichia fermentans (JZ1-1)	100^{a}	13.42 ± 1.84^{c}
Pichia fermentans (DX1-2)	100^{a}	13.40 ± 1.05^{c}
Pichia fermentans (NQ11-2)	97.22 ± 4.81^{a}	12.85 ± 1.26^{c}
Torulaspora delbrueckii (NQ4-2)	100^{a}	17.04 ± 1.80^{b}
Candida zeylanoides (DX2-2)	100^{a}	15.73 ± 1.84^{bc}
Rhodotorula mucilaginosa (RKZ1-5)	76.48 ± 15.87^{b}	8.16 ± 1.58^{d}

JZ and RKZ were obtained from the Xigaze area of Tibet; NQ from Nagqu County and DX from Damxung County, Tibet. The uppercase letters combined with numbers stand for the strain number. The concentration of washed Tibetan yeast suspension incubated into wounds on pear fruits was 1×10^{8} cells/ml. The pathogen conidial suspension was added into wounds at 1×10^{4} spores/ml. Results are presented as mean±SD (n=3). Within a column, mean values followed by different letters are statistically different at $P<0.05$ according to Duncan's multiple range test

3.3 Antagonistic efficacy of selected yeast strain *R. mucilaginosa* to *P. expansum* on pear fruits

The results of the antagonistic efficacy of *R. mucilaginosa* at different concentrations to *P. expansum* on pear fruits after 6 d of incubation are shown in Fig. 1a. Compared with the control, the incidence of decay was significantly reduced ($P<0.05$) by *R. mucilaginosa* except at the concentration of 1×10^{5} cells/ml. The higher the concentration of the yeast, the lower

was the incidence of decay. The highest biocontrol activity (the least decay) was obtained at the higher concentrations of 1×10^8 cells/ml (16.7% decay) and 1×10^9 cells/ml (2.8% decay) (Fig. 1a). The effect of *R. mucilaginosa* at different concentrations on lesion diameter was similar. However, the antagonist also had obvious efficacy on lesion diameter (P<0.05) at the low concentration of 1×10^5 cells/ml (Fig. 1b). The trend in the incidence of decay also indicated that the biocontrol activity gradually weakened with storage time, but at the high concentrations of 1×10^8 and 1×10^9 cells/ml, the rise in the incidence of decay was much slower than that at lower concentrations (Fig. 1c).

3.4 Antagonistic activity of different preparations of *R. mucilaginosa in vivo*

The biocontrol activity of different preparations of *R. mucilaginosa* is shown in Fig. 2. The washed cell suspension had higher antagonistic activity (41.7%) to control blue mold of pear fruits than the other three preparations (Fig. 2a). The same result was found for the lesion diameter. The washed cell suspension produced the smallest lesion diameter of 4.6 mm (Fig. 2b). The autoclaved cell culture had hardly any antagonistic activity.

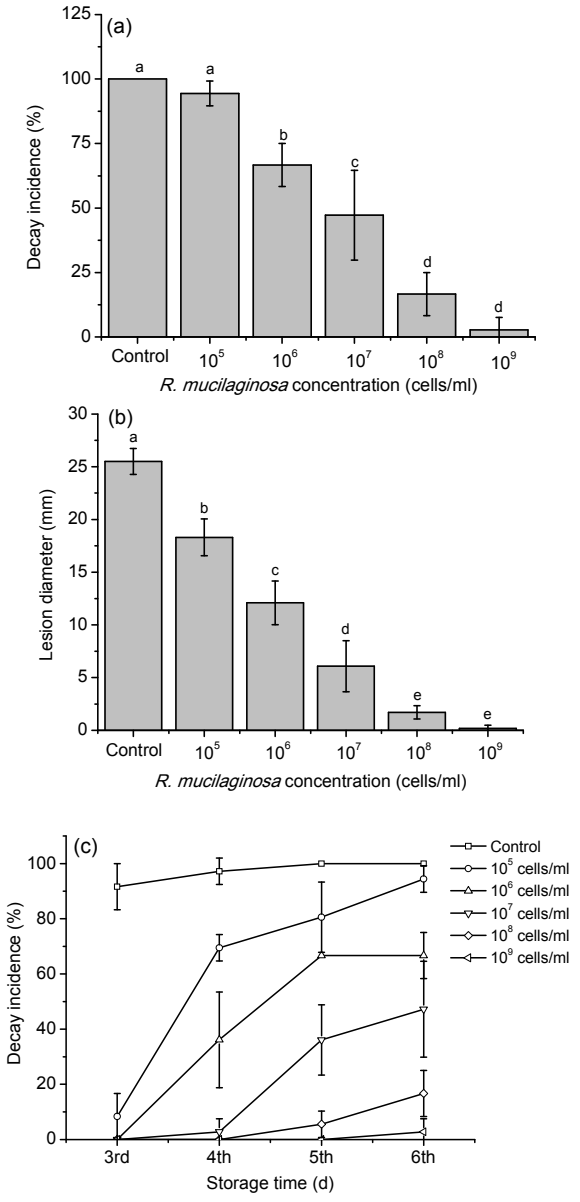

Fig. 1 Antagonistic efficacy of *R. mucilaginosa* to *P. expansum* on pear fruits

Decay incidence (a) and lesion diameter (b) were measured after 6 d storage at 20 °C. (c) The statistic of incidence trend was made by the decay incidence of each day. Data are presented as mean±SD (n=3). Data in columns with the different letters are significantly different at P<0.05 according to Duncan's multiple range test

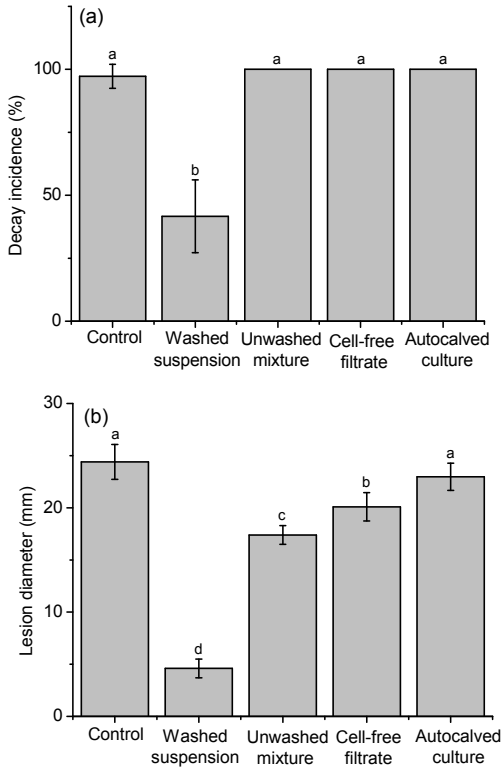

Fig. 2 Antagonistic activity of different preparations of *R. mucilaginosa in vivo*

Decay incidence (a) and lesion diameter (b) were measured after 6 d of storage at 20 °C. Data are presented as mean±SD (n=3). Data in columns with the different letters are significantly different at P<0.05 according to Duncan's multiple range test

3.5 Inhibition of spore germination of *P. expansum* by different preparations of *R. mucilaginosa*

The biocontrol activity of different preparations of *R. mucilaginosa in vitro* is shown in Fig. 3. Both washed and unwashed yeast cell suspensions were most effective at inhibiting spore germination in *P. expansum*, and the inhibition rates were 54.0% and 54.7%, respectively. The cell-free culture filtrate and autoclaved cell culture had hardly any effect on *P. expansum* spore germination, but promoted growth of the pathogen in PDB.

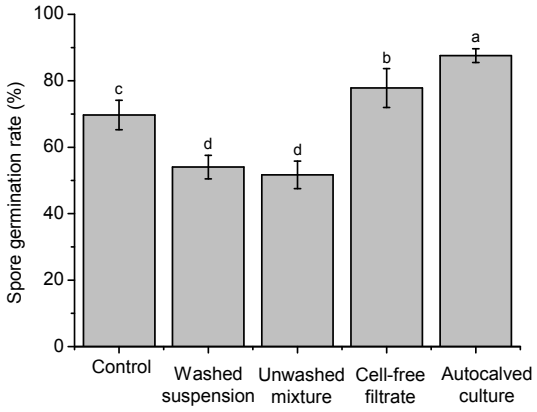

Fig. 3 Inhibition of spore germination by *R. mucilaginosa in vitro*

Spore germination (percentage of germinated spores calculated from counts at least 200 spores) was measured after 18 h of incubation. Data are presented as mean±SD (n=3). Data in columns with the different letters are significantly different at P<0.05 according to Duncan's multiple range test

3.6 Effects of *R. mucilaginosa* on fruit quality

The results of quality tests on pear fruits are shown in Table 3. After 6 d storage at 20 °C, although the titratable acidity of treated fruits increased (P<0.05), *R. mucilaginosa* had hardly any effect on the TSS or ascorbic acid content.

Table 3 Effects of *R. mucilaginosa* on pear fruit quality

Treatment	TSS (%)	Titratable acidity (% malic acid)	Ascorbic acid (mg/100 g)
Control	11.44±0.74[a]	0.061±0.0055[b]	2.70±0.08[a]
Antagonist	11.43±0.67[a]	0.079±0.0018[a]	2.53±0.21[a]

Results are presented as mean±SD (n=3). Within each column, mean values followed by different letters are statistically different at P<0.05 according to Duncan's multiple range test

3.7 Population dynamics of *R. mucilaginosa* in pear wounds

The population dynamics of *R. mucilaginosa* in pear fruit wounds at 20 °C is shown in Fig. 4. The yeast grew rapidly in the first 24 h at all three concentrations (1×10^6, 1×10^7, and 1×10^8 cells/ml). The higher the concentration, the faster was the growth. At 48 h of incubation, the population dynamics at three different concentrations reached maximum densities of 8.63×10^6, 1.71×10^7, and 3.82×10^7 cells/ml, respectively. As time went on, the populations gradually decreased, falling to 4.47×10^6, 5.46×10^6 and 1.63×10^7 cells/ml, respectively, after 96 h.

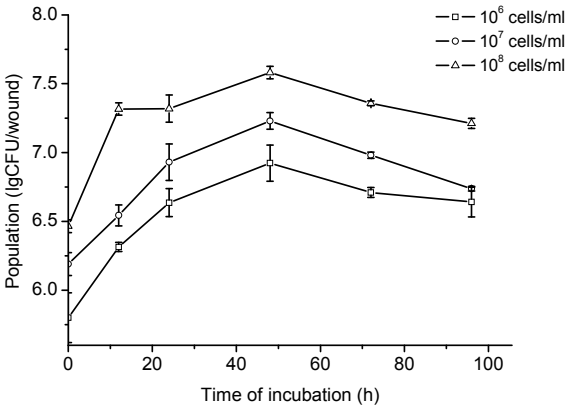

Fig. 4 Population dynamics of *R. mucilaginosa* in pear wounds at 20 °C

The population density was measured at 12, 24, 36, 48, 72, and 96 h. Data are presented as mean±SD (n=3)

4 Discussion

The use of microbial antagonists in the control of postharvest decay has been reported as an effective alternative to the use of chemical fungicides (Droby *et al.*, 2009; Wilson *et al.*, 2011). Antagonistic yeast is regarded as the most promising biocontrol agent since the inhibitory mechanism does not depend mainly on the production of antibiotics (Droby *et al.*, 1998; Zhang *et al.*, 2005; 2008; Manso and Nunes, 2011). Antagonistic yeasts are generally isolated from fruit surfaces and fruit wounds (Wilson and Wisniewski, 1989; Wilson *et al.*, 1993). The sources have recently been expended to other environments, such as sea water (Wang *et al.*, 2010) and Antarctic soil (Vero *et al.*, 2013). The main reason for exploring these new sources is that the yeasts in specific geographic areas

may have unique characteristics and perhaps be more effective as biocontrol agents against fruit pathogens (Sayago *et al.*, 2012; Vero *et al.*, 2013). Based on this, our study focused on screening antagonistic yeast from Tibet.

Through a preliminary screening test, *R. mucilaginosa* was screened out from 20 strains of yeasts isolated from local fermented products in Tibet. All the yeast isolates were identified and provided by the Key Laboratory of Dairy Biotechnology and Engineering, Ministry of Education (Inner Mongolia, China). The biocontrol activity of *R. mucilaginosa* was confirmed in further screening tests (Table 2). Although this kind of yeast has been reported previously to protect fruits after harvest (Li *et al.*, 2011; Robiglio *et al.*, 2011), this is the first study of the use of *R. mucilaginosa* from Tibet to control the blue mold of pear fruits.

From the detailed antagonistic efficacy trials, the results in Fig. 4 indicated that the biocontrol activity of *R. mucilaginosa* gradually improves with increasing concentration. The optimum concentration was 1×10^8 cells/ml, because at this concentration biocontrol activity was similar to that at 1×10^9 cells/ml. A similar phenomenon was also found in other studies of *Rhodotorula* yeasts (Castoria *et al.*, 2005; Dal Bello *et al.*, 2008; Zhang *et al.*, 2008; Robiglio *et al.*, 2011). Generally, competition with pathogen for nutrients and space in the wounds of fruits is recognized as the main biocontrol mechanism of antagonistic yeast (Ippolito and Nigro, 2000; Sharma *et al.*, 2009), so the population dynamics of *R. mucilaginosa* in wounds of pear fruits was used to study its biocontrol mechanism. The results were consistent with this theory and not only showed that *R. mucilaginosa* could rapidly colonize and grow on the pear fruits, but also explained why the higher concentration could lead to better biocontrol activity.

To further investigate the control mechanism of *R. mucilaginosa* against the blue mold of pear fruits, the efficacy of the yeasts in different preparations was evaluated *in vivo* and *in vitro*. According to the results of the *in vivo* test (Fig. 2), the washed cell suspension had the best biocontrol activity, while the other three preparations hardly had any effect on the incidence of blue mold of pear fruits. Among the other three preparations, the unwashed cell culture mixture and cell-free culture filtrate inhibited the lesion expansion of pear fruits at different levels, and the unwashed cell culture mixture had the better activity. This finding indicates that the competition of *R. mucilaginosa* for nutrients and space on pear fruits was weakened when the yeast was in a suspension full of nutrients (Zhang *et al.*, 2003; Bencheqroun *et al.*, 2007). It also demonstrates that competition for nutrients in fruit wounds is an important mechanism of yeast antagonism. The cell-free culture filtrate had some inhibiting effects on the expansion of lesions in pear fruits, showing that *R. mucilaginosa* may produce some substances that inhibit the pathogen, such as hydrolases (Zhang *et al.*, 2011; 2012; Barbey *et al.*, 2012; Calvo-Garrido *et al.*, 2013; Hershkovitz *et al.*, 2013) or rhodotorulic acid (Calvente *et al.*, 1999; Sansone *et al.*, 2005). The result of the *in vitro* test (Fig. 3) showed that both the washed and unwashed cell culture mixtures inhibited pathogen spore germination effectively. There may be other mechanisms of action by which *R. mucilaginosa* can control blue mold *in vitro*. In any case, both test results indicated that the washed cell suspension of *R. mucilaginosa* had the best biocontrol activity among the four different preparations and the major mechanism of action is likely to be the competition with pathogen for nutrients and space in wounds of the pear fruits.

The study also showed that *R. mucilaginosa* had almost no detrimental effects on the quality of pear fruits during storage at room temperature. Although the titratable acidity increased above controls after treatment, there was no apparent damage to the pears. What is more, the TSS or ascorbic acid content was not significantly changed by *R. mucilaginosa*.

In conclusion, the study indicated that the yeast *R. mucilaginosa* isolated from Tibetan fermented products had biocontrol activity against the blue mold of pear fruits during postharvest storage. Through further study, its potential as a new kind of antagonistic yeast agent to protect the pear fruits from decay can be explored.

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Compliance with ethics guidelines

Hao HU, Yang XU, Huang-ping LU, Rui XIAO, Xiao-dong ZHENG, and Ting YU declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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中文概要

题　目：西藏发酵制品中拮抗酵母对水晶梨青霉病的防治效果研究

目　的：从西藏发酵制品中分离筛选出对水晶梨采后青霉病具有较好防治效果的拮抗酵母，并对其作用机理进行研究。

创新点：首次从西藏发酵制品中筛选得到一株对水晶梨采后青霉病具有较好防治效果的拮抗酵母 *Rhodotorula mucilaginosa*，并对其作用机理进行了初步研究。

方　法：采用水晶梨果实体内筛选的方法，经过初筛与复筛，确定具有较好生防效果的拮抗酵母；通过对筛选所得拮抗酵母不同浓度、不同处理液的生防效果，果实伤口处生长动态，以及对梨果实采后品质影响的研究，初步探讨西藏发酵制品中拮抗酵母的生防作用机理。

结　论：从西藏发酵制品中筛选得到一株拮抗酵母 *R. mucilaginosa*，其对水晶梨采后青霉病具有较好的生物防治效果。研究结果发现其主要作用机理为在果实伤口处与病原菌的营养与空间竞争。通过进一步研究，可开发为梨果实采后病害防治的新型生物保鲜剂。

关键词：生物防治；采后；*Rhodotorula mucilaginosa*；西藏发酵制品；水晶梨