Food Chemistry 241 (2018) 301–307

Contents lists available at ScienceDirect

Food Chemistry

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

Yeast cell wall induces disease resistance against *Penicillium expansum* in pear fruit and the possible mechanisms involved

Cui Sun, Ming Lin, Da Fu, Jiali Yang, Yining Huang, Xiaodong Zheng, Ting Yu*

National Engineering Laboratory of Intelligent Food Technology and Equipment, Key Laboratory for Agro-Products Postharvest Handling of Ministry of Agriculture, Key Laboratory for Agro-Products Nutritional Evaluation of Ministry of Agriculture, Zhejiang Key Laboratory for Agro-Food Processing, Fuli Institute of Food Science, College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310058, People's Republic of China

ARTICLE INFO

Keywords:
Pear fruit
Cell wall
Rhodosporidium paludigenum
Penicillium expansum
Induced resistance

ABSTRACT

The results from this study showed that cell wall prepared from *Rhodosporidium paludigenum* induced strong disease resistance against blue mold rot caused by *Penicillium expansum* in pear fruit. Yeast cell wall reduced germination of *P. expansum in vitro* and in fruit wounds after 24 h of treatment. Moreover, the cell wall treatment significantly enhanced the activities of defense-related enzymes (β-1,3-glucanase and chitinase) and the genes expression of PR proteins (*PR1-like*, *endoGLU9, endoCHI-like* and *PR4*), which may be an important mechanism by which cell wall reduces the fungal disease of pear fruit caused by *P. expansum*. These findings suggest that the mechanism by which *R. paludigenum* induced fruit resistance was linked to the function of its cell wall and application of cell wall might be a useful strategy for the control of postharvest disease in pear fruit.

1. Introduction

Postharvest diseases cause considerable economic losses in fruit industry. Blue mold caused by filamentous fungi *Penicillium expansum* Link is one of the devastating postharvest fungal diseases affecting pear fruit worldwide (Holmes, 1990; Jones & Aldwinckle, 1990; Snowdon, 1990). Application of synthetic chemical fungicides is the most common method to limit postharvest decay and fungal infections during storage at present (Jatoi et al., 2017). However, the continued use of chemical fungicides is limited because of the increasing concerns toward potential problems related to public health associated with pesticide use, fungicide resistance in pathogens, and potential harmful effects on the environment (Jeong et al., 2015; Spadaro & Droby, 2016). Therefore, it is an urgent need to develop a safe and effective alternative strategies to control postharvest disease as well as increase the storage life of fruits.

Induction of fruit resistance is considered a promising strategy for control of postharvest disease owing to provide a long-lasting and systemic resistance to a broad spectrum of activity against many disease-causing organisms (He et al., 2017; Romanazzi et al., 2016; Welling, 2001). Moreover, natural induced resistance against pathogens in harvested horticultural crop could be activated by biological, chemical, or physical means (Romanazzi et al., 2016). Since the first chemical resistance activator, Probenazole, was registered in Japan as Oryzemate in 1975, many other chemical and biological activators have been developed, including *Cryptococcus laurentii*, β-aminobutyric acid (BABA), salicylic acid (SA) and methyl jasmonate, which have great efficiency in the laboratory and in a few field cases to control diseases in fruit by inducing defense resistance (He et al., 2017; Walters, Ratsep, & Havis, 2013; Yan, Hu, Lu, & Zheng, 2016; Yang et al., 2017). In pear fruit, it has been reported that induced resistance can be achieved by application of colloidal chitin (Fu, Xiang, Yu, Zheng, & Yu, 2016), γ-aminobutyric acid (Yu et al., 2014), acibenzolar-S-methyl (Cao, Jiang, & He, 2005).

The cell wall is essential for the integrity of the yeast cell, providing strength and shape to the growing cell, as well as protection against environmental insults (Ene et al., 2015). In general, the yeast cell wall consist about 20%–30% (w/w) of the total weight of a cell and mainly composed of polysaccharides, i.e., approximately 40% of mannoproteins, 60% of β-glucan, and 2% of chitin (Klis, Boorsma, & De Groot, 2006). In plants, Housaku Monogatari (HM), prepared from the yeast cell wall extract of the budding yeast *Saccharomyces pastorianus* during the beer brewing process, increased the resistance of *Arabidopsis thaliana* and *Brassica rapa* leaves against bacterial and fungal pathogens as a result of inducing early activation of jasmonate/ethylene and late activation of salicylic acid (SA) pathways (Narusaka et al., 2015). Minami, Tanaka, Takasaki, Kawamura, and Hiratsuka (2011) also found that HM might contain multiple microbe-associated molecular patterns (MAMPs) that activate systemic acquired resistance and induced systemic resistance signaling pathways at different times in

* Corresponding author at: 866 Yuhangtang Road, Hangzhou 310058, People's Republic of China.
E-mail address: yuting@zju.edu.cn (T. Yu).

http://dx.doi.org/10.1016/j.foodchem.2017.08.092
Received 13 July 2017; Received in revised form 21 August 2017; Accepted 28 August 2017
Available online 31 August 2017

Arabidopsis plant. However, cell wall has not been tested as a practical pesticide.

Previous studies indicated that *Rhodosporidium paludigenum* can significantly induce disease resistance against *P. expansum* in citrus fruit (Lu et al., 2015). However, further study on the substance basis of its function yet to be determined. Thus, in the present study, we investigate whether or not yeast cell wall made from *R. paludigenum* induces resistance against *P. expansum* in pear fruit and investigate the possible action mechanisms involved. More specifically, this study (1) assess the effect of cell wall at different concentrations on induction of disease resistance against blue mold in pear fruit. (2) evaluate the inhibitory ability of cell wall on spore germination *in vitro* and *in vivo* against *P. expansum*. (3) analyze the influence of activities of defense-related enzymes and gene expression using RT-qPCR. (4) determine the cell wall polysaccharides content of *R. paludigenum*.

2. Materials and methods

2.1. Fruit material

Pear (*Pyrus pyrifolia* Nakai. cultivar "Shuijing") fruits were harvested at commercial maturity and sorted based on uniform size, ripeness and absence of physical defects or decay. The fruits were surface-sterilized with 0.1% sodium hypochlorite solution for 2 min, thoroughly rinsed with tap water and air-dried at 20 °C.

2.2. Pathogen

Penicillium expansum was originally isolated from naturally infected pear fruit and maintained on potato dextrose agar (PDA) medium (containing the extract from 200 g of boiled potato, 20 g of glucose and 20 g of agar in 1 L of distilled water) at 25 °C. Spore suspensions were prepared by flooding the 7-day-old sporulating cultures of *P. expansum* with sterile distilled water and the spore concentration obtained was adjusted to 1×10^4 spores mL^{-1} using a hemocytometer.

2.3. Yeast

The yeast strain *Rhodosporidium paludigenum* Fell & Tallman used in the present study work was isolated from the East China Sea and identified by CABI Bioscience Identification Services (IMI 394084) (Wang et al., 2008). The yeast was maintained on nutrient yeast dextrose broth (NYDB) medium (containing 8 g of nutrient broth, 5 g of yeast extract, and 10 g of glucose in 1 L of distilled water) at 28 °C for 24 h on a gyratory shaker at 200 rpm.

2.4. Yeast cell wall preparation

Cell wall was obtained as described by Ferreira et al. (2006), with some modifications. Before disruption, the yeast was washed three times with 0.2 M sodium phosphate buffer (pH 8.0) by successive resuspension and centrifugation steps (4000 × *g*, 10 min, 4 °C). Cells were then disrupted mechanically in the same buffer with 425 μm-diameter acid-washed glass beads in tissue homogenizer (Tissuelyer-48, Shanghai Jingxin Technologies, China) by application of 5 cycles of shaking of 180 s each cycle at 70 Hz and 1 min intervals on ice between each cycle. The disruption mixture was washed by centrifugation at 4000 × *g*, once with the same buffer and three times with water. The cell wall suspension was heated at 121 °C for 20 min to inactive the residual enzymes. Cell wall were harvested by centrifugation for 20 min at 10000 × *g* and lyophilized.

2.5. Effects of cell wall at different concentrations on induction of disease resistance against blue mold in pear fruit

Six wounds were made (5 mm diameter and 3 mm deep) on each pear fruit with a sterile borer. Each wound was treated with 30 μL of CW at 0.01%, 0.1%, 0.2%, 0.5% and 1.0% (w/v), respectively. Wounds treated with sterile distilled water served as the control. After 24 h, 30 μL of a spore suspension of *P. expansum* (1×10^4 spores mL^{-1}) was inoculated into each wound. The fruit were then air dried and stored in enclosed plastic trays to maintain 90–95% relative humidity (RH) at 25 °C. Each treatment included 3 replicates and each replicate consisted of 9 pears. Each test was performed at least twice.

2.6. Effect of cell wall on spore germination of P. expansum in vitro

The effect of cell wall on spore germination of *P. expansum* was tested in potato dextrose broth (PDB). Cell wall was added into the 10 mL glass tube containing 2 mL PDB in final concentrations at 0.01%, 0.1%, 0.2%, 0.5% and 1%, respectively. At the same time, aliquots of 100 μL of spore suspensions of *P. expansum* were put into each tube to obtain a final concentration at 1×10^6 spores mL^{-1}. All treated tubes were placed on a rotary shaker (QYC 2102, Shanghai FUMA, China) at 200 rpm at 25 °C. After 12 h of incubation, approximately 150–200 spores of the pathogen per replicate were observed microscopically for germination rate. Spores were considered germinated when germ tube lengths were equal to or greater than spore lengths. Each treatment included 3 replicates and the experiment was repeated twice.

2.7. Effect of cell wall on spore germination rate of P. expansum in vivo

Each pear fruit was gently wounded as described above and each wound was treated with 30 μL of cell wall at 0.5% or sterile distilled water as a control. After 2 or 24 h, 30 μL of a conidial suspension of *P. expansum* at 1×10^7 spores mL^{-1} inoculated into each wound. The fruits were then air dried and stored as above. After 12 h of incubation, the spores were collected from the wound and approximately 150–200 spores of the pathogen were observed microscopically for germination rate. Each treatment included 3 replicates and each replicate consisted of 9 fruits for each time point.

2.8. Assay of enzyme activities of GLU and CHI in pear fruit

Each pear fruit was wounded as described above and treated with (1)30 μL sterile distilled water as the control; (2) 30 μL cell wall at 0.5%; (3) 30 μL of the *P. expansum* suspensions at 1×10^4 spores mL^{-1}; (4) 30 μL of the *P. expansum* suspensions at 1×10^4 spores mL^{-1} 24 h post-treatment with the cell wall at 0.5%. Tissue samples around the wounds were taken at a series of time intervals (0, 12, 24, 36 and 48 h) after treatment and immediately frozen in liquid nitrogen, then stored at −80 °C prior to crude enzyme extraction.

For the extraction of β-1,3-glucanase (GLU) and chitinase (CHI), frozen fresh tissues (0.9 g fresh weight) was ground with 3.6 mL of pre-cooled sodium acetate buffer (50 mM, pH 5.0) containing 1% (w/v) polyvinyl-pyrrolidone (PVP). The homogenates were centrifuged at 10,000 × *g* for 20 min at 4 °C and the supernatants were collected as the crude enzyme extracts. The protein content in the enzyme extracts was determined according to the method of Bradford (1976), using bovine serum albumin as the standard.

GLU activity was assayed with laminarin as the substrate, following the method described by Ippolito and Nigro (2000) with slight modifications. The reaction mixture containing 250 μL of enzyme preparation and 250 μL of 50 mM sodium acetate buffer (pH 5.0) containing 0.5% laminarin (w/v) was incubated at 40 °C for 4 h. The reaction was terminated by heating the sample in boiling water for 5 min after reaction with 500 μL of 3,5-dinitrosalicilate. Then, the solution was diluted to 1:4 with sterile distilled water and the amount of reducing sugar released from laminarin was measured using Microplate Reader (SpectraMax190, Molecular Devices, USA) at 500 nm. The specific activities of GLU are expressed as Units (U) mg^{-1} protein.

CHI activity was assayed with chitin azure as the substrate,