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Control of postharvest blue mold decay in pears by *Meyerozyma guilliermondii* and it's effects on the protein expression profile of pears

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ABSTRACT

This study assessed the biocontrol efficacy of *Meyerozyma guilliermondii* against blue mold decay caused by *Penicillium expansum* in pears and the possible mechanisms involved. The results indicated that *M. guilliermondii* significantly inhibited the blue mold decay caused by *P. expansum* without affecting the quality of the pears. *M. guilliermondii* rapidly colonized the wounds and surfaces of the pears at both 4 °C and 20 °C. The rapid growth in the population of *M. guilliermondii* in the wounds and surface environments of pears indicated that it has the potential to inhibit pathogens in pears. The activities of antioxidant enzymes (peroxidase and catalase) in the pear were improved after the application of the yeast. Phenylalanine ammonialyase (PAL), a key enzyme involved in lignin biosynthesis and defense related activity, was also markedly enhanced. Generally, the application of yeast induced disease resistance in the pear. The results pear proteomics profile after *M. guilliermondii* treatment showed that 17 proteins were significantly up-regulated and 13 were down-regulated in response to induction with *M. guilliermondii*. Most of the proteins were involved in defense and stress responses based on biological process. These results provided a new insight into the biocontrol mechanism of the antagonist yeast in the pear fruit.

1. Introduction

The pear is one of the world's cultivated fruits, with more than 70 countries and regions producing it (Qu et al., 2016). Postharvest diseases caused by pathogenic infections result in great losses of fruits (Xu et al., 2013). *Penicillium expansum* is the most common pathogen of pear fruit which causes blue mold decay during storage (Cao et al., 2013). Furthermore, the growth of *P. expansum* organisms tends to accumulate more patulin (4-hydroxy-4H-furo [3,2c] pyran, 2[6H]-one), which is an unsaturated heterocyclic lactone that is toxic to animals, causes intestinal injuries, and is shown to be mutagenic (Andersen et al., 2004). So far, the use of microbial agents has been an effective and safe method (Yang et al., 2017) for the biological control of pathogens. It was reported (Fu et al., 2015) that *Cryptococcus laurentii* could effectively inhibit the growth of *P. expansum* in pears, and that *Rhodotorula mucilaginosa* (Hao et al., 2015) could control blue mold decay in pear fruits.

Previous studies have shown that *M. guilliermondii* has antibacterial properties. It colonizes fruit wound surfaces (Larralde-Corona et al., 2011) and competes for nutrients and niche exclusion; these are the most important mode of action of the yeast for inhibiting pathogenic growth in fruit wounds (Spadaro and Droby, 2016). Typically, the mechanisms conferring yeast antagonists with the ability to inhibit postharvest pathogens include competition for nutrients and space (Jamalizadeh et al., 2011), induction of host resistance (Tian et al., 2011), oxidative response (Macarisin et al., 2010), and parasitism (Magallon-Andalon et al., 2012). These mechanisms of action have been reviewed by Spadaro and Droby (2016).

Among these, induction of host resistance plays an important role in postharvest biocontrol. It was reported (Lu et al., 2013) that the activity of defense-related enzymes such as β-1,3-glucanase, phenylalanine ammonialyase, peroxidase, and polyphenoloxidase of mandarins was activated by *Rhodosporidium paludigenum*. Polyphenoloxidase (PPO) and peroxidase (POD), linked to the lignification of host plant cells, were considered key enzymes in host defense reactions against pathogenic infections (Cao et al., 2008; Ma et al., 2013; Zhang et al., 2011a). Inoculation with *Yarrowia lipolytica* enhanced the PPO and POD activities in apple fruits; these enzymes are considered to play important roles in increasing disease resistance (Zhang et al., 2017). Moreover, the activities of phenylalanine ammonialyase (PAL) and catalase (CAT)

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were increased significantly following treatment with *Hanseniaspora uvarum,* which activated protective enzymes and induced disease resistance (Qin et al., 2015). In addition, when the plant host was attacked by pathogens, the defense system was activated by the expression of some pathogenesis-related (PR) proteins, such as proteinase inhibitors and plant defense proteins (Sels et al., 2008). The generation of PR proteins was an important biochemistry mechanism involved in plant resistance (Charles et al., 2009; Quaglia et al., 2011; Wang et al., 2009). PR families mainly comprise 17 groups, most often associated with the plant defense response (van Loon et al., 2006). It was reported (Chan et al., 2007) that PR proteins were involved in induction of resistance by *P. membranefaciens* in peach fruit induced. The induced expression of some proteins in the host by antagonistic yeasts could increase the resistance of the fruit against pathogens. It was reported (Nantawanit et al., 2010)that the induction of host resistance by antagonistic yeast in harvested fruits is a promising means of controlling fruit decay.

M. guilliermondii isolated from the surface of pears has been successfully applied to control postharvest pathogens on several fruits and vegetables; examples include the use of *Penicillium digitatum* on grapes, *P. expansum* on apples, and *Rhodotorula nigricans* on tomatoes (Zhang et al., 2011b). Furthermore, *M. guilliermondii* was reported to control fungal disease in the loquat (Liu et al., 2010), citrus (Lahlali et al., 2011) and cherry tomato caused by *Colletotrichum acutatum*, *Penicillium italicum* and *Botrytis cinerea* (Zhao et al., 2011), respectively. However, the biocontrol efficacy of *M. guilliermondii* against *P. expansum* is yet to be studied in the pear fruit. Furthermore, there is no information regarding the physiological and molecular mechanisms by which the control of postharvest diseases has been brought about by using *M. guilliermondii* as a biocontrol agent in the pear. The present study therefore investigated (1) the biocontrol efficacy of *M. guilliermondii* on postharvest blue mold decay caused by *P. expansum* in pears; (2) the population dynamics of *M. guilliermondii* in wounds and surfaces of pears; (3) the effects of *M. guilliermondii* on the antioxidant activity of enzymes, including catalase (CAT), peroxidase (POD), and phenylalanine ammonialyase (PAL) in pears; (4) the differentially expressed proteins of pears treated with or without *M. guilliermondii*.

2. Materials and methods

2.1. Yeast, pathogen, and fruits

The antagonist yeast isolated from the surface of rotten pears was identified as *M. guilliermondii* based on sequence analysis of the 5.8 S internal transcribed spacer (ITS) ribosomal DNA (rDNA) region. *M. guilliermondii* was then maintained at 4 °C on a nutrient yeast dextrose agar (NYDA) containing 8 g beef extract, 5 g yeast extract, 10 g glucose, and 20 g agar in 1 L of distilled water (Sangon Co., Shanghai, China). A loop of the culture was inoculated into 50 mL nutrient yeast dextrose broth (NYDB) contained in 250 mL and placed on a rotary shaker at 180 × *g* at 28 °C for 20 h. After incubation, cells were centrifuged at 7500 × *g* for 10 min and washed twice with sterile distilled water to remove the residual media. Cell pellets were then resuspended in sterile distilled water and adjusted to an appropriate concentration (1×10^8 cells/mL). *P. expansum* was isolated from rotten pears and maintained on potato dextrose agar (PDA; extract of boiled potatoes, 200 mL; dextrose, 20 g; agar, 20 g; and distilled water, 800 mL) at 4 °C. Spore suspensions were harvested by removing the spores from a 7-day-old culture and suspending in sterile distilled water. Spore concentrations were adjusted to an appropriate concentration (1×10^5 cells/mL), as required.

Pears (*Pyrus pyrifolia* Nakai."Shuijing") were harvested at commercial maturity from an orchard in Zhenjiang in the Jiangsu province.

2.2. Inhibitory efficacy of M. guilliermondii on the postharvest decay of pears caused by P. expansum

Three wounds (5 mm wide and 3 mm deep) were made at the equator of each pear. Wounds were inoculated with 30 μL of *M. guilliermondii* cell suspension that were adjusted to initial concentrations of 1×10^6 and 1×10^9 cells/mL. Pears treated with 30 μL of sterile distilled water served as controls. After two hours, 30 μL of spore suspension containing 1×10^5 spores/mL of *P. expansum* was inoculated into each wound. The pears were allowed to dry and were sealed with plastic wrappers to maintain a high humidity (20 °C, 95%). Decay incidence and lesion diameter of treated pears were measured with vernier calipers after 4 days. Each treatment process consisted of three replicates, each performed using 12 fruits; the experiment was repeated twice.

2.3. Population dynamics of M. guilliermondii on the surface of pears

Three circles (approximately 12 mm in diameter) were made on the equator of each pear with a marker pen. The *M. guilliermondii* (30 μL, 1×10^8 cells/mL) cell suspension was applied to the center of each circle and spread evenly with a coated bar. The treated pears were enclosed in plastic baskets and stored at 20 °C and 4 °C respectively. The populations of *M. guilliermondii* on the surface of stored pears with different storage durations and temperatures were measured as follows: The surface tissues of the pears were removed along the marked circles with a sterilized knife and ground in a mortar, and 30 mL of sterile distilled water was added. Subsequently, serially diluted yeast was transferred to petri dishes and cultured at 28 °C for 48 h. The number of *M. guilliermondii* was determined by the $\log_{10}$ of CFU/circle. The population of *M. guilliermondii* samples was determined by the plate count technique. The yeast population determined within 1 h was considered as that determined at 0 h. Similarly, the yeast population in the control was also determined. The treatment process was replicated thrice, using 12 fruits for each replicate; the entire experiment was repeated twice.

2.4. Population dynamics of M. guilliermondii in pear wounds

Artificially created wounds (5 mm × 3 mm) were inoculated with 30 μL of *M. guilliermondii* cell suspension (1×10^8 cells/mL). Treated fruits were enclosed in plastic baskets and stored at 20 °Cand 4 °C. Tissues around the wound of the fruit were excised with a sterilized knife that was 9-mm in diameter and 10-mm deep. The following steps were the same as in Section 2.3, pears treated with sterile distilled water served as the control. The treatment process was replicated thrice, using 12 fruits for each replicate; the entire experiment was repeated twice.

2.5. Effects of M. guilliermondii on antioxidant enzyme activity of pears

2.5.1. Fruit treatment

A previously described method (Xu et al., 2013) was used for pear treatment. Briefly, the tissues around the wounds were excised at day 0 (1 h after treatment), and day 1, 2, 3, 4 and 5 after treatment. Two grams of the above samples were put into a mortar, and 10 mL of precooled phosphate buffer (4 °C, 50 mM, pH 7.8, containing 1.33 mM EDTA and 1% PVPP) was added. A small amount of quartz sand was ground together with the pear tissues and added to the ice bath, and the ground samples were centrifuged at 10,000 × *g* for 4 min to obtain the supernatant. The experiment was conducted twice.

2.5.2. Analysis of enzymatic activity in pears

The Peroxidase (POD) activity was determined by a previously described method (Xu et al., 2013) with some modifications as follows: 0.2 mL of enzyme extract was added to 2.2 mL of 0.3% guaiacol (prepared as 50 mM in phosphate buffer with pH 6.4), and pre-heated at