Available online at www.sciencedirect.com
SCIENCE DIRECT
Postharvest Biology and Technology 39 (2006) 314–320
Postharvest Biology and Technology
www.elsevier.com/locate/postharvbio

Induction of H_2O_2-metabolizing enzymes and total protein synthesis by antagonistic yeast and salicylic acid in harvested sweet cherry fruit

Zhulong Chan [a,b], Shiping Tian [a,*]

[a] *Key Laboratory of Photosynthesis and Environmental Molecular Physiology, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China*
[b] *Graduate School of the Chinese Academy of Sciences, Beijing 100039, China*

Received 12 May 2005; accepted 14 October 2005

Abstract

The immersion of sweet cherry fruit in *Pichia membranefaciens* at a concentration of 5×10^7 cells ml^{-1} or in salicyclic acid (SA) at 0.5 mM for 10 min reduced the incidence of decay and lesion size caused by *Penicillium expansum*. Without pathogen inoculation, peroxidase (POD) activity was enhanced in yeast-treated fruit, but activities of catalase (CAT) and superoxide dismutase (SOD) showed a decrease in the same fruit. SA-treatment significantly inhibited CAT activity, but stimulated SOD and POD activities. After inoculation with *P. expansum*, CAT activity decreased and SOD activity increased in both yeast- and SA-treated fruit. No obvious difference was found in POD activity between treatments and water control. Treatments with yeast and SA changed the expression of POD isozymes. In addition, yeast and SA treatment increased total protein content of sweet cherry and up-regulated 33 and 47 kDa protein bands shown by SDS-PAGE. These results indicated that yeast- and SA-treatments induced synthesis of anti-oxidant enzymes and specific proteins, which may play a role in the resistance against postharvest blue mold.

Keywords: Antagonistic yeast; Induced resistance; Postharvest disease; Salicylic acid; Sweet cherry

1. Introduction

Recently, considerable attention has been placed on postharvest application of antagonistic agents for the inhibition of plant disease (Cook et al., 1999; Castoria et al., 2001; Droby et al., 2001; El-Ghaouth et al., 2003). Treatment with antagonistic yeasts has suggested that intensification of defense mechanisms has potential in reducing postharvest decay (El-Ghaouth et al., 1998; Fan and Tian, 2000). El-Ghaouth et al. (2003) found that, along with the induction of a systemic protection in fresh apples, *Candida saitoana* caused a rapid accumulation of chitinase (EC 3.2.1.14) and β-1, 3-glucanase (EC 3.2.1.6) activities locally in the treated wound site and systemically in tissues distant from the initial wound.

In addition, as a hormone-like substance, salicylic acid (SA) has proved to be a major component in signal transduction pathways and plays an important role in the regulation of plant growth and development, including transpiration, stomatal closure, seed germination, fruit yield, glycolysis, flowering and heat production (Klessig and Malamy, 1994; Ananieva et al., 2004). It is believed that systemic acquired resistance (SAR) is dependent on SA-mediated signalling and is associated with the production of PR proteins (Linda, 2001). In the fields of disease control, SA has received particular attention because its accumulation is essential for expression of multiple modes of plant disease resistance. Exogenous application of SA at non-toxic concentrations to susceptible plants could enhance resistance to pathogens (Murphy et al., 2000; Gális et al., 2004).

In a previous study, we observed the induced resistance of *Pichia membranefaciens* and SA against postharvest diseases. Significant changes in polyphenoloxidase (PPO, EC 1.10.3.1), peroxidase (POD, EC 1.11.1.7), phenylalanine

* Corresponding author. Tel.: +86 10 6283 6559; fax: +86 10 8259 4675.
E-mail address: tsp@ibcas.ac.cn (S. Tian).

0925-5214/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.postharvbio.2005.10.009

ammonia-lyase (PAL, EC 4.3.1.5), and β-1, 3-glucanase activities were found to be involved in the action (Fan and Tian, 2000; Qin et al., 2002, 2003; Yao and Tian, 2005). To date, although the interaction between SA and active oxygen has been characterized (Lamb and Dixon, 1997), little information is available on whether the positive effect of antagonistic yeasts on postharvest diseases is mediated by an antioxidant system. The objective of this study was to determine the relationship between antioxidant enzymes and host resistance induced by *P. membranefaciens* and SA. The time-course and magnitude of CAT (EC 1.11.1.6), POD and superoxide dismutase (SOD, EC 1.15.1.1) have been investigated after treatment with SA or antagonists in sweet cherry. Additionally, total protein metabolism was assayed.

2. Materials and methods

2.1. Fruit material

Sweet cherry (*Prunus avivum* L. cv. Hongdeng) fruit were harvested at commercial maturity from the experiment orchard of the Institute of Forest and Fruit, Beijing Academy of Agricultural Sciences in Beijing. Fruit were directly transported to our laboratory and sorted based on size and the absence of physical injuries or infections. Sweet cherries were disinfected with 2% (v/v) sodium hypochlorite for 2 min, washed with tap water, and air-dried prior to use.

2.2. Antagonistic yeast and pathogen

P. membranefaciens was isolated from the wounds of peach fruit in our previous work (Fan and Tian, 2000) following the method of Wilson and Chalutz (1989) and identified by CABI Bioscience Identification Services (International Mycological Institute, UK). The yeast was cultured in 250 ml conical flasks containing 50 ml of nutrient yeast dextrose broth (NYDB: 1 g of beef extract, 10 g of glucose, 5 g of soya peptone, 5 g of NaCl, and 5 g of yeast extract in 1000 ml water) on a rotary shaker at 200 rpm for 48 h at 28 °C. Yeast cells were centrifuged at 3365 × *g* for 10 min, resuspended in sterile distilled water, and adjusted to a concentration of 5×10^7 cells ml^{-1} with a haemocytometer.

Penicillium expansum was obtained from infected apple fruit and cultured on Potato Dextrose Agar (PDA) for 14 days at 25 °C. A spore suspension of the pathogen was prepared by flooding PDA cultures with sterile distilled water containing 0.05% Tween 80. Spore concentration of the pathogen was adjusted to 1×10^4 spores ml^{-1} with sterile distilled water, using a haemocytometer.

2.3. Antagonistic yeast and SA treatments

Sweet cherries were immersed in the suspension of antagonistic *P. membranefaciens* at 5×10^7 cells ml^{-1} or SA solution at 0.5 mM for 10 min according to our previous experiments (Qin et al., 2003). Fruit treated with sterile distilled water were used as the controls. All treated fruit were then divided into three groups. The first group was dried in the air and directly put into 200 mm × 130 mm × 50 mm plastic boxes with plastic film to maintain a high relative humidity (about 95%), then stored at 25 °C. The second and third groups were air-dried for 2 h, and then a uniform 4 mm deep × 3 mm wide wound was made at the equator of fruit using a sterile nail. Aliquots of 15 μl of 1×10^4 spores ml^{-1} *P. expansum* were inoculated into each wound site. After air-drying, all fruit were put in 200 mm × 130 mm × 50 mm plastic boxes as described above. Each treatment contained three replicates of 10 fruit and the entire experiment was repeated twice.

2.4. Evaluation of fruit decay

Fruit with pathogen inoculation from the third group were used for decay evaluation. Disease incidence and lesion diameters caused by *P. expansum* were determined at 24, 48, and 72 h after treatment. Fruit with no infection were not counted for lesion size measurements. There were three replicates in each treatment, and the experiment was conducted twice.

2.5. CAT, POD and SOD enzyme assay

At various time intervals (0, 24, 48, and 72 h) after treatment, flesh samples from 10 fruit in the first and second groups were obtained for the enzyme assays and protein extraction. A flesh sample of 5 g from the middle part (the first group) or near the wound (the second group) was collected using a sampler (5 mm deep and 7 mm in diameter) in each treatment and homogenized in 25 ml of ice-cold sodium phosphate buffer (50 mM, pH 7.8) and 0.5 g polyvinyl pyrrolidone (PVPP) with a Kinematica tissue grinder (Crl-6010, Kriens-LU, Switzerland). The homogenate was centrifuged at 6730 × *g* for 40 min at 4 °C and the resulting supernatants were used directly for enzyme assays. There were three replicates in each treatment for enzyme assays, and the experiment was conducted twice.

CAT activity was determined by adding 0.2 ml of enzyme preparation to 2.8 ml of 40 mM H_2O_2 (dissolved with 50 mM sodium phosphate buffer, pH 7.0) as a substrate (Wang et al., 2004). The decomposition of H_2O_2 was measured by the decline in absorbance at 240 nm with UV-160 spectrophotometer (Shimadzu, Japan). The specific activity was expressed in units per mg protein, where one unit of catalase converts 1 μmol of H_2O_2 per minute.

POD activity was analyzed using guaiacol as substrate (Yao and Tian, 2005). The reaction mixture consisting of 0.5 ml of crude extract, 2 ml of guaiacol substrate (100 mM sodium phosphate, pH 6.4 and 8 mM guaiacol) was incubated for 5 min at 30 °C. The increase in absorbance at 460 nm was spectrophotometrically assayed after 1 ml H_2O_2 (24 mM) was added. Enzymatic activities were defined as the increase