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Effect of preharvest application of *Hanseniaspora uvarum* on postharvest diseases in strawberries

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ABSTRACT

This study was conducted to evaluate the efficacy of preharvest applications of *Hanseniaspora uvarum* against postharvest mold decay of strawberry (*Fragaria ananassa*) fruit and quality maintenance during storage at $2 \pm 1\,^{\circ}C$ and 90–95% RH. Results showed that the treatment significantly reduced postharvest mold decay ($P < 0.05$), maintained fruit firmness and total soluble solids content, and did not impair pH and surface color during postharvest storage. Morever, it was found that the activities of some defense-related enzymes, peroxidase, superoxide dismutase, catalase, polyphenoloxidase, phenylalanine ammonia-lyase, β-1,3-glucanase and ascorbate peroxidase, increased and malondialdehyde content, a decomposition product of polyunsaturated fatty acid hydroperoxides, was reduced, in response to preharvest application of *H. uvarum*. Overall, preharvest application of *H. uvarum* has potential for controlling postharvest decay of strawberry.

1. Introduction

In recent years, the use of biocontrol agents has become a new way for controlling postharvest diseases of fruit and vegetables. Among them, antagonistic yeasts are attracting research focus, with their wide antimicrobial spectrum, good antagonitic effects, genetic stability, low nutrition requirements and high security (Fan et al., 2009a; Zong et al., 2010; Mekbib et al., 2011; Yu et al., 2012). Yeasts that are naturally present on. fruit surfaces represent the major group and they have also been isolated from other sources, such as the phyllosphere, roots, soil, and sea water (Liu et al., 2013). The mechanisms of action of most biocontrol agents of postharvest diseases are poorly understood and it is generally assumed that they involve a complex interaction between host, pathogen, antagonists and environment (Nunes, 2012). The basic mechanism of action for most of antagonistic yeasts is competition for available nutrients and space (Liu et al., 2013), which often occurs in the first 24 h after the yeast cells come into contact with fruit surface. Other modes of action of yeast antagonists against specific fungal pathogens are: induction of host defense (Zheng and Chen, 2009; Xu et al., 2013), attachment and lytic enzyme secretion (Chan and Tian, 2005), adjustment of population density (McGuire, 2000; Fiori et al., 2012), morphology change (Fiori et al., 2012), reactive oxygen species (ROS) tolerance (Liu et al., 2011a), iron depletion (Saravanakumar et al., 2008), alleviation of oxidative damage of the fruit host (Xu et al., 2008), and induction of ROS production in the host (Macarisin et al., 2010).

Strawberry (*Fragaria* × *ananassa Duch.*) is a non-climacteric fruit with a very short postharvest life. Loss of quality in this fruit is mostly due to its relatively high metabolic activity and sensitivity to fungal decay, and the decay is mainly caused by gray mold (*Botrytis cinerea* Pers.) and rhizopus rot (*Rhizopus stolonifer* (Ehrenb.) Vuill.) (Romanazzi et al., 2001). Strawberry is also susceptible to water loss, bruising and mechanical injuries due to their soft texture and lack of a protective rind (Hernández-Muñoz et al., 2006). Several antagonistic yeasts against fruit rot in strawberry have been studied. El-Neshawy and Shetaia (2003) found the yeast *Candida oleophila* followed by *Candida fructus* provided significant decay control, with the greatest effect from *C. fructus* in restricting visual rating of mold development on strawberry and maintaining quality parameters, including firmness, soluble solid content, anthocyanin content, titratable acidity (TA), pH and surface color. Fan et al. (2009b) found a novel edible bio-film containing *Cryptococcus laurentii* significantly reduced microbial decay, decreased weight loss and maintained the firmness of strawberries. Karabulut et al. (2004) tested the yeast *Metschnikowia fructicola* for control of preharvest and postharvest rots of strawberry fruit and found it reduced the incidence of fruit rot significantly. Long and Yuan (2009) found preharvest treatment with the yeast *Kloeckera apiculata strain (34-9)* was the most effective to control *B. cinerea* on strawberry, while

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postharvest and sumilex treatment equally reduced the incidence of decay. Zhang et al. (2007a) sprayed strawberry fruit with the antagonist *Rhodotorula glutinis* and found the higher the concentration of the antagonist, the lower the disease incidence. Zhang et al. (2007b) also found that the yeast *C. laurentii* was effective in controlling Rhizopus rot of strawberries and if combined with short hot water dips, the effect would be greater.

Although most postharvest diseases appear in the packinghouse, infections often begin in the field (Palou et al., 2002). Preharvest application can enhance the biocontrol system, because it will allow the antagonist to have longer interaction with the pathogen and to colonize tissues before the arrival of the pathogen, such as happens in latent and incipient infections occurring through wounds resulting from the harvesting period (Nunes, 2012). Therefore, spraying antagonistic yeasts before harvest to suppress fruit and vegetables postharvest storage diseases will be one of the important areas of the application of antagonistic microorganisms. *Hanseniaspora uvarum* was an effective antagonist against gray mold of grape and had inhibitory effect on spore germination and lesion growth of *B. cinerea* and reduced the natural decay development of grape berries, and did not impair quality parameters (Liu et al., 2010b). The objective of the present work was to assess the efficacy of preharvest application of the antagonistic yeast *H. uvarum* in inducing resistance against postharvest diseases and its effects on quality parameters of strawberries during storage.

2. Materials and methods

2.1. Preparation of suspensions of antagonist

H. uvarum (CGMCC 2.3970), isolated from strawberry fruit and identified based on a similarity analysis of its physiological–biochemical characteristics and 26S rDNA D1/D2 domain sequence, was cultured in 250 mL Erlenmeyer flasks with 100 mL of PDB (1 L distilled water containing potato 200 g and glucose 20 g) on a gyratory shaker at 180 rpm for 24 h at 28 °C. The cells were harvested by centrifuging at 10,000 rpm for 15 min at 4 °C, then washed with sterile-distilled water two times and resuspended in sterile distilled water. Cells concentration was counted with a haemocytometer and diluted to 1×10^8 CFU mL^{-1} with sterile-distilled water containing 0.05% Tween-20 as required.

2.2. Preharvest treatment of strawberry

Strawberries, cv. HongYan, were grown in a greenhouse located in Yuhua district, Nanjing city, Jiangsu province, China. Diurnal temperature in the greenhouses ranges from 8 °C to 25 °C. At 3 days before harvest in December, the fruit were sprayed with distilled water (as the control) and the yeast cell suspensions (1×10^8 CFU mL^{-1}) using a 500 mL watering can, until all fruit were wet to run-off. The watering can was soaked with 75% alcohol for 10 min and dried out an a clean bench before using. To ensure sufficient number of fruit for each treatment, no less than 400 were given different treatments and were separated by non-treated fruit. Commercially mature fruit were harvested after three days. Fruit free of wounds and homogeneous in maturity and size were selected, and then placed in polyethylene-lined plastic boxes and transported to storage room at 2 ± 1 °C and 90–95% RH. Each treatment contained three replications with 90 single berries per replication.

2.3. Effect of antagonist H. uvarum on decay index of strawberries

Fruit decay was determined on 40 fruit per sample according to a five-point scale, where 0 = no decay, 1 = very slight decay, covering <10% of the fruit surface, 2 = slight decay, covering >10% but <25% of the fruit surface, 3 = moderate decay, covering >25% but <40% of the fruit surface, and 4 = severe decay, covering >40% of the fruit surface. The decay index was calculated using the following formula:

$$\text{Decay index} = \frac{(1 \times N_1 + 2 \times N_2 + 3 \times N_3 + 4 \times N_4)}{4 \times N}$$

where N was the total number of fruit measured and N_1, N_2, N_3 and N_4 were the numbers of fruit showing the different severities of decay.

2.4. Effect of antagonist H. uvarum on fruit quality

Quality parameters were measured after storage on three replicates of ten fruit each. The testing methods are described below:

Fruit firmness was measured at two points on the equatorial region by using a TA-XT2i texture analyzer (Stable Micro Systems Ltd., UK) with a P_{50} cylinder plunger probe and the maximum force was recorded as fruit firmness (N). Test conditions used for measurements were: pre-test speed of 5.0 mm s^{-1}, test speed of 1.0 mm s^{-1}, and post-test speed of 5.0 mm s^{-1}, penetration distance of 4 mm.

Total soluble solids (TSS) were determined by measuring the refractive index of the strawberry fruit juice with a hand-held refractometer (WYT-4, Top instrument Co., Ltd., China) and the results expressed as percentages (g per 100 g fruit weight).

Surface color was measured at two points around the equatorial zone of the fruit by a Minolta CR-400 Chronometer (Konica Minolta Sensing, Osaka, Japan), using the CIE (Commission International de l'Eclairage) color space L^*, a^* and b^* values. Values of lightness L^* (ranging from 0, black to 100 white) and a^* (positives values for red, negative values for green) were measured.

pH was measured by using a pH meter (DELTA 320, Top instrument Co., Ltd., China) and strawberry juice filtered through one layer of cheesecloth was prepared for the measurement.

2.5. Effect of antagonist H. uvarum on MDA and defense-related enzyme activities of strawberry

At various time intervals (0, 3, 6, 9,12 and 15 days) after harvest, fruit peels were removed from five fruit in each treatment using a sterile scalpel to cut off peel of about 2 mm thickness, the achenes were discarded, and the skinless pulp tissues were frozen until assayed.

2.5.1. MDA content

Lipid peroxidation was determined in terms of malondialdehyde (MDA) content by the TBA reaction as described by Du and Bramlage (1992). Tissues (1 g) prepared were ground with 7 mL (V_1) 5% (w/v) cold trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 rpm for 15 min at 4 °C. The solution contained 2 mL (V_2) of the resulting supernatant and 2 mL 0.67% (w/v) TBA was heated at 100 °C for 5 min, and quickly cooled in an ice-bath. The absorbance of the supernatants was recorded at A_{532} and A_{600}. MDA content was calculated according to the following formula: $C_{MDA}(\text{nmol g}^{-1}) = (A_{532} - A_{600}) \times V_1 / 1.55 \times 10^{-1} \times V_2 \times m$, where 1.55×10^{-1} was the molar extinction coefficient of MDA and m (g) was the weight of tissues prepared for measured.

2.5.2. SOD, POD and APX activity

For the determination of peroxidase (POD), superoxide dismutase (SOD) activity and ascorbate peroxidase (APX), skinless pulp tissues (2 g) prepared were ground with in 8 mL cold 50 mmol L^{-1} phosphate buffered saline solution (pH 7.8) containing 1% (w/v) polyvinyl–polypyrrolidone (PVPP). The homogenate was centrifuged at 10,000 rpm for 15 min at 4 °C, and 1 mL resulting supernatants were used as crude enzyme extracts for assaying