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A combination of marine yeast and food additive enhances preventive effects on postharvest decay of jujubes (*Zizyphus jujuba*)

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ABSTRACT

We investigated the effects of marine yeast *Rhodosporidium paludigenum* in combination with a food additive, carboxymethylcellulose sodium (CMC-Na), on prevention of postharvest decay and food quality of Chinese winter jujubes. *R. paludigenum* (1×10^8 cells/ml) combined with CMC-Na (0.3%) significantly increased the inhibition of black rot on jujubes at 25 °C when compared with *R. paludigenum*-alone treatment (5.8% vs. 20%, $p < 0.05$). The combination also reduced natural rot from 86% (control) to 56%. The combination caused transient changes in enzyme activities or contents of some oxidation reactive markers such as peroxidase (POD), superoxide dismutase (SOD), and malondialdehyde (MDA) of jujubes. The combination had no significant effect on the food qualities such as colour (chroma and hue angle), total soluble solid (TSS) and titratable acidity (TA) of the fruit. While enhancing these effects, CMC-Na did not affect the survival of *R. paludigenum* in nutrient yeast dextrose agar (NYDA) culture. Thus, we conclude that the combination of *R. paludigenum* and CMC-Na is a promising formulation to control postharvest decay of Chinese winter jujubes.

1. Introduction

Chinese winter jujube (*Zizyphus jujuba* Mill. cv. Dongzao) is a tasty and high nutritious fruit in China. It is rich in vitamin C and amino acids (Sun, Liu, Zhu, & Wang, 2007). Postharvest losses of Chinese winter jujubes are attributed to postharvest browning and decay caused by *Alternaria alternata* (Wang, Wang, Li, & Yi, 2005). Currently, the main preventive approaches for postharvest decay of the fruit are chemical fungicides and cold storage. Chemical fungicides not only cause health problems, but also result in pathogen resistance. Therefore, it is urgently needed to find alternative strategies for control of postharvest decay (Janisiewicz & Korsten, 2002). While cold storage can postpone postharvest decay, a cold chain system for postharvest fruit is not available everywhere in many countries including China (http://www.clb.org.cn/zt/cfls-zt/index.html). Hence, safer biocontrol agents have drawn wide attention. Amongst them, antagonistic yeasts have been regarded as one of the safe alternatives to fungicides (Droby, Wisniewski, Macarisin, & Wilson, 2009).

However, the effectiveness of yeast used alone on postharvest decay control is not comparable to fungicides under changeable postharvest environments (Tian, Qin, & Xu, 2004). More and more studies have been focusing on enhancing its pathogen-inhibition capability. Additives such as thickeners, stickers, diluents, and protectors have been incorporated into biocontrol agents to improve the biocontrol efficacy (Cañamás et al., 2008a). A great effort has also contributed to integrate food additives and antagonists in order to enhance biocontrol effects; however, scientific data in this area remain insufficient to support commercialisation (Cañamás et al., 2008b).

A good biocontrol agent requires multi-mechanisms of actions in antagonizing pathogens. The abilities to improve its stability in a real environment and to induce fruit resistance to pathogens are some other important factors. Induction of disease resistance responses by antagonistic yeasts has been reported in apples, pears, and many other fruits (Janisiewicz & Korsten, 2002). Some strains were also reported to cause a transient increase or decrease in markers for oxidative stress such as peroxidase (POD), malondialdehyde (MDA), superoxide dismutase (SOD), and chitinase (Yu, Zhang, Li, & Zheng, 2008; Yu et al., 2007).

Yeast suspensions can naturally precipitate, which affects their applications in postharvest protection of fruit. It is, therefore, necessary to develop a formulation to enhance the stability of biocontrol agent suspensions (Droby et al., 2009). Carboxymethylcellulose sodium (CMC-Na) is a traditional food additive to enhance the stability of suspensions in food industry. It is also a main composition of edible packaging film, which was widely used in fruit preservation. Bancroft reported that a fruit coating (TAL Pro-long) containing CMC-Na applied with biocontrol agents reduced fruit decay (Bancroft, 1995). Unfortunately, there is little information about the influence of CMC-Na alone on antagonistic yeasts.

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To our best knowledge, no information is available about the role of a combination of *R. paludigenum* and CMC-Na on prevention of postharvest decay and food quality of fruit. The purpose of this study was to examine the effect of *R. paludigenum* in combination with CMC-Na in controlling decay development of Chinese winter jujubes and the influence of such a combination on fruit colour and quality.

2. Materials and methods

2.1. Preparation of R. paludigenum and CMC-Na

The marine yeast, *R. paludigenum*, was previously isolated from seawater (Wang et al., 2008). Yeast cells were grown in 50 ml of nutrient yeast dextrose broth (NYDB) in a 250-ml flask at 28 °C and 200 rpm for 24 h, and the fresh colonies were centrifuged (KA1000, Shanghai Anke, Shanghai, China) at 7000 g for 5 min and were resuspended twice in sterile distilled water to remove the fermentation liquid. *R. paludigenum* suspension was counted with a haemocytometer and adjusted with sterile distilled water or CMC-Na solutions (0.1% or 0.3%) to meet a final concentration of 1×10^8 cells/ml. CMC-Na (Zhengjian ShengXiao Chemicals CO., Ltd.) solutions (0%, 0.1%, and 0.3%) were prepared with sterile distilled water and filtered through a 0.2-μm polycarbonate membrane filter.

2.2. Fruit and pathogen

Chinese winter jujubes (*Zizyphus jujuba* Mill. cv. Dongzao) were obtained from a orchard in Tianjin (China) and used immediately after arrived in the laboratory. For each experiment, jujubes without any injury and with similar size and in green ripeness stage were selected. The pathogen strain *A. alternata* (CGMCC 3.4578) was purchased from IMCAS (Institute of Microbiology, Chinese Academy of Sciences, China). *A. alternata* was maintained on potato dextrose agar (PDA) at 28 °C for 1–2 weeks. Spores were gently rubbed off the potato dextrose agar (PDA) medium with a sterile glass rod and then suspended in sterile distilled water. The spore suspension was determined by using a haemocytometer and diluted with sterile distilled water to obtain a final concentration of 5×10^4 spores/ml (Wang et al., 2009).

2.3. Evaluation of the combination treatment on inhibition of black rot

Jujubes were dipped into 0.5% sodium hypochlorite solution for 30 s, washed with running water, air-dried, and then wounded with a sterile cork-borer (about 5 mm in diameter and 3 mm deep). The jujubes were treated with (A) 0.3% CMC-Na, (B) 1×10^8 cells/ml *R. paludigenum* (C) 1×10^8 cells/ml *R. paludigenum* suspended in 0.3% CMC-Na, and sterile distilled water was used as control. An aliquot of 30 μl of these treatments was piped into each wound. After 4 h, each wound was inoculated with 15 μl of *A. alternata* (5×10^4 spores/ml). After air-drying, samples were put in a plastic box ($40 \times 20 \times 8$ cm), packaged with polyethylene bags to maintain high humidity, and kept in darkness at 25 °C. The number of the infected fruit wounds was examined after a 5-day storage. Each treatment contained 20 samples. The experiment was repeated twice and each treatment was comprised of three replicates.

2.4. Evaluation of the combination treatment on inhibition of natural rot

To investigate the influence of the combination of *R. paludigenum* and CMC-Na on the control of natural decay development, 50 intact fresh jujubes were dipped into various treatments at room temperature for 30 s. After air-drying, samples were placed in a plastic box, wraped in polyethylene bags, and stored in darkness at 25 °C for 10 days. Rot classification of the fruit is expressed in four ranks: 0 (no rot), 1 (⩽25%), 2 (25–50%), 3 (⩾50%). The rot index was calculated with the formula: $\sum$ (Rank × Quantity)/$4 \times 50 \times 100\%$. Three replicates were used for each treatment and the experiment was repeated twice.

2.5. Evaluation of the combination treatment on the fruit quality

2.5.1. Fruit colour

Before treatment and after storage, colour parameters of fruit surface were tested with a chromameter (WSC-S, Shanghai, China). Each sample was examined at the opposite sites around the equatorial region and average values were recorded. Eight samples were randomly selected for each treatment and a^*, b^* and L^* readings were obtained. Values a^* and b^* were converted into C* (Chroma), and h° (hue angle) (Lancaster, Lister, Reay, & Triggs, 1997). Three replicates were used for each treatment and the experiment was repeated twice.

2.5.2. Other markers for fruit quality

For total soluble solid (TSS) and titratable acidity (TA) analyses, juice was extracted from six fruits in each treatment. TSS content was tested with a WYT-4 refractometer (Quanzhou Zhongyou Optical Instrument Co., Ltd., China). TA content was measured based on the method described by Zhang et al. (2008) and expressed as a concentration of malic acid. Ascorbic acid (AA) content was determined directly by the method of AOAC (Association of Official Analytical Chemists) procedures (AOAC, 1995). Three replicates were used for each treatment and the experiment was repeated twice.

2.6. Influence of the combination treatment on enzyme activities of jujubes

To examine whether the combination treatment of *R. paludigenum* and CMC-Na affects the oxidative reaction markers of jujubes, activities of peroxidase (POD) and superoxide dismutase (SOD) in jujubes were tested according to Yu et al. (2008) with slight modification. Pericarp tissues (3 g) from six fruits of each treatment were taken at various time intervals (0, 1, 2, 3, 5, and 7 d), mashed in 5 ml of 0.05 mM sodium phosphate buffer (pH 7.8) containing 1% (w/v) polyvinyl-polypyrrolidone at 4 °C. After centrifuging at 12,000*g* and 4 °C for 10 min, the supernatant was collected as the crude enzyme extract for assay. With guaiacol as a substrate, POD activity was measured in a reaction mixture containing 3 ml of 50 mM sodium phosphate buffer (pH 7.8), 220 μl of 0.3% (v/v) guaiacol, and 20 μl of crude enzyme extract. The absorbance at 470 nm was measured every 30 s after 60 μl of 0.3% (v/v) H_2O_2 was added (Spectra max plus 384, Molecular Devices Limited, USA). One unit of enzyme activity is defined as the amount of a change of 0.01 in absorbance per min. For SOD activity assay, the 3 ml reaction mixture contained 50 mM sodium phosphate butter (pH 7.8), 12.37 mM methionine, 1.33 mM EDTA, 71.3 μM nitroblue tetrazolium, 2 μM riboflavin, and 50 μl of crude enzyme extract. Samples were illuminated under two 15-W fluorescent lamps and the assay was stopped by turning off the light. Non-illuminated samples were used as control. The absorbance at 560 nm was read and one unit of SOD is defined as the amount of enzyme that inhibits NBT reduction by 50%. Three replicates were used for each treatment and the experiment was repeated twice.