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Effect of sodium dehydroacetate on the development of sour rot on Satsuma mandarin

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ABSTRACT

Sour rot, caused by *Geotrichum citri-aurantii*, is one of the most devastating diseases in citrus fruits. This disease is difficult to control due to the lack of registered fungicides. In this study, sodium dehydroacetate (SD), a common food preservative, was evaluated to control postharvest sour rot of citrus fruits through *in vivo* and *in vitro* experiments. Results demonstrated that SD dose-dependently inhibited the mycelial growth of *G. citri-aurantii*, with a minimum inhibitory concentration (MIC) and a minimum fungicidal concentration (MFC) of both 0.80 g/L. The application of various SD concentrations (1 × , 2 × , and 4 × MFC) to citrus fruits inoculated with *G. citri-aurantii* significantly ($P < 0.05$) decreased the incidence of sour rot during the entire storage period. After 8 d of storage, the decay incidences in SD (4 × , 2 × or 1 × MFC)-treated fruits were only 10%, 30%, 60%, respectively, in contrast to 100% of the control fruits. In addition, SD treatment induced an increase in the activities of SOD, POD, and PAL but not CAT. Meanwhile, SD treatment retained the fruit quality of citrus because it has no negative effect on pH, coloration index, total soluble solids, vitamin C content, firmness, and the weight loss rate. Our results suggest that SD can be considered as a good alternative to conventional fungicides in controlling the decay of citrus fruits.

1. Introduction

Fungal diseases are the major cause of postharvest rots of fresh citrus fruits during storage and transport. Sour rot, caused by *Geotrichum citri-aurantii*, despite less common than green mold and blue mold, has been reported as an important postharvest disease of citrus fruits from most areas of the world, especially during wet and rainfall seasons (Feng, Wu, Li, Jiang, & Duan, 2011; Karim et al., 2016; Talibi et al., 2012a,b; Zhou, Tao, & Jia, 2014). This pathogen cannot be efficiently controlled by registered fungicides widely used in controlling green mold or blue mold. The application of sodium *o*-phenylphenate was found to be effective against *Geotrichum citriaurantii*, whereas its current use has been limited due to some adverse damages toward the fruits (Regnier, Combrinck, Veldman, & Du Plooy, 2014). Furthermore, the use of fungicides is increasingly becoming restricted owing to stringent regulation, carcinogenicity, high and acute residual toxicity, long degradation period, environmental pollution, and growing public concern about chemical residual in fruits (Du Plooy, Regnier, & Combrinck, 2009; Talibi, Boubaker, Boudyach, & Ait Ben Aoumar, 2014). Therefore, there is a need for alternative approaches for effective management of sour rot of citrus fruits.

Recently, some naturally occurring substances, such as essential oil or their volatile compounds, has emerged as an effective and safe strategy to control postharvest citrus sour rot due to their notable antifungal activity (Liu et al., 2009; Regnier et al., 2014; Talibi et al., 2012a; Wuryatmo, Able, Ford, & Scott, 2014; Zhou et al., 2014). Some plant extracts were also demonstrated to be effective in inhibiting the mycelial growth or spore germination of *G. citri-aurantii* (Hao, Li, Hu, Yang, & Rizwan-ul-Haq, 2011; Karim et al., 2016). However, low efficacies, instability or complexity of operation for these approaches limits their use (Feng et al., 2011).

In the past decades, some organic and inorganic salts serving as commercial food additives have been determined to be efficient in controlling green mold or blue mold of citrus fruits (Palou, Usall, Smilanick, Aguilar, & Vinas, 2002; Palou, Smilanick, & Droby, 2008; Valencia-Chamorro, Palou, Del Rio, & Pérez-Gago, 2011; Youssef, Ligorio, Nigro, & Ippolito, 2012a; Youssef, Ligorio, Sanzani, Nigro, & Ippolito, 2012b; Youssef, Sanzani, Ligorio, Ippolito, & Terry, 2014). In another report, immersing citrus fruits in 1% potassium

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sorbate or sodium bicarbonate solutions at 50 °C for 30 s could also reduce the sour rot from 94.5% to 37.0% and 15.7%, respectively (Smilanick, Mansour, Gabler, & Sorenson, 2008). Therefore, these types of substances might be a good alternative to conventional fungicides in controlling postharvest disease of citrus fruits.

Sodium dehydroacetate (SD) is a novel safe food preservative widely used in food industry (Yan, Zhang, Dong, Hou, & Guo, 2013). Previously, we have observed that it dramatically inhibited the mycelial growths of *Penicillium digitatum* and *Penicillium italicum in vitro*, with the MFC values of 0.40 g/L. *In vivo* tests demonstrated that various SD concentrations (2 ×, 4 ×, and 8 × MFC) significantly reduced the incidence of green and blue molds in citrus fruits up to 3 and 5 d at 25 ± 2 °C, respectively. Meanwhile, it had minor effect on fruit quality except a reduction in weight loss rate (Duan, Jing, Fan, & Tao, 2016). To the best of our knowledge, however, limited information is available regarding the effect of SD on sour rot of citrus. Therefore, the objective of present work is to evaluate the effectiveness of SD in controlling postharvest citrus sour rot through *in vitro* and *in vivo* experiments. The effects of SD treatment on fruit quality and the activities of some antioxidant enzymes [e.g., catalase (CAT), superoxide dismutase (SOD), as well as peroxidase (POD)] and defense-related enzymes [e.g., phenylalanine ammonia lyase (PAL)] will also be determined.

2. Materials and methods

2.1. Chemicals

Sodium dehydroacetate (Dehydroacetic acid sodium salt, 99%) was obtained from Crystal Pure Biochemical Technology Co., Ltd. (Shanghai, China).

2.2. Fungal strain

The fungal pathogen *G. citri-aurantii* used in this study was isolated from infected citrus fruits (Zhou et al., 2014) and preserved on potato dextrose agar (PDA) at 28 ± 2 °C.

2.3. Fruits

The mature fruits of Satsuma mandarin (*Citrus unshiu* Marc. cv. Miyagawa Wase) were harvested on November 4, 2014 from a local orchard near Xiangtan University, China. Defect-free fruits with uniform sizes were chosen for the experiments.

2.4. In vitro experiments

The effects of SD on mycelial growth of *G. citri-aurantii* were evaluated by the poisoned food technique (Tao, Fan, Jia, & Zhang, 2014) with some modifications. An aliquot of 10 μL SD stock solution was added to each sterilized PDA medium to generate a final concentration of 0, 0.05, 0.10, 0.20, 0.40, 0.80, and 1.60 g/L. These amended PDA media were immediately poured into sterilized Petri dishes (90 mm in diameter). Then a 6 mm diameter disc of inoculum, taken from the edge of 7-d-old cultures of *G. citri-aurantii*, was placed at the center of each Petri dish. The plates were cultured for 5 d at 28 ± 2 °C. The diameter (mm) of colony zone was measured using a G102-123-101 caliper (Shanghai Measuring and Counting Tools Co., Ltd, Shanghai, China). All of the tests were performed in triplicate. The lowest concentration that completely prevented the growth of the fungus after 48 h of incubation at 28 ± 2 °C was regarded as the minimum inhibitory concentration (MIC) (Zhou et al., 2014). The minimum fungicidal concentration (MFC) was defined as the lowest concentration that inhibited pathogen growth after 96 h of incubation, indicating fungicidal activity >99.5% of the original inoculums (Talibi et al., 2012a).

2.5. In vivo assays

For *in vivo* assays, all fresh citrus fruits were surface-sterilized by immersing in 1% (v/v) sodium hypochlorite solution for 2 min, rinsing twice in sterile distilled water, and then drying in ambient air. Thereafter, two wounds (length of 3 mm and depth of 3 mm) were made evenly around each fruit equator using a sterilized scalpel. The spore suspension of *G. citri-aurantii* was prepared and adjusted to 1×10^7 spore/mL by using a hemocytometer with sterile distilled water. Each incision was inoculated with 20 μL of conidial suspension (Chen & Zhu, 2011). Fruits were then stored at room temperature for 4 h. The fruits were immersed in various SD solutions (1 ×, 2 × or 4 × MFC) for 30 s and stored in a sealed incubator at 25 ± 2 °C and 85–90% relative humidity (RH). A single replicate comprised 30 fruits, and each treatment was performed in triplicate. The incidence rate of each treatment during storage time was measured by counting the number of sour rot contaminated fruits by the following formula:

Disease incidence (%) = (number of rotten fruits/ number of total fruits) × 100

2.6. Fruit quality parameters

After storage at an interval of 2 d, fruit pulp samples were collected from three fruits randomly chosen from each group. The pH value was determined by a Delta-320 pH-meter (MettlerToledo, Greifensee, Switzerland). Total soluble solid (TSS) content was analyzed using a LB 32T hand refractometer (Mingrui Electron Science-Technology Co., Ltd, Guangzhou, China). Vitamin C (Vc) content was determined by 2, 6-dichlorophenolindor-henol titration (Tao et al., 2014). Firmness was determined by a fruit hardness tester (Mingrui Electron Science-Technology Co., Ltd, Guangzhou, China). The coloration index was determined by using a Minolta CR-330 chromameter (Minolta Co., Ltd., Osaka, Japan) (Fan, Tao, Jia, & He, 2014). Fruit weight loss rate was determined in lots of 30 fruits per treatment. The fruits were weighted on the day of harvest and at each storage period. Cumulative weight loss rates were expressed as percentage of initial weight loss.

2.7. Determining CAT, SOD, POD, and PAL activities

The fruit peel was homogenized in a grinder and used for further analysis. All of the experiments were conducted in triplicate for each treatment per sample. All the enzyme activities were determined by photometric assay using a UV-2450 UV/Vis spectrophotometer [Shimadzu (China) Co., Ltd., Shanghai, China]. CAT and POD activities were estimated using our previously described method (Lemoine, Chaves, & Martínez, 2010). SOD and PAL activities were measured according to the method proposed by Sellamuthu, Sivakumar, Soundy, and Korsten (2013). The specific activities of the targeted enzymes were expressed in U/g fresh weight (FW).

2.8. Statistical analysis

Each assay was performed in triplicate, and the data were processed by an analysis of variance (ANOVA). Daily analysis results of the treatments were compared at $P = 0.05$ according to Duncan's multiple range tests.