Postharvest Biology and Technology 88 (2014) 96–102

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Combination of hot water, *Bacillus amyloliquefaciens* HF-01 and sodium bicarbonate treatments to control postharvest decay of mandarin fruit

Peng Hong [1], Weining Hao [1], Jianjun Luo, Shaohua Chen, Meiying Hu [*], Guohua Zhong [*,1]

Key Laboratory of Natural Pesticide and Chemical Biology, Ministry of Education, South China Agricultural University, Guangzhou 510642, PR China

ARTICLE INFO

Article history:
Received 15 August 2013
Accepted 19 October 2013

Keywords:
Bacillus amyloliquefaciens
Mandarin fruit
Biocontrol
Sodium bicarbonate
Hot water treatment
Postharvest diseases

ABSTRACT

An antagonistic isolate *Bacillus amyloliquefaciens* HF-01, sodium bicarbonate (SBC) and hot water treatment (HW) were investigated individually and in combination against green and blue mold and sour rot caused by *Penicillium digitatum*, *P. italicum* and *Geotrichum citri-aurantii* respectively, in mandarin fruit. Populations of antagonists were stable in the presence of 1% or 2% SBC treatment, and spore germination of pathogens in potato dextrose broth was greatly controlled by the hot water treatment of 45 °C for 2 min. Individual application of sodium bicarbonate at low rates and hot water treatment, although reducing disease incidence after 8 weeks or 4 weeks of storage at 6 °C or 25 °C respectively, was not as effective as the fungicide treatment. The treatment comprising *B. amyloliquefaciens* combined with 2% SBC or/and HW (45 °C for 2 min) was as effective as the fungicide treatment and reduced decay to less than 80% compared to the control. *B. amyloliquefaciens* HF-01 alone or in combination with 2% SBC or/and HW significantly reduced postharvest decay without impairing fruit quality after storage at 25 °C for 4 weeks or at 6 °C for 8 weeks. These results suggest that the combination of *B. amyloliquefaciens* HF-01, SBC and HW could be a promising method for the control of postharvest decay on citrus while maintaining fruit quality after harvest.

1. Introduction

'Wuzishatangju' mandarin is a citrus cultivar derived from a bud sport variation of the seedy 'Shatangju' cultivar found in Guangdong Province, South China in the 1980s (Ye et al., 2006). Significant postharvest decay in *Citrus reticulate* (mandarin) can occur in arid and sub-tropical climates primarily due to green mold, caused by *Penicillium digitatum*, and secondarily by blue mold and sour rot caused by *P. italicum* and *Geotrichum citri-aurantii* (*syn. G. candidum*), respectively (Palou et al., 2001). Synthetic fungicides such as imazalil, thiabendazole, pyrimethanil, and prochloraz are generally used on packing as the first line of defense against postharvest green and blue molds of citrus. But within a few years of their introduction, resistance to such fungicides is very common and thus compromises their efficacy (Mavroeidi and Shaw, 2005). Therefore, resistance has become an important factor in limiting the effectiveness and useful lifetime of fungicides, which are being developed at increasingly high costs (Kinay et al., 2007). Meanwhile, the use of synthetic fungicides is also becoming more restricted due to health and environmental concerns, thus it is necessary to develop alternative treatments for replacing fungicides and avoiding environmental risk and satisfying consumer demands.

Biological control using microbial antagonists is increasingly becoming an effective alternative to chemical control, that shows effectiveness in controlling postharvest diseases of citrus (Lai et al., 2012). *Bacillus* spp. have been considered as potential biocontrol agents due to their high spore production ability, resistance and ability to survive desiccation, heat, UV irradiation, and organic solvents (Romero et al., 2007). Successful control of infections caused by a number of postharvest pathogens using *Bacillus* spp. and other biological control agents have been reported on mandarin fruit (Abraham et al., 2010; Arrebola et al., 2010a). Our previous study also showed that the strain HF-01 of the bacterium *Bacillus amyloliquefaciens*, isolated from mandarin fruit surfaces, is an effective antagonist to the major postharvest pathogens of citrus (Hao et al., 2011). Microbial antagonists when applied individually, usually do not bring about 100% control of postharvest diseases of fruit and vegetables, but their activity can be enhanced by manipulation of the environment, using mixtures of beneficial organisms, physiological and genetic enhancement of the biocontrol mechanisms, and integration of biocontrol with other methods such as low doses of fungicides and other chemicals (Qin and Tian, 2004). Biological

* Corresponding authors. Tel.: +86 20 85280308; fax: +86 20 85280292.
E-mail addresses: humy@scau.edu.cn (M. Hu), guohuazhong@scau.edu.cn (G. Zhong).
[1] Both authors contributed equally to this work.

0925-5214/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.postharvbio.2013.10.004

control agents in combination with selected chemicals such as calcium chloride (Tian et al., 2002), sodium bicarbonate (Obagwu and Korsten, 2003), ammonium molybdate (Wan and Tian, 2005), essential oils (Arrebola et al., 2010b), tea saponin (Hao et al., 2011), and chitin (Ahmed et al., 2003) have been demonstrated to provide synergistic effects in controlling fruit decay.

Sodium bicarbonate (SBC, $NaHCO_3$), is a good candidate used in combination with other chemical, physical, or biological methods for the integrated control of postharvest citrus diseases (Smilanick et al., 2005, 2006). In addition to considerable antimicrobial activity, SBC is inexpensive, readily available, and can be used to manage citrus postharvest decay with a minimal risk of injury to the fruit. SBC has also been found very successful when used with microbial antagonists such as *Pantoea agglomerans* (Torres et al., 2007; Usall et al., 2008), *Candida oleophila* (Porat et al., 2002), or *Bacillus subtilis* (Carla et al., 2010) for controlling postharvest diseases of fruit.

Hot water treatment, a completely safe technique based solely on the use of heat, has acquired increasing interest as a natural means to control fruit postharvest decay in recent years (Fallik, 2004). Integration of antagonists with hot water treatments could enhance the bioefficacy of microbial antagonists such as *B. subtilis* (Obagwu and Korsten, 2003), *Pseudomonas syringae* (Conway et al., 2005), *Cryptococcus laurentii* (Zhang et al., 2007b), *P. agglomerans* (Torres et al., 2007) and *Pichia guilliermondii* (Liu et al., 2010). However, the effect of this combined treatment on 'Wuzishatangju' Mandarin fruit has not yet been studied.

The objectives of this study were to: (1) evaluate the effect of SBC on the growth of *B. amyloliquefaciens in vivo*; (2) determine the efficacy of hot water against citrus green and blue mold and sour rot *in vitro*; (3) evaluate the influence of sodium bicarbonate or hot water treatment on the performance of *B. amyloliquefaciens* HF-01 against *P. italicum, P. digitatum and G. citri-aurantii*; and (4) determine the *in vivo* activity of combination treatments of *B. amyloliquefaciens* with sodium bicarbonate and/or hot water on the efficacy of inoculated and naturally infected 'Wuzishatangju' mandarin fruit in different storage conditions.

2. Materials and methods

2.1. Chemicals and fruit

Sodium bicarbonate (Guangzhou Chemical Reagent Company, China) was used at a concentration of 1%, 2% or 4% (weight/volume, w/v). Imazalil (95%, w/v, technical grade, Gengreen Ltd., China) was used at 300 $\mu g\,mL^{-1}$.

Mandarin (*Citrus reticulate* Blanco cv. Wuzishatangju) fruit used were harvested from an orchard of Sihui City, Guangdong Province, South China. Fruit were classified according to uniformity of size and maturity, without wounds or rots. All fruit were surface-disinfected by immersion for 2 min in 2% sodium hypochlorite, rinsed with tap water, and allowed to air-dry at room temperature (25 °C).

2.2. Pathogen culture

Three isolates were originally isolated from infected 'Wuzishatangju' mandarins from citrus packinghouses in Sihui, City. *P. italicum* and *P. digitatum* isolates were cultured for 1–2 weeks on potato dextrose agar (PDA) at 25 °C whereas *G. citri-aurantii* was cultured for 3 days on PDA at 25 °C. Conidia of *P. italicum*, *P. digitatum* and arthroconidia of *G. citri-aurantii* were collected by adding 5 mL of sterile, de-ionized water (diH_2O) containing 0.05% Triton X-100, to the Petri dish. Spore concentrations were determined with a haemocytometer, and adjusted to 1×10^8 conidia/arthroconidia mL^{-1} for the experiments.

2.3. Antagonist

The antagonist used in the study, *B. amyloliquefaciens* (HF-01), was originally isolated from the surface of mandarin (*C. reticulata* Blanco cv. Wuzishatangju) fruit. It was identified based on the carbon substrate oxidation pattern using the standard protocols (BIOLOG, Hayward, CA, USA) and 16S rDNA sequences analysis. The *B. amyloliquefaciens* HF-01 was cultured in 250 mL Erlenmeyer flasks containing 100 mL nutrient broth (NB) at 28 °C on a rotary shaker at 80 rpm for 48 h. Cells were harvested by centrifuging at 12,000 rpm for 20 min. The pellet was re-suspended in sterile distilled water and centrifuged for a second time. Washed cells (pellets) were suspended in quarter strength Ringer's (Merck) and adjusted to a concentration of 1×10^9 cells mL^{-1}. Required concentrations for different experiments were adjusted using a haemocytometer.

2.4. Effect of SBC on the population of B. amyloliquefaciens HF-01 in wounds

In this experiment, all fruit were wounded (2 mm wide and 2 mm long) on the equatorial zone with the tip of a sterile dissecting needle. Each wound was inoculated with 20 μL of *B. amyloliquefaciens* HF-01 at 1×10^7 cells mL^{-1}, individually or in combination with 1%, 2% or 4% SBC. Treated fruit were stored in air at 25 °C and 6 °C, respectively. Fruit samples were taken as described by Janisiewicz et al. (1992) at different time intervals after treatment, with some modifications. The resulting cylinders of excised tissue (5 mm deep × 5 mm wide) from five fruit were placed in a mortar with 5 mL of sterile distilled water and ground with a pestle. 100 μL of the serial 10-fold dilutions was plated on fresh NA plates. The plates were incubated at 28 °C for 72 h and the colonies were counted. Colony counts were expressed as $\log_{10}$ colony forming units (CFU) $wound^{-1}$. Each treatment was repeated three times and the entire experiment was performed twice.

2.5. Effect of antagonist and sodium bicarbonate in controlling pathogens in vivo

All fruit were wounded as described in Section 2.4. Each wound was inoculated with 20 μL of the treatment suspensions as follows: CK (sterile distilled water), *B. amyloliquefaciens* (1×10^8 cells mL^{-1}), *B. amyloliquefaciens* (1×10^8 cells mL^{-1}) + 2% SBC, 2% SBC, Imazalil (300 $\mu g\,mL^{-1}$). Three hours later, the fruit were inoculated with 15 μL of a spore suspension containing either 1×10^5 conidia mL^{-1} of *P. italicum*, *P. digitatum* or 1×10^5 arthroconidia mL^{-1} of *G. citri-aurantii*, respectively. After application as described above, all treated fruit were placed into trays and stored for 5 days at 25 °C. Disease incidence and lesion diameter were recorded after 5 days. Each treatment included three replicates of 20 fruit.

2.6. Effect of hot water treatment on spore germination of citrus pathogens in vitro

According to the experimental method of Zhang et al. (2008a), glass tubes containing 5 mL of potato-dextrose broth (PDB) had 100 μL aliquots of spore suspensions added containing either 5×10^7 conidia mL^{-1} of *P. italicum*, *P. digitatum* or 5×10^7 arthroconidia mL^{-1} of *G. citri-aurantii*, respectively. The treated tubes were put in a water bath with hot water treatments as follows: 40 °C for 1 min, 40 °C for 2 min, 45 °C for 1 min, 45 °C for 2 min, 50 °C for 1 min, 50 °C for 2 min and room temperature water for 2 min (CK). All tubes were normalized in water at room temperature immediately and then were put on a rotary shaker (75 rpm) at 25 °C for 18 h. Spores were considered to be germinated when germ tubes exceeded the spore diameter, and about 100 spores