Postharvest Biology and Technology 138 (2018) 46–55

Contents lists available at ScienceDirect

Postharvest Biology and Technology

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

Pseudomonas graminis strain CPA-7 differentially modulates the oxidative response in fresh-cut 'Golden delicious' apple depending on the storage conditions

Cyrelys Collazo[a], Jordi Giné-Bordonaba[b], Ingrid Aguiló-Aguayo[b], Ismael Povedano[a], Ariadna Bademunt[a], Inmaculada Viñas[a,*]

[a] Food Technology Department, University of Lleida, XaRTA-Postharvest, Agrotecnio Center, Rovira Roure 191, 25198 Lleida, Catalonia, Spain
[b] IRTA, XaRTA-Postharvest, Edifici Fruitcentre, Parc Científic i Tecnològic Agroalimentari de Lleida, Parc de Gardeny, 25003, Lleida, Catalonia, Spain

ARTICLE INFO

Keywords:
Antioxidant enzymes
Oxidative stress
Antagonist
Fruit defense response

ABSTRACT

The oxidative response in fresh-cut antioxidant-treated 'Golden delicious' apples during chilling storage was differentially modified by the biopreservative bacterium *Pseudomonas graminis* CPA-7 depending on the storage conditions (passive modified atmosphere packaging (MAP) or air). Results showed that inoculation with CPA-7 had no influence on fruit quality parameters in any of the conditions tested. During the first 24 h both in air and in MAP, ascorbate peroxidase (APX) activity triplicated the initial level in response to CPA-7, reaching up to 4-fold the activity of non-inoculated fruit (control). From 24 h of storage in MAP, polyphenol oxidase (PPO) activity was sharply enhanced in response to CPA-7 attaining values up to 8-fold higher than that of the control at the end of the experiment, yet it was not paired to an increase in browning incidence. Concomitantly, at 24 h of storage in MAP, CPA-7 suppressed peroxidase (POX) and catalase (CAT) activities. Subsequently, after 3 d in such conditions, superoxide dismutase (SOD) and PPO activities were almost duplicated in the presence of CPA-7 compared to the control. On the other hand, when stored in air, POX showed a biphasic induction in response to CPA-7 after 1 d and 6 d of incubation. On day 6, this enzyme duplicated its activity in inoculated samples compared to the control regardless of storage conditions. Inoculation with CPA-7 led to the slowdown of the decline of antioxidant capacity in air, which contrasted with the response upon MAP conditions. These results suggest that CPA-7 may trigger the activation of the fruit defense-response thereby mitigating its oxidative damage. Such activation may play a role as a putative biocontrol mechanism against foodborne pathogen infections.

1. Introduction

Given the growing demand for ready-to-eat products and the concern of consumers about the use of chlorine for decontamination in the food industry, the use of biocontrol agents (BCA) is a bio-friendly method which has been envisaged as an alternative (Belak and Maraz, 2015; Leverentz et al., 2006). However, more research is needed to enlarge the information about the mode of action of BCA in order to obtain their approval and registration for industrial application.

The processing of fresh produce not only increases the risk for contamination with human and plant pathogens but also impacts on physicochemical quality due to the disruption of constitutive barriers and the leakage of cell components. During this process, the cutting of fruit flesh compromise compartmentalization in nearby cells allowing phenolic compounds located in vacuoles to get in contact with polyphenol oxidase (PPO) located in plastids, triggering the reaction known as enzymatic browning (Holderbaum et al., 2010). Thus, ensuring the microbiological safety and physicochemical quality of fresh-cut products implies the need for combined strategies which may include biological (biocontrol agents), chemical (antimicrobial, anti-browning, texture-maintaining products) and physical methods (refrigerated storage and modified atmosphere packaging) which in turn, modulate fruit physiology and specifically, its oxidative metabolism (Reviewed by Parish et al., 2003; Spadaro and Droby, 2016).

Cold storage has shown to induce the accumulation of reactive oxygen species (ROS) in harvested fruit (Chiriboga et al., 2013). However, differential responses have been observed regarding the production of hydrogen peroxide (H_2O_2) and the induction of ROS scavenging enzymes in processed apples under chilling conditions according to the type of preservative chemical compound used

* Corresponding author.
E-mail address: ivinas@tecal.udl.cat (I. Viñas).

https://doi.org/10.1016/j.postharvbio.2017.12.013
Received 25 August 2017; Received in revised form 22 November 2017; Accepted 25 December 2017

(Larrigaudiere et al., 2008). Nevertheless, scarce information is available about the oxidative changes in fresh-cut fruit at the metabolic level after treatment with biocontrol agents.

Besides PPO, other antioxidant enzymes such as peroxidase (POX) have also been linked to polyphenol-associated browning through a coordinated mechanism involving the accumulation of POX substrate, hydrogen peroxide (H_2O_2), stimulated by the PPO-mediated generation of quinones (Jiang and Miles, 1993). Concomitantly, H_2O_2 is highly oxidant and may damage membrane and other cellular components through several mechanisms. Thus, in both, fresh-cut and intact fruit, its removal is rapidly activated in order to protect plant cells from these damages. H_2O_2- scavenging mechanisms include enzymatic reactions which involve superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) found in the cytosol, chloroplasts and mitochondria of higher plants (Hung et al., 2005) as well as non-enzymatic antioxidants including phenolic compounds (vitamin E, flavonoids, phenolic acids among others); nitrogen compounds (alkaloids, amino acids and amines), carotenoids and chlorophyll derivatives (Panda, 2012).

The control of enzymatic browning in the fresh- cut produce industry is currently mainly based on the modulation of PPO activity through antioxidant formulations that commonly contain reducing compounds such as ascorbic acid and its derivatives as well as cysteine and glutathione (Rupasinghe et al., 2005). These chemical compounds are hypothesized to control PPO activity either by reducing quinones to the native diphenols or by reacting irreversibly with o- quinones to form stable colourless products (Nicolas et al., 1994; He and Luo, 2007).

In addition, plant oxidative metabolism is also activated in response to biotic stress as a part of induced defense mechanisms (Pieterse et al., 2000, 2014). Under the light of the increasing knowledge about plant-microorganism interactions, induced resistance has arisen as a method for controlling postharvest diseases (Reviewed by Da Rocha and Hammerschmidt, 2005; Walters and Fountaine, 2009). Several studies have evidenced the protective effect of biotic agents applied in the roots (plant growth promoting rhizobacteria, PGPR) or in the green parts of the plant, through the activation of Induced Systemic Resistance (ISR) against plant pathogens invading distant organs (Attitalla et al., 2001; Verhagen et al., 2010). Moreover, the application of strategies combining antagonists and chemical or physical methods have shown to reduce disease severity to fungal plant pathogens in cherry tomato fruit and peach (Yao and Tian, 2005; Zhao et al., 2009) .The investigation of these processes has revealed that biocontrol agents (BCA) activate defense-related enzymes such as phenylalanine ammonia lyase (PAL), POX, PPO and chalcone synthase among others. BCA have also shown to stimulate oxidative burst and lignin deposition in mechanically damaged fruit (Chen et al., 2000; Valentines et al., 2005). The activation of such mechanisms might play an important role in priming fruit active defense response allowing higher tolerance to a subsequent pathogen attack (Alkan and Fortes, 2015; Jain et al., 2012; Lim et al., 2014).

Pseudomonas graminis strain CPA-7 is an aerobic epiphyte bacillus which was isolated from whole apple surface (Alegre et al., 2013b). Its efficacy as biocontrol agent against foodborne pathogens such as *Escherichia coli* O157:H7, *Salmonella enterica* and *Listeria monocytogenes* has been observed on fresh cut apples, peaches and melons (Abadias et al., 2014; Alegre et al., 2013a, 2013b). Although attempts to elucidate the mechanisms underlying its antagonistic activity have been carried out, they still remain poorly understood (Collazo et al., 2017). Thus, it was hypothesized that a putative mode of action for CPA-7 could be the activation of non-host pathogen oxidative-related defense mechanisms in fresh-cut fruit, which could eventually lead to the control of pathogens populations, as it has been reported for several BCA (Reviewed by Spadaro and Droby, 2016). Accordingly, enzymatic and non-enzymatic oxidative metabolism of fresh-cut apples treated with an anti-browning compound and inoculated with CPA-7 were investigated in two different scenarios: i) aerobic conditions, more suitable for the antagonist growth and ii) semi-commercial conditions (modified atmosphere packaging, MAP) more stressful for the antagonist growth.

2. Materials and methods

2.1. Fruit processing

Apples (*Malus domestica* L. cv. Golden Delicious) were obtained from local packing-houses (Lleida, Catalonia, Spain). Prior to experimental assays, the fruit were washed with running tap water, surface disinfected with 700 mL L^{-1} ethanol, peeled with an electric fruit peeler and cut into eight wedges with a handheld corer/slicer. Wedges were kept in chilled (5 °C) chlorinated tap water (pH 6) until subsequent treatment.

2.2. Antagonist culture conditions

For the antagonist inoculum preparation, a single colony of *Pseudomonas graminis* strain CPA-7, grown in tryptone soy agar plates (TSA, Biokar, Beauveais, France) during 48 h at 30 °C, was inoculated into 50 mL of tryptone soy broth (TSB, Biokar, Beauveais, France) and incubated in agitation (15.71 rad s^{-1}), in aerobic conditions at 25 °C for 24 h. Bacterial cells were harvested by centrifugation at 9800 × *g* for 10 min at 10 °C and re-suspended in sterile distilled water. The concentration of the suspension was checked by plate count of appropriate 10-fold dilutions in saline peptone (SP, 8.5 g L^{-1} NaCl, 1 g L^{-1} peptone) onto TSA plates after incubation at 30 °C for 48 h.

2.3. Inoculation, sampling and microbiological analysis

Suspensions of CPA-7 at a concentration of 10^7 CFU mL^{-1} were prepared in an antioxidant aqueous cold solution (4 °C) containing 60 g L^{-1} NatureSeal® AS1 (AgriCoat Ltd., Great Shefford, UK), a calcium ascorbate-based food grade antioxidant agent. Fruit wedges were dipped-inoculated at a ratio of 1:2 (weight of fruit: volume of suspension) for 2 min in agitation (15.71 rad s^{-1}) in a tabletop orbital shaker (Unimax 1010, Heidolph, Germany). Non-inoculated antioxidant solution was included as control treatment.

After the drainage of the excess of water, samples were packaged (120 g per replicate) in 400 mL polyethylene terephthalate trays (ShelfMaster™ Pronto™, PlusPack, Denmark). Each tray was considered as a replicate and three replicates per treatment and sampling time were used. Each tray was thermosealed with 181.7 cm^2 of a 3-holed (60 − 80 µm diameter, 75 mm spacing) multilayered microperforated film (PDS Group, Murcia, Spain) composed of polyester anti-fog film (OALF, 14 µm of thickness) + oriented polypropylene film (OPP, 20 µm of thickness), to achieve passive modified atmosphere. In a parallel set of trays the film was macro perforated (nine extra holes per tray) in order to attain aerobic conditions.

Trays were stored at 5 °C in darkness and biochemical and microbiological analyses were performed at 0, 1, 3 and 6 d post-treatment. For biochemical tests, about 70 g of each sample was frozen with liquid nitrogen, grinded in a commercial grinder (Minimoka 6R-020, Coffeemotion, Lleida, Spain) and stored at −80 °C until analysis. For microbiological analysis, 10 g of apple from each tray was homogenized in 90 mL of buffered peptone water (BPW, Biokar, Beauvais, France) and tested by viable cells count at each sampling time. In inoculated samples, CPA-7 populations were analyzed on TSA plates incubated at 30 °C for 48 h. In non-inoculated samples, total mesophilic bacteria populations were determined on plate count agar plates (PCA, Biokar, Beauveais, France) after incubation at 25 °C for 72 h.

2.4. Physicochemical quality parameters

Firmness, color and pH of fresh-cut wedges were determined initially and at each sampling time as described elsewhere (Alegre et al.,