Food Microbiology 33 (2013) 139–148

Contents lists available at SciVerse ScienceDirect

Food Microbiology

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

Antagonistic effect of *Pseudomonas graminis* CPA-7 against foodborne pathogens in fresh-cut apples under simulated commercial conditions

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ARTICLE INFO

Article history:
Received 6 April 2012
Received in revised form
10 September 2012
Accepted 18 September 2012
Available online 9 October 2012

Keywords:
Escherichia coli O157:H7
Salmonella
Listeria monocytogenes
Minimally processed apples
Biopreservation
Biocontrol

ABSTRACT

Recently, we reported that the application of the strain CPA-7 of *Pseudomonas graminis*, previously isolated from apple, could reduce the population of foodborne pathogens on minimally processed (MP) apples and peaches under laboratory conditions. Therefore, the objective of the present work was to find an antioxidant treatment and a packaging atmosphere condition to improve CPA-7 efficacy in reducing a cocktail of four *Salmonella* and five *Listeria monocytogenes* strains on MP apples under simulated commercial processing. The effect of CPA-7 application on apple quality and its survival to simulated gastric stress were also evaluated. Ascorbic acid (2%, w/v) and N-acetyl-L-cysteine (1%, w/v) as antioxidant treatments reduced *Salmonella*, *L. monocytogenes* and CPA-7 recovery, meanwhile no reduction was observed with NatureSeal® AS1 (NS, 6%, w/v). The antagonistic strain was effective on NS-treated apple wedges stored at 10 °C with or without modified atmosphere packaging (MAP). Then, in a semi-commercial assay, efficacy of CPA-7 inoculated at 10^5 and 10^7 cfu mL^{-1} against *Salmonella* and *L. monocytogenes* strains on MP apples with NS and MAP and stored at 5 and 10 °C was evaluated. Although high CPA-7 concentrations/populations avoided *Salmonella* growth at 10 °C and lowered *L. monocytogenes* population increases were observed at both temperatures, the effect was not instantaneous. No effect on apple quality was detected and CPA-7 did not survived to simulated gastric stress throughout storage. Therefore, CPA-7 could avoid pathogens growth on MP apples during storage when use as part of a hurdle technology in combination with disinfection techniques, low storage temperature and MAP.

1. Introduction

Recently, there has been an increasing market demand for minimally processed (MP) fruits and vegetables due to their fresh-like character, convenience, and human health benefits, and, in particular, fresh-cut apples have recently emerged as popular snacks in food service establishments, school lunch programs, and for family consumption (Gorny, 2003a).

In spite of the low pH of many fruits, including apples and peaches, foodborne pathogens (FBP) such as *Escherichia coli* O157:H7, *Salmonella* and *Listeria monocytogenes* could be present and cause public health problems. The incidence and/or survival/growth of these FBP in MP apples and peaches has been demonstrated (Abadias et al., 2006, 2008, 2009; Alegre et al., 2010a, 2010b; Harris et al., 2003; Liao and Sapers, 2000). In addition, outbreaks linked to fresh-cut fruit have been reported (CDC, 2007; Harris et al., 2003).

There are several processing steps in the fresh-cut produce production chain and many points for potential microbial contamination exist in each of these steps (Nguyen-The and Carlin, 1994). The only step for reducing microorganisms during processing is washing. A variety of disinfectants (including chlorine, hydrogen peroxide, organic acids and ozone) have been used to reduce bacterial populations on fruit and vegetables (Beuchat, 1998; EU Scientific Comitee on Food, 2002). However, besides their potential toxicity, they have proved incapable of completely removing or inactivating microorganisms on fresh produce (Koseki and Itoh, 2001; Park et al., 2001). Washing raw fruit and vegetables removes only a portion of pathogenic and spoilage microorganisms as some of them may escape contact with washing or sanitizing agents attaching to the surface of fruit and vegetables and tending to locate in protected binding sites (Allende et al., 2008; Sapers et al., 2001; Takeuchi and Frank, 2001).

In addition, reducing/controlling the native microbial populations by washing and sanitizing can allow human pathogens to flourish on produce surface (Brackett, 1992) as it reduces competition for space and nutrients thereby providing growth potential

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http://dx.doi.org/10.1016/j.fm.2012.09.007

for pathogenic contaminants. Chemical synthetic additives can reduce decay rate, but consumers are concerned about chemical residues in the product, which could affect their health and cause environmental pollution (Ayala-Zavala et al., 2008; Roller and Lusengo, 1997). Therefore alternative methods for controlling fresh-cut fruit decay are required.

Biological control fits well with this new tendency. Some bioprotective microorganisms have already shown its potential for application in MP apples. For example, the strain L-59-66 of *Pseudomonas syringae* prevented the growth of *E. coli* on apple wounds (Janisiewicz et al., 1999). Growth of *L. monocytogenes* and *Salmonella* on fresh-cut apple was reduced by strains of *Gluconobacter asaii*, *Candida* spp., *Dicosphaerina fagi* and *Metschnikowia pulcherrima* (Leverentz et al., 2006). The postharvest biocontrol agent *Candida sake* CPA-1 reduced *E. coli* growth on apple wounds, but not in MP apples (Abadias et al., 2009). Lactic acid bacteria were also reported to be inhibitory of *L. monocytogenes* on wounded apples (Trias et al., 2008). Recently, we have demonstrated the ability of *Pseudomonas graminis* CPA-7, isolated from whole apple surface, to reduce *E. coli* O157:H7, *Salmonella* and *Listeria innocua* on MP apples and peaches (Alegre et al., in press). However, none of these studies were performed under realistic conditions for MP apples.

Beyond microbiological contamination, development of fresh-cut apple slices has been hampered by the rapid oxidative browning of apple flesh. Browning can be delayed by reducing agents. For example, ascorbic acid has long been applied in combination with organic acids and calcium salts to prevent enzymatic browning of fruits (Gorny et al., 1998, 2002; Pizzocaro et al., 1993; Sapers et al., 1989; Soliva-Fortuny et al., 2001, 2002). Several studies have shown that NatureSeal® products can reduce browning in fresh-cut fruit slices (Abbott et al., 2004; Bhagwat et al., 2004; Rößle et al., 2009; Rupasinghe et al., 2005; Toivonen, 2008) and some natural thiol-containing compounds, such as N-acetylcysteine, have also been investigated as an alternative method to control enzymatic browning (Gorny et al., 2002; Molnar-Perl and Friedman, 1990; Oms-Oliu et al., 2006; Rojas-Grau et al., 2006; Son et al., 2001).

Enzymatic browning of apple slices can also been delayed by the use of modified atmosphere packaging (MAP) with very low oxygen levels (Gorny, 2003b); but extremely low O_2 levels pose the risk of anaerobic respiration and consequent off-flavours (Luo and Barbosa-Canovas, 1996) and, potentially, the growth of microaerophilic human pathogens, such as *E. coli* O157:H7, *Salmonella* spp. and *L. monocytogenes* (Buck et al., 2003; Gunes and Hotchkiss, 2002).

The objective of this study was to test the efficacy of the antagonistic strain *P. graminis* CPA-7 against a cocktail of four *Salmonella* strains and five *L. monocytogenes* strains on MP 'Golden Delicious' apples under simulated commercial conditions throughout storage at 5 and 10 °C. Different antioxidant treatments and modified atmospheres were tested. The effect of antagonist application on quality of MP apple was also evaluated. In addition, the ability of *Salmonella*, *L. monocytogenes* and *P. graminis* CPA-7 to survive to simulated gastric stress conditions following storage was studied.

2. Materials and methods

2.1. Fruit

'Golden Delicious' apples were obtained from local packinghouses in Lleida (Catalonia, Spain). Prior to the experimental studies, apples were washed in running tap water and let to dry at room temperature. Apples were cut in 10 skin-on wedges using an apple slicer/corer.

2.2. Bacterial strains

The bacterial strains used in this work are listed in Table 1. The antagonistic strain of *P. graminis* CPA-7 was isolated from apple surface in our laboratory (Alegre et al., in press). CPA-7 strain was grown in tryptone soy broth (TSB, Oxoid, UK) for 20–24 h at 30 °C. *Salmonella* strains were adapted to grow on tryptone soy agar (TSA, Oxoid, UK) supplemented with 100 μg mL^{-1} of streptomycin sulphate salt (St, Sigma, Germany) thereby enabling detection on a selective medium (TSA-St) in the presence of the antagonist and the natural microbial flora associated with apples. The strains were grown individually in TSB supplemented with streptomycin (TSB-St) medium for 20–24 h at 37 °C. *L. monocytogenes* strains were grown individually in TSB supplemented with 6 g L^{-1} of yeast extract (tryptone yeast extract soy broth, TYSEB) for 20–24 h at 37 °C. Bacterial cells were harvested by centrifugation at 9820 × *g* for 10 min at 10 °C and then resuspended in sterile distilled water (CPA-7) or saline solution (SS; 8.5 g L^{-1} NaCl, *Salmonella* and *L. monocytogenes*). The four *Salmonella* concentrated suspensions were mixed, as well as the five *L. monocytogenes* concentrated suspensions.

For the inoculum preparation, bacterial concentration was estimated using a spectrophotometer set at $\lambda = 420$ nm according to standard curves, and a volume of each of the bacterial concentrated suspensions was added to deionized water with or without antioxidant to obtain approximately 10^5 cfu mL^{-1} or 10^7 cfu mL^{-1}. Inoculum concentration was checked by plating appropriate dilutions onto TSA-St for *Salmonella*, onto Palcam agar (Palcam Agar Base with selective supplement, Biokar Diagnostics, Beauvais,

Table 1
Bacterial strains used in this study.

Number	Microorganism	Serovar	Source
CPA-7	*Pseudomonas graminis*		Apple surface (isolated in our laboratory, Alegre et al., in press)
ATCC BAA-707	*Salmonella enterica* subsp. *enterica*	Agona	Alfalfa sprouts
ATCC BAA-709	*Salmonella enterica* subsp. *enterica*	Michigan	Cantaloupe
ATCC BAA-710	*Salmonella enterica* subsp. *enterica*	Montevideo	Clinical (patient with salmonellosis associated with tomatoes)
ATCC BAA-711	*Salmonella enterica* subsp. *enterica*	Gaminara	Orange juice
CETC 4031/ATCC 15313	*Listeria monocytogenes* (Murray et al., 1926) Pirie, 1940	1a	Rabbit
CECT 933/ATCC 19113	*Listeria monocytogenes* (Murray et al., 1926) Pirie, 1940	3a	Human
CECT 940/ATCC 19117	*Listeria monocytogenes* (Murray et al., 1926) Pirie, 1940	4d	Sheep
CECT 4032	*Listeria monocytogenes* (Murray et al., 1926) Pirie, 1940	4b	Cheese
LM230/3	*Listeria monocytogenes*	1/2a	Fresh-cut iceberg lettuce (isolated in our laboratory, (Abadias et al., 2008))