Postharvest Biology and Technology 74 (2012) 118–124

Contents lists available at SciVerse ScienceDirect

Postharvest Biology and Technology

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

An *Enterobacteriaceae* species isolated from apples controls foodborne pathogens on fresh-cut apples and peaches

Isabel Alegre[a], Inmaculada Viñas[a], Josep Usall[b], Marina Anguera[b], Marian J. Figge[c], Maribel Abadias[b,*]

[a] University of Lleida, XaRTA-Postharvest, Rovira Roure, 191, 25198 Lleida, Catalonia, Spain
[b] IRTA, XaRTA-Postharvest, Rovira Roure, 191, 25198 Lleida, Catalonia, Spain
[c] Netherlands Culture Collection of Bacteria, CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands

ARTICLE INFO

Article history:
Received 10 April 2012
Accepted 5 July 2012

Keywords:
Escherichia coli O157:H7
Salmonella
Listeria innocua
Biopreservation
Minimally processed fruit
Antagonist

ABSTRACT

Currently, chlorine is the most widely used decontaminant in the minimally processed (MP) food industry. However, it does not achieve more than a 1–2 log reduction in bacterial populations. Efficient decontamination of MP produce could create a less competitive environment in which pathogens can multiply without restriction. Therefore, our objective was to test the efficacy of the biopreservative bacterial strain CPA-6 isolated from MP apples to control a non-pathogenic strain of *Escherichia coli* O157:H7, *Salmonella* and *Listeria innocua* on MP apples and peaches. Apple and peach plugs were co-inoculated with a suspension containing one of the pathogens (10^5 colony forming units (cfu) plug^{-1}) and CPA-6 (10^6 cfu plug^{-1}) and incubated at 20 °C or 5 °C. CPA-6 effectively inhibited the growth of, or reduced, in some cases to below the limit of detection, pathogen populations on both fruit incubated for 2 days at 20 °C and of *E. coli* on both fruit incubated at 5 °C, compared with the pathogen inoculated alone. The minimum effective dose required to inhibit any of the pathogens tested was 10^6 cfu plug^{-1} on both fruit and at both temperatures and it did not cause a hypersensitive reaction on tobacco plants. Finally, CPA-6 could not be assigned to any of the recognised species within the family *Enterobacteriaceae* based on phenotypic and 16S rRNA results. Therefore, this strain may be a suitable microorganism to use as a biopreservative culture to control the growth of food borne pathogens on MP fruit.

1. Introduction

Although the consumption of fresh produce is beneficial for optimal health, these foods may be associated with foodborne illness. Outbreaks of foodborne illness related to the consumption of fresh and minimally processed (MP) fruit, primarily due to *Escherichia coli* O157:H7 and *Salmonella*, have increased dramatically since the 1970s (Harris et al., 2003; CDC, 2007). The contamination of fresh fruit with human pathogens can occur at several points during growing, harvesting, processing and handling, and although pH is thought to be a limiting factor, the growth of *E. coli*, *Salmonella*, *Listeria innocua* and *Listeria monocytogenes* has been previously reported on, for example, fresh-cut apples and peaches (Janisiewicz et al., 1999b; Conway et al., 2000; Dingman, 2000; Leverentz et al., 2003, 2006; Abadias et al., 2009; Alegre et al., 2010a,b).

There are limited tools available for prolonging the shelf life of MP produce. Modified-atmosphere packaging and refrigeration can be utilised to slow down physiological degradation (King et al., 1991). The use of a decontamination method is another tool, but it should be mild enough to not impair the fresh or fresh-like attributes of MP produce (Gómez-López et al., 2005). Currently, chlorine is the most widely used among the washing and sanitising agents available for fresh produce. However, published data indicate that the most that can be expected at permitted concentrations is a 1–2 log reduction in the bacterial population (Beuchat, 1998; Brackett, 1999; Abadias et al., 2008). Therefore, there is still a need for the efficient and sustained decontamination of ready-to-eat produce that takes into account that efficient decontamination creates a less competitive environment in which pathogens can multiply (Carlin et al., 1996; Li et al., 2002).

The use of protective cultures, bacteriophages and bacteriocins may be alternatives to chemical treatments to reduce foodborne pathogens on fresh and fresh-cut fruit (Janisiewicz and Conway, 1999a; Leverentz et al., 2001, 2003, 2006). The native microbial community that is naturally present on the surfaces of fresh produce is assumed to play an important role in maintaining the health-supporting status of MP produce (Nguyen-The and Carlin, 1994) by out-competing pathogens for physical space and nutrients and/or producing antagonistic compounds that reduce the viability of pathogens (Liao and Fett, 2001; Parish et al., 2003). These organisms have the advantage of being part of the natural microbial community already established on the target produce, which may

* Corresponding author. Tel.: +34 973 003 430; fax: +34 973 238 301.
E-mail address: isabel.abadias@irta.cat (M. Abadias).

0925-5214/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.postharvbio.2012.07.004

facilitate their colonisation of, and survival on, the produce when applied in appropriate numbers (Leverentz et al., 2006). Therefore, there is potential for the use of native microflora to reduce pathogen growth and survival on fruit and vegetables.

The antagonist *Pseudomonas syringae* L-59-66, used for controlling the postharvest decay of pome fruit and commercialised as BioSave by EcoScience Corp. (Orlando, FL), can also prevent the growth of *E. coli* O157:H7 on wounded apple tissue (Janisiewicz and Conway, 1999a). *Gluconobacter asaii* (T1-D1), *Candida* spp. (T4-E4), *Dicosphaerina fagi* (ST1-C9) and *Metschnikowia pulcherrima* (T1-E2) inhibit the growth or reduce the populations of either or both *L. monocytogenes* and *Salmonella enterica* serovar Poona inoculated on 'Golden Delicious' apple plugs and stored at 10 °C and 25 °C (Leverentz et al., 2006). Although *Candida sake* CPA-1 reduced *E. coli* populations on 'Golden Delicious' apple wounds stored at 25 °C by approximately 1 log unit, it did not affect the survival of *E. coli* on fresh-cut apples (Abadias et al., 2009). Lactic acid bacteria (LAB) are considered food-grade microorganisms and generally recognised as safe (GRAS) and have historically been used to preserve meat and dairy products and to bioprotect fermented vegetables (Ruiz-Barba et al., 1994; Stiles and Holzapfel, 1997). Trias et al. (2008) tested six LAB strains as bioprotective agents against *E. coli*, *S. enterica* serovar Typhimurium and *L. monocytogenes* in apple wounds. LAB interfered with the growth of *S. Typhimurium* and *L. monocytogenes* but showed little effect against *E. coli*. The inhibition had a bactericidal effect against *L. monocytogenes* that could be related to bacteriocin production. Recently, the application of the probiotic strain *Lactobacillus rhamnosus* GG reduced the growth of *L. monocytogenes* on fresh-cut apples (Alegre et al., 2011).

The objective of this study was to evaluate the effectiveness of an antagonistic bacterial strain, CPA-6, isolated from fresh-cut apples to prevent the growth of *E. coli* O157:H7, *Salmonella* and *L. innocua* on fresh-cut apples and peaches. In addition, the minimum effective dose and phytopathogenicity were determined. Finally, partial 16S rRNA sequence analysis and phenotypic tests were performed to identify isolate CPA-6.

2. Materials and methods

2.1. Fruit

'Golden Delicious' apples and 'Royal Glory', 'Elegant Lady' and 'Merry O'Henry' peaches were used in experiments. Different varieties of peaches were used due to the high seasonality and low storage capacity of these fruit. Fruit that had not received any postharvest treatment was obtained from the IRTA Experimental Station and from packinghouses in Lleida (Catalonia).

The fruit was washed in running tap water and surface disinfected with 70% ethanol. Then, the fruit was cut in half, and plugs 1.2 cm in diameter and 1 cm in length were taken using a cork borer. The plugs were placed into sterile glass test tubes.

2.2. Bacterial strains

The antagonistic strain CPA-6 used in this study was isolated from minimally processed 'Golden Delicious' apples and was selected because it demonstrated an antagonistic effect against *E. coli* O157:H7 in previous studies (data not shown).

A non-pathogenic strain of *E. coli* O157:H7 (NCTC 12900) and a pathogenic strain of *S. enterica* subsp. *enterica* (Smith) Weldin serotype Michigan (BAA-709 ATCC) were used. Both strains were adapted to grow on tryptone soy agar (TSA, Oxoid, UK) supplemented with 100 μg mL^{-1} of streptomycin sulphate salt (St, Sigma, Madrid, Spain), thereby enabling their detection on selective medium (TSA-St) in the presence of CPA-6 and the natural microbial flora associated with apples and peaches. The strains were grown in tryptone soy broth (TSB, Oxoid, UK) supplemented with 100 μg mL^{-1} streptomycin (TSB-St) for 20–24 h at 37 °C. The CECT-910 strain of *L. innocua* was used as a microbial surrogate for *L. monocytogenes* because previous studies have demonstrated that it is a valid model for *L. monocytogenes* behaviour (Francis and O'Beirne, 1997). *L. innocua* was grown overnight in TSB supplemented with 6 g L^{-1} of yeast extract (Biokar Diagnostics, Beauvais, France, Tryptone soy broth yeast extract, TSBYE) at 37 °C.

E. coli O157:H7, *Salmonella* and *L. innocua* cells were harvested by centrifugation at 9820 × *g* for 10 min at 10 °C and then resuspended in a sterile 8.5 g L^{-1} NaCl solution (SS) to obtain a concentrated suspension. The concentration was estimated using a spectrophotometer set at λ = 420 nm according to previously determined standard curves.

2.3. Antagonistic effect of CPA-6 on minimally processed apples and peaches

CPA-6 was grown on nutrient yeast dextrose agar plates (NYDA, 8 g L^{-1} nutrient broth, Biokar Diagnostics, 5 g L^{-1} yeast extract, 10 g L^{-1} dextrose, VWR International Eurolab S.L., Spain, and 15 g L^{-1} agar, Industrias Roko S.A., Spain) overnight at 25 ± 1 °C. Colonies were scraped from the medium, and a suspension of 30 ± 5% transmittance (λ = 420 nm), which corresponded to approximately 1 × 10^8 cfu mL^{-1}, was prepared in 5 mL of sterile deionised water. Then, a volume of the *E. coli* O157:H7, *Salmonella* or *L. innocua* concentrated suspension (approximately 50 μL) was added to the 30 transmittance antagonist suspension to obtain a pathogen concentration of approximately 1 × 10^7 cfu mL^{-1}. The antagonist and pathogen concentrations in the prepared suspensions were confirmed for each assay. The antagonist and pathogen suspension was pipetted (15 μL) onto apple and peach tissue plugs, and the fruit plugs were stored at 20 ± 1 °C for 2 days and at 5 °C for up to 10 days (for *E. coli* O157:H7 only). Control treatments consisted of a pathogen suspension without antagonist. For pathogen recovery, each fruit plug was placed into a sterile plastic bag (Bagpage 80 mL, Interscience BagSystem, St. Nom La Breteche, France), and 9 mL of saline peptone (SP, 8.5 g L^{-1} NaCl and 1 g L^{-1} peptone) was added. The sample was homogenised in a stomacher for 120 s at high speed (Bagmixer 100 Minimix, Interscience, Weymouth, MA). Aliquots of the mixture were serially diluted and spread onto TSA-St plates for *E. coli* O157:H7 and *Salmonella* or on Palcam agar plates (Palcam Agar Base with Palcam selective supplement, Biokar Diagnostics) for *L. innocua*. The agar plates were incubated overnight at 37 ± 1 °C. The initial pathogen populations on apple and peach plugs were also determined following the same methodology. Three replicate fruit plugs were assessed per treatment and sampling time.

To evaluate the results, the populations of the pathogen inoculated alone or in the presence of the antagonist were compared. The reduction of the foodborne pathogen (FBP) population was calculated as follows:

$$\text{Reduction} = \log N_{\text{FBP}} - \log N_{\text{FBP+CPA-6}}$$

where N_{FBP} is the FBP population in the control treatment (FBP alone, cfu plug^{-1}) after the storage period and $N_{\text{FBP+CPA-6}}$ is the FBP population (cfu plug^{-1}) after the storage period in the presence of the antagonist.

2.4. Determination of the lowest effective antagonist dose

CPA-6 was grown in TSB for 20–24 h at 30 °C. Then, the cells were harvested by centrifugation at 15,344 × *g* for 15 min at 10 °C and resuspended in sterile SS. The concentration was estimated using