Food Microbiology 73 (2018) 275–281

Contents lists available at ScienceDirect

Food Microbiology

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

Inactivation of *Escherichia coli* O157:H7 and *Salmonella* during washing of contaminated gloves in levulinic acid and sodium dodecyl sulfate solutions

Marilyn C. Erickson [a, *], Jye-Yin Liao [a], Mussie Y. Habteselassie [b], Jennifer L. Cannon [c]

[a] Center for Food Safety, Department of Food Science and Technology, University of Georgia, 1109 Experiment St., Griffin, GA, 30223-1797, USA
[b] Department of Crop and Soil Sciences, University of Georgia, 1109 Experiment St., Griffin, GA, 30223-1797, USA
[c] CDC Foundation, 600 Peachtree St. NW, Atlanta, GA, 30308, USA

ARTICLE INFO

Article history:
Received 22 August 2017
Received in revised form
22 November 2017
Accepted 23 January 2018
Available online 3 February 2018

Keywords:
Harvesting
Latex
Nitrile
Canners
Inactivation
Cross-contamination

ABSTRACT

Field workers often wear gloves harvesting ready-to-eat produce; however, fields are not sterile environments and gloves may become contaminated numerous times during a working shift. This study explored the potential for inactivation of *Escherichia coli* O157:H7 and *Salmonella* when contaminated gloves were washed in levulinic acid (LV) and sodium dodecyl sulfate (SDS) solutions. Washing nitrile gloves with increasing concentrations of LV above 1.0% led to a decreased prevalence of glove contamination by *Salmonella* ($P = 0.0000$). A higher level of prevalence occurred for solid agar-cultured pathogens than liquid broth-cultured pathogens after nitrile gloves were washed in LV/SDS ($P = 0.0000$). Pathogens residing on latex gloves were more likely to be completely inactivated by washing in 0.5% LV/0.1% SDS solutions than nitrile or Canners gloves that exhibited inconsistent responses dependent on the pathogen strain. However, drying after washing nitrile gloves in 0.5% LV/0.1% SDS led to additional pathogen inactivation ($P = 0.0394$). Pathogen transfer from gloves to produce was implied as the pathogen prevalence on cantaloupe rind handled by LV/SDS-washed gloves was not statistically different from the prevalence on gloves ($P = 0.7141$). Hence, the risk of produce contamination may still exist but would be reduced by washing gloves in LV/SDS.

1. Introduction

Fresh and fresh-cut produce is a recognized rich source of many nutrients and leads to numerous health benefits. Despite these benefits, the proportion of outbreaks attributed to this commodity group is significant (Lynch et al., 2009). As evidence of this statement, during the period 2004–2012, a total of 377 and 198 produce-associated outbreaks were reported in the United States and European Union, respectively (Callejón et al., 2015).

Although trace-back to the farm has occurred in several produce-associated outbreaks, identification of the vehicle responsible for contamination in the field is usually not determined. Instead, several points of potential entry have been recognized including improperly composted animal manure, contaminated irrigation waters, wild or domestic animal encroachment, and the hands of workers harvesting the crop (Doyle and Erickson, 2012). In regards to this latter source of contamination, current regulations by the United States and good agricultural practices advocate that field workers who have contact with ready-to-eat crops be trained on proper hand hygiene and be provided access to facilities where they may execute those activities (US FDA, 2017). Similar guidelines are also present in the US Food Code for workers in retail food establishments; however, an additional stipulation that is recommended in the Food Code for those workers is that they wear gloves to serve as an additional barrier to prevent transfer of enteric pathogens from sick or asymptomatic workers to the food (US FDA, 2013).

Consequently, it is not surprising to have many commercial produce farms mandate that their workers wear gloves when harvesting the crop. Towards that goal, several glove types have been used for field harvesting. For example, single-use latex and nitrile gloves of moderate thickness (10–12 mil, respectively) are popular gloves whose tensile properties allow freedom of movement and are less prone than vinyl gloves to developing micro-tears

* Corresponding author.
E-mail address: mericks@uga.edu (M.C. Erickson).

https://doi.org/10.1016/j.fm.2018.01.024
0740-0020/

or pinholes with prolonged usage. Price is the chief advantage of latex gloves; however, allergens, to which many people are susceptible, may be present. In addition, latex gloves are chemically more unstable than nitrile gloves (Korniewicz et al., 2004). In contrast, Canners gloves are also latex-based but are manufactured to be chemically stable and hence, are promoted as being reusable (Todd et al., 2010). However, when gloves are soiled in the retail food establishment, they are discarded and a clean pair applied. Unfortunately, gloves worn by field workers have many more opportunities than gloves worn by retail food workers to come into contact with materials other than the crop or food that may be contaminated (i.e., contaminated soil or wildlife fecal deposits). Under those circumstances, changing gloves every time the gloves are suspected to have interacted with potential contamination sources would likely be cost prohibitive. Alternatively, the potential for disinfecting gloves, whether they are thick single-use gloves or reusable gloves, may provide a solution that could reduce the risk of produce contamination.

The most common disinfectant used in the produce industry within the United States is chlorine given the chemical's broad spectrum of antimicrobial activity, minimal effect on product quality, and relatively low cost compared to other disinfectants (Sapers, 2014). However, one of the major drawbacks to using chlorine as a disinfectant is that its effectiveness is reduced when organic material is also present in the solution (Chaidez et al., 2012). In contrast, a bactericide containing levulinic acid and sodium dodecyl sulfate (SDS) has been previously judged to be an effective sanitizer in the presence of organic matter (Zhao et al., 2009, 2011). Given that FDA has designated levulinic acid (US FDA, 2008, 21 CFR, 172.515) and SDS (US FDA, 2007, 21 CFR 172.822) as being generally recognized as safe for specific uses in foods, this study sought to examine the effectiveness of these chemicals for disinfecting gloves contaminated with either *Escherichia coli* O157:H7 or *Salmonella*.

2. Materials and methods

2.1. Pathogen strains

Multiple strains of *E. coli* O157:H7 and *Salmonella enterica* were used for this study and had been labeled using the method of Sambrook et al. (1989) with either a green fluorescent protein (GFP) plasmid or a red fluorescent protein (RFP) plasmid that also contained an ampicillin-resistant marker. The virulent strains included one RFP-labeled strain of *E. coli* O157:H7 (USDA 5, unknown origin) and four GFP-labeled strains of *E. coli* O157:H7 (F4546, alfalfa sprout outbreak; K3995, 2006 spinach outbreak; K4492, 2006 Taco Bell outbreak; and E0122, cattle feces). Two RFP-labeled avirulent strains of *E. coli* O157:H7 (MD56 and MD58) were also used and had been derived from the F4546 and K4492 virulent strains, respectively, by knocking out the Shiga toxin and intimin genes (Webb et al., 2014). The GFP-labeled virulent strains of *Salmonella* included three strains of serovar Enteritidis (ME18, H4717, and Benson 1, all of unknown origin) and one strain of serovar Newport (11590, beef). In addition, an avirulent strain of *Salmonella* Typhimurium (χ3985 Δcrp-11, Δcya-12), courtesy of Roy Curtiss III (Washington University, St. Louis, MO), had been labeled with GFP.

2.2. Lettuce sap preparation

During harvesting of lettuce, an exudate, known as latex, is released from the cut tissue that would likely be transferred to glove surfaces. Consequently, this exudate could serve as a medium for retaining any pathogens coming into contact with gloves. As it is conceivable that this medium could also affect the susceptibility of that pathogen to stress and any chemical disinfectants, a concentrated extract (lettuce sap) was prepared, that based on carbon and nitrogen analysis, closely mimicked that of iceberg head latex (data not shown). A key component to preparation of this latex substitute was using only the core and outer leaves of iceberg lettuce heads. These materials were ground in the presence of sterile deionized water (1:2 w/v) for 30 s in a One-Touch chopper NC 306 (Black and Decker Corp., Towson, MD). This homogenate was then filtered through a double layer of cheesecloth into a sterile Corning petri dish (145 mm diameter, 17 mm height). The dish and its contents were then held at 37 °C for three to five hours to evaporate the majority of the liquid followed by reconstitution of the mixture to a concentration that would be comparable if the latex had been exuded or squeezed from the fresh plant tissue. The material was then frozen following distribution of the lettuce sap into small vials. Upon thawing a vial, the lettuce sap was then used immediately to either suspend or dilute the pathogen.

2.3. Pathogen culture and strain mixture combinations

Both liquid broth (tryptic soy broth containing 100 μg/ml ampicillin [TSB-amp]) and solid media (tryptic soy agar containing 100 μg/ml ampicillin [TSA-amp]) were used to culture the pathogen strains for a period of 18–24 h at 37 °C. After this time period, cells were recovered by centrifugation (4050 × *g*, 25 min, 4 °C) from liquid cultures, whereas cells from solid media cultures were recovered by scraping of the agar surface using a 1 μl plastic inoculating loop. At this point, recovered cells were treated similarly in that they were washed three times in sterile 0.1% peptone water and centrifugation used to separate washed cells from the rinse. The final pellet of each isolate was suspended in lettuce sap to give a stock culture of ca. 10^9 CFU/ml.

Cocktails of two to four pathogen strains were prepared for individual experiments by combining equal volumes of each strain's stock culture. The strains used in these cocktail mixtures have been identified both in a column within Table 3 and in the footnotes of Tables 1, 2 and 4. After stock mixtures were prepared, they were diluted with lettuce sap to the working inoculum population and used immediately for inoculation of gloves. This requirement was implemented to minimize metabolic changes to the cells that could occur if left in an environmental state (i.e., liquid suspension) that would not have been representative of those culture conditions (i.e., solid media) in which the cells were generated.

2.4. Glove preparation and inoculation

Three brands of gloves were used in this study (UniSeal 12 mil textured latex gloves, American Healthcare Products, Inc., Alhambra, CA; UniSeal 10 mil nitrile gloves, American Healthcare Products, Inc.; Canners 20 mil latex gloves, Ansell, Iselin, NJ). Prior to their inoculation, the glove surfaces were soiled by grinding a sandy loamy soil containing 4.5% organic matter onto the outside surface of the palm. To accomplish this objective, both gloves were placed on a pair of hands and then ca. 2 g of soil scooped into the left hand. The two hands were clamped together and the palms rubbed firmly together for 10 s to grind the dirt onto the gloves. To remove any loosely adhering dirt, the gloved hands were patted together before removing the gloves and placing them on a benchtop, palm-side facing up. Following this operation, 100 μl of either non-inoculated or inoculated lettuce sap was applied to each glove in the palm area and the sap spread by folding the palm area over onto itself several times. The glove was then spread again onto the benchtop, with palm-side facing up, allowing the sap on the