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Inactivation of *Escherichia coli* O157:H7 and *Salmonella* deposited on gloves in a liquid state and subjected to drying conditions

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ABSTRACT

Gloves are worn by workers harvesting ready-to-eat produce as a deterrent for contaminating the produce with enteric pathogens that may reside on their hands. As fields are not sterile environments, the probability for gloves to become contaminated still exists and therefore it is critical to understand the conditions that affect the survival of pathogens on gloves. Both *Escherichia coli* O157:H7 and *Salmonella* deposited on glove surfaces in a liquid state survived longer when the pathogen had been suspended in lettuce sap than when suspended in water. Despite this protection, pathogens deposited on clean single-use gloves were more likely to survive during drying than pathogens deposited on dirty gloves (a film of lettuce sap had been applied to the surface prior to pathogen application and soil had been ground into the gloves). Survival of both *E. coli* O157:H7 and *Salmonella* was biphasic with the greatest losses occurring during the first hour of drying followed by much slower losses in the ensuing hours. Pathogens grown in rich media (tryptic soy broth) versus minimal media (M9) as well as those cultured on solid agar versus liquid broth were also more likely to be resistant to desiccation when deposited onto gloves. Although survival of *E. coli* O157:H7 on nitrile gloves was in general greater than it was on latex gloves, the relative survival of *Salmonella* on the two glove types was inconsistent. Due to these inconsistencies, no one glove type is considered better than another in reducing the risk for contamination with enteric pathogens. In addition, the extended survival of what are generally referred to as stress-resistant pathogens suggests that gloves either be changed frequently during the day or washed in a disinfectant to reduce the risk of glove contamination that could otherwise contaminate product handled with the contaminated gloves.

1. Introduction

In the United States, Canada, and European Union, produce consumption has increased in recent decades but accompanying this growth has been an increased number of outbreaks associated with fresh-cut produce items (Anderson et al., 2011; Callejón et al., 2015; Da Silva Felício et al., 2015; Kozak et al., 2013) and a heightened awareness by the public of this food safety issue. In light of these outbreaks, recent studies have been conducted in several countries (Canada, Egypt, Japan, Portugal, Spain, Turkey, United States) for the detection of *Salmonella* and/or *Escherichia coli* O157:H7 in several produce items; however, the prevalence of contamination was found to be low (< 1%) or absent (Arthur et al., 2007; Greve et al., 2015; Gunel et al., 2015; Khalil et al., 2015; Koseki et al., 2011; Oliveira et al., 2010; Santos et al., 2012).

To reduce the number of outbreaks when prevalence of pathogen contamination of produce is typically low, the industry as well as researchers have critically evaluated operations and risk factors throughout the continuum of production, harvesting, and packing using traditional biological indicators such as *E. coli* (Heredia et al., 2016) and other microbial source tracking tools, such as *Bacteroidales* (Ravaliya et al., 2014). Based on these studies, it has been suggested that hand contamination contributes to the final microbial profile and load on produce (Heredia et al., 2016; Ravaliya et al., 2014).

Hands are typically colonized by microorganisms in numbers ranging from 2 to 6 log/cm^2 but these resident microorganisms are not usually the causal agent of foodborne illness (Rediers et al., 2008). Instead, transient fecal pathogens that may cause foodborne illness, such as *Salmonella*, *E. coli* O157:H7, norovirus, and *Shigella*, may also be present on hands of asymptomatic carriers (Todd et al., 2008b). Proper

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hand washing, which is defined by the US FDA as scrubbing hands with soap and rinsing with potable water followed by hand drying with single-use paper or clean cloth towels (US FDA, 2013), reduces the levels of these pathogens (Charbonneau et al., 2000; Curtis and Cairncross, 2003; Montville et al., 2002), but a significant amount of bacteria may still remain on the hands of individuals (Charbonneau et al., 2000; de Aceituno et al., 2016). Consequently, due to this limitation and the low infectious dose of many of these enteric pathogens (Todd et al., 2008a), the Federal Food Code has advocated that in the retail segment of the food chain, food handlers should use gloves to serve as a barrier between skin and food and discard the gloves when damaged or soiled (US FDA, 2013). As evidence of the efficacy of gloves to reduce risk of contamination, outbreaks involving food workers who had worn gloves were fewer then the number of outbreaks that occurred when food workers had not worn gloves (Todd et al., 2010). Moreover, another benefit to the use of gloves is that they may be monitored by management and regulatory agencies.

In the field, some mechanical harvesting of produce is conducted but human labor is still utilized frequently. Since contact between hands and produce should be avoided in this setting as it is in retail, many harvesting crews have their workers wear gloves (both single-use and reusable gloves) to minimize cross-contamination. Unfortunately in field harvesting operations, the materials handled by the field worker's gloves (i.e., produce and soil) may already be contaminated and could provide numerous opportunities for the gloves, unbeknownst to the field worker, to become contaminated. Under these conditions, the worker would likely continue to wear the gloves for extended periods of time.

Both reusable and disposable gloves with varying degrees of film thicknesses are options for use in contacting food items. Latex gloves are probably the most common and cheapest and offer several advantages including excellent tactile properties, decreased resistance to perforations compared to vinyl gloves, and their tensile properties allow freedom of movement (Korniewicz et al., 2004). Limitations of latex gloves are that proteins within the gloves often trigger allergic reactions (Sussman et al., 2002) and they may also be degraded by chemical oxidants and alcohols. In contrast, although still sensitive to alcohol degradation, nitrile gloves are considered more chemically stable, contain fewer preexisting pinhole defects, are not allergenic to the user, but are more expensive than latex gloves (Ameratunga et al., 2008).

Many field harvesting operations take place in the early morning hours when dew may be present on the plants and at those times pathogen transfer could be in the presence of an aqueous medium. However, as the day progresses and temperature increase, glove surfaces would dry. Concurrently, any pathogens that were resident on the moist glove surfaces would be subject to desiccation stress that could lead to their loss of viability. This mode of inactivation has been addressed to a limited extent in a cross-contamination study employing latex gloves and *Salmonella* (Brar and Danyluk, 2013). However in the current study, *Salmonella* and *Escherichia coli* O157:H7 were included as pathogens of interest. In addition, additional variables (glove type, pathogen culture conditions, type of other glove contaminants and length of drying) were included.

2. Materials and methods

2.1. Pathogen strains

Five virulent *E. coli* O157:H7 isolates (1997 alfalfa sprout outbreak isolate; 2006 spinach outbreak isolate; 2006 Taco Bell lettuce outbreak isolate; an isolate from cattle feces; and one isolate from USDA of unknown origin), four virulent *Salmonella* isolates (*Salmonella* Enteritidis ME18; *Salmonella* Enteritidis H4717; *Salmonella* Newport 11590K; and *Salmonella* Enteritidis Benson 1), one avirulent *E. coli* O157:H7 strain (MD36) lacking the *stx*- and *eae*-genes (Webb et al., 2014), and one avirulent strain of *Salmonella* Typhimurium (χ3985 Δcrp-11, Δcya-12) (Curtiss and Kelly, 1987), courtesy of Roy Curtiss III (Washington University, St. Louis, MO), were used in this project. Using a calcium chloride heat shock transformation method described by Ma et al. (2011), each of these strains had been inserted with either the Clontech Gfpuv green- or Clontech dsRed red-fluorescent plasmid that also contained an ampicillin-resistant marker.

2.2. Pathogen culture

Frozen cultures of each pathogen were thawed and streaked individually onto tryptic soy agar plates containing 100 μg/ml ampicillin (TSA-amp). After incubating the plates for 24 h at 37 °C, individual colonies were transferred into tryptic soy broth containing 100 μg/ml ampicillin (TSB-amp), grown at 37 °C for 18–24 h with agitation (150 rpm) to give a culture in stationary phase, and then cells recovered by centrifugation (4050 × *g*, 25 min, 4 °C). The pellet was washed by suspending in sterile 0.1% peptone water and centrifuging three consecutive times. The final pellet was suspended in either sterile deionized water, sterile 0.1% peptone water (pH 7.0), or a lettuce sap mixture (described below) to give ca. 10^9 CFU/ml.

Alternative methods for culture of strains were also employed to potentially generate strains with different phenotypes or metabolic states that could respond differently to desiccation. In one scenario, strains were cultured to the stationary phase in M9 minimal media instead of TSB-amp. In that case, cells were recovered and resuspended in a similar manner to that used when cultured in TSB-amp. In a second scenario, cells were cultured on TSA-amp and the cells were scraped off the solid media, washed in sterile 0.1% peptone water, and then resuspended in a similar manner as to those cells cultured in liquid broth. In both scenarios, the recovered cells were immediately used for inoculation to avoid changes to their metabolism prior to their being suspended and spot inoculated onto glove surfaces.

For the majority of the experimental trials conducted in this study, the responses of *E. coli* O157:H7 and *Salmonella* to drying were evaluated in separate experiments. As such, equal portions of each of the 4 *E. coli* O157:H7 strains were combined to form a stock *E. coli* O157:H7 cocktail for inoculation and the *Salmonella* strains were likewise mixed together to give a stock *Salmonella* cocktail for inoculation. However, in one experimental trial (experiment 4 described in Section 2.6), two green fluorescent-labeled *Salmonella* strains (virulent *S.* Newport and avirulent. *S.* Typhimurium) and two red fluorescent-labeled *E. coli* O157:H7 strains (the virulent *E. coli* O157:H7 USDA strain and the avirulent *E. coli* O157:H7 MD58 strain) were mixed together in a single cocktail for inoculation and evaluation of the pathogen's responses made concurrently. In any event, all stock inocula cocktails were diluted to varying extents with the suspension media used to prepare the stock inoculum prior to spot inoculation onto gloves or glove pieces.

2.3. Lettuce sap preparation

To mimic the lettuce sap that is exuded during harvest of iceberg lettuce heads, different sections of the lettuce head (core, outer leaves, inner leaves) were mixed with sterile deionized water in a 1:2 w:v ratio and ground for 30 s in One-Touch Chopper, HC 306 (Black and Decker Corp., Towson, MD). This homogenate was filtered through a double layer of cheesecloth (Mainstays™ Projects, imported by TUFCO, LP Manning, SC) with filtrate collected in a sterile glass Pyrex petri dish (135 mm diameter, 20 mm height, 200 ml capacity). This dish was placed in a 37 °C incubator and the solution stirred slowly for three to five hours in order to evaporate the liquid bringing it to the concentration it would be had it been directly exuded or squeezed from the plant material. Following carbon and nitrogen analysis on this concentrated extract and lettuce sap (latex) collected from freshly harvested iceberg lettuce heads, it was confirmed that sap made from the cores and outer lettuce leaves had similar C:N ratios, whereas sap made