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Salmonella enterica growth and biofilm formation in flesh and peel cantaloupe extracts on four food-contact surfaces

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ABSTRACT

Salmonella enterica is responsible for the highest number of foodborne disease outbreaks pertaining to cantaloupe industry. The objective of this study was to examine the growth and biofilm formation by outbreak strains of *S. enterica* ser. Poona (*S.* Poona), *S. enterica* ser. Stanley (*S.* Stanley) and *S. enterica* ser. Montevideo (*S.* Montevideo) on different food-contact processing surfaces in cantaloupe flesh and peel extracts at 22 °C and 10 °C. The generation time of all *S. enterica* strains tested was shorter in the high concentration (50 mg/ml) of cantaloupe extract and high temperature. In 50 mg/ml of cantaloupe flesh or peel extract, the populations of *S. enterica* were increased by 5 log CFU/ml in 24 h at 22 °C and 1 log CFU/ml in 72 h at 10 °C. In 2 mg/ml of cantaloupe flesh or peel extracts, the populations of *S. enterica* were increased by 3.5 log CFU/ml in 56 h at 22 °C, but there were no changes in 72 h at 10 °C. The biofilm production of *S. enterica* was greater at 50 mg/ml of cantaloupe extract and 22 °C, but no major differences ($P \geq 0.05$) were found among the strains tested. In 50 mg/ml cantaloupe extract, *S. enterica* produced 5–6 log CFU/cm^2 biofilm in 4–7 d at 22 °C and approximately 3.5–4 log CFU/cm^2 in 7 d at 10 °C. In 2 mg/ml of cantaloupe extract, *S. enterica* produced 4–4.5 log CFU/cm^2 biofilms in 4–7 d at 22 °C and 3 log CFU/cm^2 in 7 d at 10 °C. Biofilm formation by *S.* Poona (01A4754) was lowest on buna-n rubber compared to stainless steel, polyethylene and polyurethane surfaces under the majority of conditions tested. Overall, these findings show that *S. enterica* strains can grow rapidly and form biofilms on different cantaloupe processing surfaces in the presence of low concentrations of cantaloupe flesh or peel extracts.

1. Introduction

Salmonella enterica is a Gram-negative, non-spore forming, rod-shaped bacterium that is recognized as a potential human health hazard when present in food (Jones et al., 2008). There are > 2500 serovars of *S. enterica*, but three of these serovars, namely *S.* enterica ser. Enteritidis, *S. enterica* ser. Newport and *S. enterica* ser. Typhimurium account for > 37% of the confirmed *Salmonella* cases in 2016 in the USA (Marder et al., 2017). In European countries, the most frequently reported human *S. enterica* isolates belong to *S.* Enteritidis and *S.* Typhimurium (EFSA and ECDC, 2016). In African countries, the most prevalent in human *S. enterica* serovars include Enteritidis, Typhimurium, Livingstone, Corvallis, Typhi and Braenderup while in Asian countries, Enteritidis, Weltevreden and Typhi are the serovars that are responsible for more than half of the human salmonellosis cases (Hendriksen et al., 2011).

Eggs and poultry products are the main sources of *S. enterica* infection (Painter et al., 2013), but plant-based food products are responsible for a significant number of *S. enterica* outbreaks around the world (Bennett et al., 2015; Kozak et al., 2013). Fruits and vegetables such as cantaloupe, cilantro, broccoli, cauliflower, lettuce, tomato, and watermelon have been reported as vehicles of *S. enterica* (Buck et al., 2003; Scolforo et al., 2017). As a fruit grown close to the ground, cantaloupe has a high risk of contamination with pathogens that are associated with soil and animals. Cantaloupe may also be contaminated by pathogenic microorganisms during harvesting, processing from contaminated water and equipment, transportation and distribution (Bowen et al., 2006; Castillo et al., 2004; Nyarko et al., 2018). In 2012, a multistate outbreak of *S.* Newport and *S.* Typhimurium infections was linked to whole cantaloupes, leading to 261 illnesses (94 hospitalizations, 3 deaths) in 24 states within the United States. *S.* Newport, *S. enterica* ser. Poona, and *S. enterica* ser. Javiana are associated with

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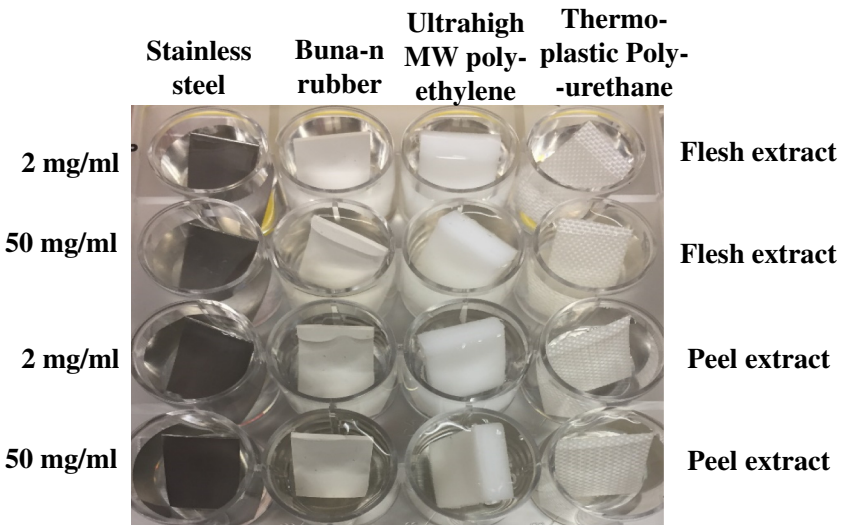

Fig. 1. *S. enterica* biofilm experimental setup with four food contact surfaces (stainless steel, buna-n rubber, ultra-high molecular weight polyethylene and thermoplastic polyurethane) in the presence of low and high concentrations of cantaloupe flesh and peel extracts in a 24-well plate.

melon related outbreaks (Zarecki et al., 2013). The investigations undertaken by the United States Food and Drug Administration (FDA) suggested that submerging warm cantaloupes in cool dump tank water may have facilitated their contamination by infusion of dump tank water contaminated with *Salmonella* into the cantaloupe tissue via stem scars or surface pores.

Several studies have indicated that peel and pulp of the fruits can allow microbial cells to attach and grow for an extended period of time (Berger et al., 2010; Collignon and Korsten, 2010; Leff and Fierer, 2013; Ukuku and Sapers, 2007). Growth and biofilm formation is further influenced by the type of pathogen, microbial competition and temperature (Richards and Beuchat, 2004; Scolforo et al., 2017). Annous et al. (2005) reported that *S.* Poona and *S. enterica* ser. Michigan were able to form biofilm on the cantaloupe rind surface after 24 h which was difficult to remove using commercially available sanitizers. Electron microscopic images (Cryo-SEM) indicated that *S. enterica* and *L. monocytogenes* formed biofilms on cantaloupe rind surfaces after 2–12 h of incubation (Fu et al., 2017). Moreover, treating with higher concentrations of lauroyl arginate ethyl (LAE) was unable to completely remove the attached cells from the cantaloupe rind surface. Such biofilm formation can cross-contaminate other food-contact and non-food contact surfaces, thus leading to food safety hazards (Dhowlaghar et al., 2018; Carpentier and Cerf, 1993 and Bernbom et al., 2011).

Growth and biofilm formation of *S. enterica* has been well characterized using standard broth models that contain optimal levels of all the necessary nutrients. In cantaloupe processing environments, *S. enterica* is exposed to nutrients that are leaked from cantaloupe. Also, cantaloupe residues can form deposits on the food contact and non-food contact surfaces when inadequately washed that may support the growth and biofilm formation of *S. enterica* (Food and Drug Administration, 2013). Currently, there are limited data on the role of cantaloupe extracts on the growth and biofilm formation of *S. enterica* strains. The objectives of the present study were to examine the effect of strain, temperature, nutrient level and food-contact surface on the growth and biofilm formation by outbreak strains of *S.* Poona, *S. enterica* ser. Stanley and *S. enterica* ser. Montevideo in cantaloupe flesh and peel extracts.

2. Materials and methods

2.1. Bacterial strains and culture conditions

S. Poona (01A4754, 00A3279, 01A242, 00A3208) strains associated with the 2011 cantaloupe outbreak were obtained from Dr. Cathy C. Webb, University of Georgia. Other two strains, *S.* Stanley (H1256 strain from Alfalfa sprout outbreak, Center for Disease Control) and *S.* Montevideo (G4639 strain from Tomato outbreak, Center for Disease Control) were obtained from Poultry Science Department, Mississippi State University. These strains were kept frozen in Tryptic soy broth containing 0.6% yeast extract (TSB-YE, pH 7.2; BD Bio sciences, San Jose, CA) that was supplemented with 16% glycerol at −80 °C. For preparing working stocks, each *S. enterica* strain was streaked with 10 µl loopful of frozen stock onto xylose lysine deoxycholate agar (XLD, BD Difco™, San Jose, CA) and incubated at 37 °C for 24 h. These plates were stored at 4 °C for up to 4 weeks. The overnight culture of each strain was prepared by inoculating one colony from XLD into 10 ml of tryptic soy broth containing 0.6% yeast extract (TSBYE, pH 7.2; BD Bio sciences, San Jose, CA) at 37 °C for 18 to 20 h with shaking at 150 rpm (Imperial III incubator, Lab-Line Instrument Inc., IL, USA).

2.2. Food-contact surfaces preparation

Four types of food-contact surfaces (table tops, equipment surfaces, conveyer belt, cutting board), including a stainless steel (304 #4) (Thyssen Krup, Thickness 0.018), nitrile buna-n rubber (Warco Nitrite, thickness 0.031), thermoplastic polyurethane (Habasit, thickness 0.01) and ultrahigh molecular weight polyethylene (Sibe Automation, thickness 0.125) were used in this study (Fig. 1). Sheets made with these materials were cut into 1 × 1.5 cm^2 size coupons and soaked in an alkali detergent for 1 h and washed thoroughly with deionized water. Stainless steel coupons were autoclaved at 121 °C for 15 min. Buna-n rubber, ultra-high molecular weight polyethylene and thermoplastic polyurethane coupons were sterilized by submersion in 100% alcohol for 15 min, followed by a sterile water rinse to eliminate alcohol residues. Sterile coupons were surface dried inside a biosafety cabinet prior to use.

2.3. Preparation of cantaloupe extracts

Cantaloupe (*Cucumis melo* L. var. reticulatus) fruits were obtained from a local supermarket and washed under running tap water for 1 min. The peel was then removed and the flesh was deseeded. In order to prepare cantaloupe flesh and peel extracts, 100 g of cut cantaloupe flesh or peel was blended with 400 ml of deionized water for 30 s using a juice extractor (Hamilton beach). The blended flesh and peel extracts were then filtered using a filter bag (BA6141/STR) and centrifuged at a