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Short communication

Sonication reduces the attachment of *Salmonella* Typhimurium ATCC 14028 cells to bacterial cellulose-based plant cell wall models and cut plant material

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ABSTRACT

This study investigated the removal of bacterial surface structures, particularly flagella, using sonication, and examined its effect on the attachment of *Salmonella* Typhimurium ATCC 14028 cells to plant cell walls. *S.* Typhimurium ATCC 14028 cells were subjected to sonication at 20 kHz to remove surface structures without affecting cell viability. Effective removal of flagella was determined by staining flagella of sonicated cells with Ryu's stain and enumerating the flagella remaining by direct microscopic counting. The attachment of sonicated *S.* Typhimurium cells to bacterial cellulose-based plant cell wall models and cut plant material (potato, apple, lettuce) was then evaluated. Varying concentrations of pectin and/or xyloglucan were used to produce a range of bacterial cellulose-based plant cell wall models. As compared to the non-sonicated controls, sonicated *S.* Typhimurium cells attached in significantly lower numbers (between 0.5 and 1.0 log CFU/cm^2) to all surfaces except to the bacterial cellulose-only composite without pectin and xyloglucan. Since attachment of *S.* Typhimurium to the bacterial cellulose-only composite was not affected by sonication, this suggests that bacterial surface structures, particularly flagella, could have specific interactions with pectin and xyloglucan. This study indicates that sonication may have potential applications for reducing *Salmonella* attachment during the processing of fresh produce.

1. Introduction

Fresh produce is recognized as an important vehicle for the transmission of human pathogens such as *Salmonella*, *Listeria monocytogenes* and *Escherichia coli* O157:H7 (Berger et al., 2010; Takeuchi et al., 2000). Minimally processed fresh fruits and vegetables are particularly susceptible to contamination with pathogenic microorganisms due to the lack of significant antimicrobial interventions which may damage the quality and safety of the product (Doyle and Erickson, 2008; Golberg et al., 2011). Between 1992–2006, a study done in England and Wales reported that out of 2274 of the reported foodborne outbreaks, 4% were linked to cut and prepared salad (Little and Gillespie, 2008).

Many factors contribute to the spread of human pathogens on fresh produce. Fresh produce can be contaminated both pre-harvest and post-harvest. Pre-harvest contamination sources include soil, dust, faeces, reconstituted fungicides and insecticides, contaminated irrigation water, raw or insufficiently composted manure, wild or domestic animals and human handling (Doyle and Erickson, 2008; Newell et al., 2010; Olaimat and Holley, 2012; Tauxe, 1997). Primary reservoirs of human pathogens present in soil and manure can also contaminate water systems through sewage systems that are not functioning properly, sewage overflows, polluted storm water run-off and the run-off from animal pastures (Tang et al., 2012). Post-harvest fresh produce processing, which entails cutting and shredding, also exposes cut surfaces of fresh produce to contamination by human foodborne pathogens during storage, rinsing, cleaning, cutting and transporting (Berger et al., 2010). These cut surfaces lack the waxy cuticle which hinders bacterial attachment and repels water which could carry pathogens (Aruscavage et al., 2006; Kroupitski et al., 2009). In addition cross-contamination occurs during these fresh-cut processing operations with a small lot of contaminated produce having the potential to cause the contamination of a large batch (Doyle and

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Erickson, 2008). Cross-contamination occurs through direct contact with the contaminated product or through indirect contact with contaminated equipment surfaces or contaminated water (Doyle and Erickson, 2008; Kaneko et al., 1999; Moore et al., 2003).

Chlorine is currently widely used in the fresh-cut industry in many countries for the sanitation of fresh produce (Aruscavage et al., 2006; López-Gálvez et al., 2010; Parish et al., 2003). Chlorine, however, has limited decontamination effectiveness and is generally only effective in keeping the wash water free of contaminants and for preventing cross-contamination (Aruscavage et al., 2006; Delaquis et al., 2002; Takeuchi et al., 2000). In addition, chlorine-based sanitizers are corrosive, cause skin and respiratory tract irritations and may form carcinogenic by-products (Ölmez and Kretzschmar, 2009). The use of chlorine is of environmental concern and has been banned in some European countries (São José et al., 2014). There is therefore a need to find better methods to decontaminate fresh produce.

Sonication (also known as ultrasonication) is a technology which has been used to clean tools and remove biofilms in the health industry (Rediske et al., 1998) and in the food industry (Lillard, 1994; Sams and Feria, 1991) but it has not seen widespread use in the fresh produce industry (Rodgers and Ryser, 2004). During the sonication process, ultrasonic waves with a frequency of 20 kHz or more are produced and this creates regions of alternating compression and expansion in the liquid medium (Piyasena et al., 2003). Cavitation occurs and the implosive collapse of gas bubbles creates shock waves of very high temperature and pressure which can reach up to 5500 °C and 50000 kPa (Piyasena et al., 2003). These ultrasonic shock waves can disrupt cell membranes, break cell walls and cause damage to DNA by producing free radicals (Butz and Tauscher, 2002; Mason et al., 2003). Sonication has also been used to detach bacterial surface structures including flagella and fimbriae (Jimenez-Sanchez et al., 2012; Sojar et al., 1997).

In our study, we investigated the attachment of *S.* Typhimurium ATCC 14028 cells, which had their flagella and other surface structures removed through sonication, to bacterial cellulose (BC[1])-based plant cell wall (PCW[2]) models and cut plant materials. The use of these PCW models to investigate bacterial attachment to cut PCWs has been established in an earlier study (Tan et al., 2013). This study also evaluated the potential of sonication, as a proof of concept, for use in processing in the fresh produce industry.

2. Materials and methods

2.1. Bacterial strains

Salmonella enterica subspecies *enterica* serovar Typhimurium ATCC 14028 and *Gluconacetobacter xylinus* ATCC 53524 were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). *S.* Typhimurium ATCC 14028 was grown aerobically at 37 °C on tryptic soy agar (Merck, Darmstadt, Germany) or in tryptic soy broth (Merck, Darmstadt, Germany) for 18 h under shaking incubation (150 rpm) (Lab Companion SK-600 benchtop shaker, Medline, UK). The *Gluconacetobacter* strain was grown as described below.

2.2. Production of BC-based PCW models

A primary inoculum of *G. xylinus* ATCC 53524 was prepared and then used for the scale-up production of all BC composites as described in an earlier study (Tan et al., 2016). The primary inoculum was prepared by culturing the *G. xylinus* strain at 30 °C for 72 h in Hestrin and Schramm (HS[3]) broth medium containing 2% (w/v) glucose, 0.5% (w/v) peptone, 0.5% (w/v) yeast extract, 0.27% (w/v) Na_2HPO_4 and 0.115% (w/v) citric acid and adjusted to pH 5.0 (Hestrin and Schramm, 1954). Scale-up production of the different BC composites were prepared individually and grown at the same time. Triplicates of each type of BC composite were grown for each attachment assay. These composites were produced in enclosed plastic containers (1.5 cm × 1.5 cm × 1.5 cm) incubated statically at 30 °C for 72 h depending on the HS medium composition. Harvested BC composites (1.5 cm × 1.5 cm, ~2 mm thickness) were purified by rinsing in 6 mM $CaCl_2$ at 100 rpm for 1 h to remove media components. The 4 different types of PCW models produced were:

- BC-only composite without the addition of pectin or xyloglucan
- BC-Pectin (BCP[4]) composite produced by adding 0.5% w/v apple pectin with a degree of methyl esterification of about 30% (Herbstreith & Fox, Neuenbürg, Germany) to the HS medium and 12.5 mM calcium chloride (R&M Chemicals, Malaysia) was added to allow incorporation of pectin into the composite.
- BC-Xyloglucan (BCX[5]) composites produced by adding 0.5% w/v xyloglucan (Megazyme, County Wicklow, Ireland) to the HS medium.
- BC-Pectin-Xyloglucan (BCPX[6]) composites produced by adding 0.25% w/v pectin, 0.25% w/v xyloglucan and 12.5 mM $CaCl_2$ to the HS medium.

2.3. Preparation of cut plant material

Potato (*Solanum tuberosum*) tuber, apple (*Malus domestica*) fruit and lettuce (*Lactuca sativa*) leaves were obtained from a retail outlet in Selangor, Malaysia. These 3 plant tissues were used since they represent a range of different plant species that *Salmonella* have been isolated from and have also been associated with cases of salmonellosis (Beuchat, 2002; Quiroz-Santiago et al., 2009). The outer surfaces of the potato, apple and lettuce leaves were lightly wiped down with 70% ethanol solution before cutting out approximately 1.5 cm × 1.5 cm pieces of the plant materials with a sterile scalpel. The surfaces of the lettuce leaves were lightly grazed to expose the upper and lower epidermis to resemble cut surfaces of fresh produce after fresh-cut processing. However, physical abrasion of the leaf surface which removes leaf waxes may not be fully representative of commercially cut and processed mature leaves which allow microbial access to the veins and interstitial spaces within the leaf structure.

2.4. Preparation of bacterial suspensions and sonication treatment

Early stationary phase cultures of the *S.* Typhimurium ATCC 14028 strain (grown for 18 h) were pelleted by centrifugation at 5500× *g* (Hettich D-78532, Tuttlingen, Germany) for 10 min at 4 °C. The pellet was washed twice with phosphate buffer saline (PBS) (pH 7.4) (1st BASE, Singapore) and suspended in PBS to an optical density at 600 nm (UV/Vis spectrophotometer, Shimadzu UV mini-1240, USA) which corresponds to ~8 log CFU/mL.

Universal bottles each with a 10 mL *S.* Typhimurium ATCC 14028

[1] BC: bacterial cellulose.
[2] PCW: plant cell wall.
[3] HS: Hestrin and Schramm.
[4] BCP: bacterial cellulose-pectin.
[5] BCX: bacterial cellulose-xyloglucan.
[6] BCPX: bacterial cellulose-pectin-xyloglucan.