Listeria monocytogenes Biofilm Formation on Silver Ion Impregnated Cutting Boards

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ABSTRACT

Listeria monocytogenes is a human pathogen that can be a member of a biofilm community attached to surfaces in poultry processing plants. When present as a biofilm on product contact surfaces, this organism can cross contaminate ready-to-eat meat. Plastic cutting boards can be formulated to include antibacterial agents such as silver ions. In this study we compared the ability of L. monocytogenes to attach and form a biofilm on identical plastic cutting boards manufactured with and without silver ions. Cutting boards were cut into 2 by 2 cm squares and inoculated with a poultry plant isolate of L. monocytogenes known to effectively form biofilms. Boards were inoculated by submersion in a cell suspension of approximately 108 cells per mL PBS for 2 hours at 25°C. All pieces were then rinsed in PBS to remove unattached cells and incubated in dilute (1/10) brain heart infusion broth for 24 hours at 25°C. Unattached cells were again removed by rinsing in PBS. The surface was sampled using a pre-moistened sterile cotton swab either immediately after removal of unattached cells or after a 24-hour dry exposure of attached cells to the board formulation at 25°C. The experiment was replicated three times, using five cutting board squares for each treatment in each replication (n = 15). When surfaces were sampled immediately after rinsing, similar numbers of L. monocytogenes were recovered from treated and untreated boards: 6.83 and 6.86 log CFU/cm2, respectively. A twenty-four-hour dry time reduced the number of attached L. monocytogenes on both types of cutting boards to the same degree; silver ion impregnated boards had 3.95 log CFU/cm2, while untreated control boards had 3.97 log CFU/cm2. Under the conditions of these tests, silver ion impregnation did not lessen the ability of L. monocytogenes to form a biofilm on the surface of plastic cutting boards.

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INTRODUCTION

Listeria monocytogenes is a human pathogen that can cause foodborne disease. Meat and poultry have been implicated in foodborne outbreaks of listeriosis (4, 9). This organism is ubiquitous in nature and able to grow at relatively low temperatures. Therefore, L. monocytogenes is of particular concern in the poultry processing arena because after chilling, most of the plant and all products are maintained at a cool temperature. L. monocytogenes has been isolated from raw poultry products and in plants in which poultry undergoes further processing (2).

L. monocytogenes can enter a poultry processing plant with raw meat (2). Once in the facility, this organism can become a long-term resident, showing particular ability to persist for months and even years in floor drains (2). The inner surface of floor drains is an environment for complex biofilm communities, including multiple bacterial genera. Spoilage organisms such as Pseudomonas putida can also form biofilms and may be present in tandem with pathogenic bacteria. L. monocytogenes has been shown to attach especially well to moist low-nutrient surfaces previously colonized with P. putida (5).

Bacteria present as long-term residents in a further processing plant floor drain have the potential to escape this niche and cross contaminate other surfaces, including those that contact product. L. monocytogenes can attach and form biofilms on a variety of processing plant surfaces (1); plant persistent subtypes are especially capable biofilm formers (7). Once on a product contact surface, L. monocytogenes can be transferred to meat (10, 12).

Plastic composite cutting boards, counters, trays and conveyors are common product contact surfaces in many poultry further processing plants. L. monocytogenes can attach to and survive on such plastic surfaces (11). Once established as an attached community, L. monocytogenes is more difficult to eradicate with heat or surface sanitizing treatments (3). Sanitizers have been shown to lessen but not eliminate L. monocytogenes when it is attached to plastic cutting board surfaces (13). Silver ions have an antibacterial affect that is well reviewed by Kampmann et al. (6).

Silver ions can be immobilized in plastics and have been reported to have utility to lessen bacterial contamination associated with treated surfaces (6).

The objective of this study was to test the ability of L. monocytogenes and P. putida to attach, grow and form stable biofilms on plastic cutting boards with and without silver ions added to the formulation.

MATERIALS AND METHODS

Cutting boards

Plastic (polyethylene) cutting boards with and without silver ion treatment were purchased from the manufacturer (Bio-guard Plastics, Meadota, MN 55120). Control cutting boards were identified by a different color, but were otherwise identical to the treated boards. Each cutting board was cut into squares 2 cm by 2 cm. Squares were disinfected prior to inoculation by rinsing with 70% ethanol and then being air dried under germicidal ultra-violet light on sterile paper towels in a biological safety cabinet.

Listeria monocytogenes culture and inoculation

The L. monocytogenes culture used in this study was originally recovered from a poultry further processing plant and found to be capable of forming a biofilm. An overnight broth culture of L. monocytogenes was plated onto ten plates of BHI agar (Oxoid Ltd., Basingstoke, Hampshire, England) using a cotton tipped applicator to result in a lawn of growth; plates were incubated for 18–24 h at 37°C. Culture was removed from each of ten plates and suspended in 300 ml of PBS (pH 7). Serial dilutions and subsequent plating on BHI agar (18–24 h, 37°C) revealed a cellular density ranging from 2 to 7 × 10^8 cells L. monocytogenes per ml PBS. Ten 50 ml conical tubes (Tyco Healthcare Group LP, Mansfield, MA 02048) were filled with 25 ml aliquots of the cell suspension. One square of treated cutting board was then removed from PBS and placed into each of the remaining five tubes. An uninoculated control tube of PBS was included for each type of cutting board to assure there was no contamination present on the cutting board squares prior to inoculation.

Squares remained in the PBS cell suspension at 25°C for 2 h. Following incubation, each square was aseptically removed from the cell suspension and placed into a fresh tube with 25 ml sterile PBS. Each tube was inverted 4 times to remove unattached cells. Each square was then removed from PBS and placed into another fresh tube with sterile 1:10 strength BHI broth (Oxoid Ltd., Basingstoke, Hampshire, England). Dilute BHI broth was used to simulate the limited nutrients expected on a rinsed food contact surface. Squares were allowed to incubate in the dilute BHI broth for 24 h at 25°C.

In the first set of experiments, squares were removed from the growth phase in dilute BHI, rinsed in a tube of PBS by inversion 4 times as previously described, and analyzed immediately. In the second set, the squares were removed from the dilute BHI, rinsed, placed on sterile paper towels and allowed to air dry for one hour under a biological safety cabinet before being transferred to a dry sterile 50 ml tube and subjected to 24 h of dry incubation at 25°C. Thus attached cells were exposed to the silver ion treatment for an additional 24 h. Prior to sampling, the dried squares were subjected to another rinse procedure as already described.

Pseudomonas putida culture and inoculation

The P. putida culture used was selected because it is known to be an excellent biofilm producer (5). The growth conditions and inoculum preparation methods for P. putida were similar to those previously described for L. monocytogenes with a few differences. The inoculum cell suspension ranged from 1.4 to 1.5 × 10^9 cells P. putida per ml PBS. P. putida was used only in the second set of experiments, with a 24-h post growth dry incubation period to allow attached cells to remain in contact with the antimicrobial plastic cutting boards. No P. putida biofilms were analyzed immediately after the growth phase.

Detection of biofilm cells

To remove biofilm cells from the cutting board squares, one cotton tipped
TABLE 1. Mean log CFU attached L. monocytogenes and P. putida per cm² polyethylene cutting board formulated with or without antimicrobial silver ions (n = 15 per treatment)

<table>
<thead>
<tr>
<th></th>
<th>L. monocytogenes</th>
<th>P. putida</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cutting board</td>
<td>0 h dry time</td>
<td>24 h dry time</td>
</tr>
<tr>
<td>Control</td>
<td>6.86&lt;sub&gt;x&lt;/sub&gt;</td>
<td>3.97&lt;sub&gt;x&lt;/sub&gt;</td>
</tr>
<tr>
<td>Silver ion</td>
<td>6.83&lt;sub&gt;y&lt;/sub&gt;</td>
<td>3.95&lt;sub&gt;y&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>time at 25°C between rinsing away unattached cells and sampling surface with cotton-tipped applicator

<sup>x</sup><sup>y</sup>values within columns are not significantly different by t test (P > 0.05)

<sup>x</sup><sup>y</sup>values within row with different superscripts are significantly different by t test (P < 0.01)

applicator was moistened by dipping into PBS and used to rub one flat surface of each square. The swab was rubbed over the entire square, the square was rotated 90 degrees and re-sampled with the same swab, the square was again rotated 90 degrees and the same swab was used a third time to rub over one flat surface. Each swab was then again placed into 9 ml PBS. The number of L. monocytogenes cells was estimated by plating 0.1 ml of serial dilutions onto the surface of duplicate modified oxford (MOX) agar (Remel, Lenexa, KS 66215). All MOX plates were incubated at 35°C for 24 h before small black colonies characteristic of L. monocytogenes were counted. P. putida cells were enumerated by direct plating 0.1 ml from serial dilutions onto the surface of duplicate BHI agar plate incubated at 35°C for 24 h.

**Experimental design and statistical analysis**

Three replications of each experiment were conducted for each organism, with five duplicate cutting board squares per replicate (n = 15). The mean number of colonies per ml was converted to log CFU/cm² and geometric means were calculated. Differences were determined by Students t test, with significance assigned at P < 0.01.

**RESULTS AND DISCUSSION**

Bacterial count data on the surface of cutting boards are presented in Table 1. L. monocytogenes attached to the surface of the plastic cutting boards and grew to a density of more than 10<sup>6</sup> cells per cm² regardless of the presence of silver ions. When attached, L. monocytogenes cells were left on the cutting board for 24 h, the numbers were substantially lower, less than 10<sup>4</sup>/cm², than when sampling occurred immediately after the growth phase. This was observed on cutting boards with or without silver ions.

It was assumed that P. putida, an efficient biofilm producer, would perform at least as well as L. monocytogenes; therefore, no testing was done immediately after the growth phase. Following twenty-four hours of dry incubation after unattached cells had been rinsed away, more than 10<sup>6</sup> cells P. putida could be found per cm² plastic cutting board. As was the case for L. monocytogenes, the silver ion treatment did not affect the numbers of P. putida detected attached to the surface.

The current data provide conclusions that differ from those of other published work. MacKeen et al. (8) found that silver-containing fiber filters placed in liquid medium significantly lowered the number of surviving Escherichia coli and Pseudomonas aeruginosa. This decline in numbers was found to be significant at 26°C and faster at 37°C. Our studies did not employ ideal bacterial growth temperatures, because it is assumed that surfaces in a poultry further processing plant would be kept cool; as such, our treatment temperature of 25°C represents a worse-case scenario.

In previous research, silver-containing polystyrene caused a dramatic decrease in bacterial numbers within 24 h at 35°C and within 72 h at 5°C (6). The same researchers examined refrigerator lining material formulated with silver ions and reported that the treated refrigerators and food stored therein had lower levels of bacterial contamination than levels for untreated control refrigerators. These conclusions, however, were based on visual observation of growth media and not quantitative data subjected to statistical analysis (6).

Gram positive species were proposed to be less sensitive to silver ion treatment than Gram negative species (6). However, in the current work, we found no difference between the effects of silver on a Gram positive (L. monocytogenes) and a Gram negative (P. putida) bacteria. The presence of silver ions in a plastic cutting board did not affect the attachment, growth or survival of either organism on the surface.

Our data suggest that L. monocytogenes cells attached to plastic cutting boards are more susceptible to drying than are cells of P. putida. It may be that L. monocytogenes becomes uncultureable (especially on the selective medium used in the current study) because of dry stress, while P. putida is more resistant to drying. However, another explanation is that L. monocytogenes cells in a dry biofilm are less well attached, making them prone to removal during pre-sample rinsing. This possibility is supported by an earlier report showing that an L. monocytogenes biofilm is more likely to cause transfer to a secondary contact surface when the biofilm has been subjected to drying (10).

**CONCLUSIONS/RECOMMENDATIONS**

Although silver ion surface treatment has been reported to have antimicrobial utility in other circumstances and
situations, the silver impregnated cutting boards tested in the current study did not inhibit the formation of biofilms by either \textit{L. monocytogenes} or \textit{P. putida} at 25°C.

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**REFERENCES**