*Food Protection Trends, Vol. 30, No. 3, Pages 168–171*
International Association for Food Protection
6200 Aurora Ave., Suite 200W, Des Moines, IA 50322-2864



# *Listeria monocytogenes* Biofilm Formation on Silver Ion Impregnated Cutting Boards

MARK E. BERRANG,[1*] JOSEPH F. FRANK[2] and RICHARD J. MEINERSMANN[1]
[1]USDA-Agricultural Research Service, Russell Research Center, Athens, GA 30605, USA;
[2]University of Georgia, Dept. of Food Science and Technology, Athens, GA 30602, USA

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

## 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 $10^8$ 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/cm$^2$, respectively. A twenty-four hour dry time reduced the number of viable attached *L. monocytogenes* on both types of cutting boards to the same degree; silver ion impregnated boards had 3.95 log CFU/cm$^2$, while untreated control boards had 3.97 log CFU/cm$^2$. 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.

A peer-reviewed article

*Author for correspondence: Phone: 706.546.3551; Fax 706.546.3018;
Email: mark.berrang@ars.usda.gov

## 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 cooking and further 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 espcially 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, Mendota, 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 $7 \times 10^8$ 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 placed into each of five tubes, and one square of control board was placed in 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 $1.5 \times 10^8$ 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)**

| | *L. monocytogenes* | | *P. putida* |
|---|---|---|---|
| **Cutting board** | **0 h dry time[1]** | **24 h dry time** | **24 h dry time** |
| Control | 6.86[a,x] | 3.97[a,z] | 6.46[a,y] |
| Silver ion | 6.83[a,y] | 3.95[a,z] | 6.53[a,y] |

[1]time at 25°C between rinsing away unattached cells and sampling surface with cotton-tipped applicator

[a]values within columns are not significantly different by *t* test ($P > 0.05$)

[x,y,z]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 $10^6$ 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 $10^4$/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 $10^6$ 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 unculture-able (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 *L. monocytogenes* or *P. putida* at 25°C.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge expert technical assistance by Eric S. Adams.

### REFERENCES

1. Beresford, M. R., P. W. Andrew, and G. Shama. 2001. *Listeria monocytogenes* adheres to many materials found in food-processing environments. *J. Appl. Microbiol.* 90:1000–1005.
2. Berrang, M. E., R. J. Meinersmann, J. F. Frank, D. P. Smith, and L. L. Genzlinger. 2005. Distribution of *Listeria monocytogenes* subtypes within a poultry further processing plant. *J. Food Prot.* 68:980–985.
3. Frank, J. F., and R. A. Koffi. 1995. Surface-adherent growth of *Listeria monocytogenes* is associated with increased resistance to surfactant sanitizers and heat. *J. Food Prot.* 53:550–554.
4. Frye, D. M., R. Zweig, J. Sturgeon, M. Tormey, M. LeCavalier, I. Lee, L. Lawani, and L. Mascola. 2002. An outbreak of febrile gastroenteritis associated with delicatessen meat contaminated with *Listeria monocytogenes*. *Clin. Infect. Dis.* 35:943–949.
5. Hassan, A. N., D. M. Birt, and J. F. Frank. 2004. Behavior of *Listeria monocytogenes* in a *Pseudomonas putida* biofilm on a condensate-forming surface. *J. Food Prot.* 67:322–327.
6. Kampmann, Y., E. De Clerck, S. Kohn, D. K. Patchala, R. Langerock, and J. Kreyenschmidt. 2008. Study on the antimicrobial effect of silver-containing inner liners in refrigerators. *J. Appl. Microbiol.* 104:1808–1814.
7. Lunden, J. M., M. K. Miettinen, T. J. Autio, and H. J. Korkeala. 2000. Persistent *Listeria monocytogenes* strains show enhanced adherence to food contact surface after short contact times. *J. Food Prot.* 63:1204–1207.
8. MacKeen, P. C., S. Person, S. C. Warner, W. Snipes, and S. E. Stevens. 1987. Silver-coated nylon fiber as an antibacterial agent. *Antimicrob. Agents Chemother.* 31:93–99.
9. Mead, P. S., E. F. Dunne, L. Graves, M. Wiedmann, M. Patrick, S. Hunter, E. Salehi, F. Mostashari, A. Craig, P. Mshar, T. Bannerman, B. D. Sauders, P. Hayes, W. Dewitt, P. Sparling, P. Griffin, D. Morse, L. Slutsker, and B. Swaminathan. 2006. Nationwide outbreak of listeriosis due to contaminated meat. *Epidemiol. Infect.* 134:744–751.
10. Rodriguez, A., W. R. Autio, and L. A. McLandsborough. 2007. Effect of biofilm dryness on the transfer of *Listeria monocytogenes* biofilms grown on stainless steel to bologna and hard salami. *J. Food Prot.* 70:2480–2484.
11. Silva, S., P. Teixeira, R. Oliveira, and J. Azeredo. 2008. Adhesion to and viability of *Listeria monocytogenes* on food contact surfaces. *J. Food Prot.* 71:1379–1385.
12. Vorst, K. L., E. C. Todd, and E. T. Rysert. 2006. Transfer of *Listeria monocytogenes* during mechanical slicing of turkey breast, bologna, and salami. *J. Food Prot.* 69:619–626.
13. Yang, H., P. A. Kendall, L. C. Medeiros, and J. N. Sofos. 2009. Efficacy of sanitizing agents against *Listeria monocytogenes* biofilms on high-density polyethylene cutting board surfaces. *J. Food Prot.* 72:990–998.

# 2010-2011 Secretary Election

**MARIA TERESA DESTRO**

**DONALD W. SCHAFFNER**

The following page contains biographical information for the 2010–2011 Secretary Candidates. This information is provided to help you make your selection of the next IAFP Secretary.

Members with valid E-mail addresses will receive election notices and a unique personal identification number via E-mail from IAFP's election service provider. Members without E-mail addresses, or invalid E-mail addresses, will be sent their unique personal identification number via postal service. Voting will take place on a Web site hosted by Survey & Ballot Systems (SBS), an independent, external organization who is conducting the IAFP election. Safeguards are in place to insure each Member votes only once.

The election Web site will be open from January 28 to March 16. Election results will be reported directly from SBS to the IAFP Teller who will report directly to President Vickie Lewandowski. Watch for the election results on the IAFP Web site in April and also in the April *IAFP Report* and the May issue of *Food Protection Trends*.

If you have questions about the election process, contact David W. Tharp, CAE, Executive Director at +1 800.369.6337; +1 515.276.3344 or E-mail: dtharp@foodprotection.org.

International Association for Food Protection®