Heat Resistance of an Outbreak Strain of *Listeria monocytogenes* in Hot Dog Batter

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ABSTRACT

The heat resistance of a strain of *Listeria monocytogenes* responsible for a listeriosis outbreak in hot dogs was not higher than the heat resistance of other *L. monocytogenes* strains when tested in tryptic soy broth and in laboratory-prepared hot dog batter. For the thermal death time experiments, the cells were grown to stationary phase or were starved in phosphate-buffered saline, pH 7, for 6 h at 30°C. Starvation increased the heat resistance of *L. monocytogenes* in broth but not in hot dog batter. *D*-values in hot dog batter were higher than in broth. For the hot dog formulation used in this study, cooking the hot dog batter for 30 s at 71.1°C (160°F), or its equivalent using a *z*-value of 6°C (11°F), would inactivate 5 logs of *L. monocytogenes*. In late 1998, an outbreak of listeriosis was traced to hot dogs produced by a manufacturer in Michigan. The factor that initially led to recognition of the outbreak was the unique genetic profile of the *Listeria monocytogenes* strain. The pulsed-field gel electrophoresis analysis profile (pattern E) is relatively rare among clinical isolates of *L. monocytogenes*. Consequently, it was somewhat easy to link sporadic cases in 22 states to a single source. The events that actually resulted in the finished product being contaminated with *L. monocytogenes* may never be known. One hypothesis is that construction in the postprocessing environment led to contamination of product contact surfaces and subsequently to postprocessing contamination of product (9). The unusual nature of the strain’s pulsed-field gel electrophoresis pattern also led to speculation that the pathogen may have had atypical heat resistance characteristics, survived the hot dog cooking process, and thus contaminated the finished product. Because *L. monocytogenes* is among the pathogens that the hot dog cooking process is designed to destroy, the heat resistance of the strain was tested to determine if the adequacy of the hot dog cooking process could have been overcome.

While most laboratory studies on microbial heat resistance have been performed using stationary-phase cells, recent studies have shown that *L. monocytogenes* demonstrates an increased heat resistance after exposure to stress conditions (6, 8). *L. monocytogenes* cells subjected to sublethal treatments of osmotic shock (4), heat shock (5), ethanol, or acid (6) demonstrated increased heat resistance values. This adaptation or tolerance does not seem to be specific to a particular stress and may induce cross-protection to other factors.

Starvation is a stress condition that may induce increased heat resistance properties in *L. monocytogenes*. A laboratory-induced starvation treatment is analogous to cells surviving cleaning and sanitizing operations on raw meat processing equipment and being deprived of nutrients long enough to induce the stress response. Consequently, stressed cells also warranted evaluation.

The purpose of this study was to compare the heat resistance of the *L. monocytogenes* pattern E strain to that of a composite of other strains, after stationary phase of growth and starvation, in laboratory media and hot dog batter. The results presented also may help hot dog processors validate their cooking process.

MATERIALS AND METHODS

Bacterial strains and culture conditions. *L. monocytogenes* pattern E strain was obtained from Dr. Bala Swaminathan of the Centers for Disease Control and Prevention, Atlanta, Ga. *L. monocytogenes* strains from the National Food Processors Association’s culture collection were used for comparison and included strain N-7004 (Scott A), a clinical isolate of *L. monocytogenes* (serotype 4b); strain N-7285, a clinical isolate (serotype 1/2a); and four strains related to meat and poultry processing: N-7278, an isolate from beef wiener batter; N-7203, isolated from hard salami; and strain N-7175, an environmental isolate from a meat plant (serotype 1/2b). Working cultures of the strains were maintained as slants on tryptic soy agar (TSA; Difco Laboratories, Detroit, Mich.) at 4°C. For a stationary-phase inoculum, the strains were individually grown overnight (16 to 18 h) at 30°C in tryptic soy broth (TSB; Difco) supplemented with 0.2% dextrose and 0.1% yeast extract. Starved cells were prepared by centrifuging the overnight cultures, washing, and resuspending the pellets in phosphate-buffered saline, pH 7, for 6 h at 30°C (1). The outbreak strain was tested separately. The comparison strains were composited by combining equal volumes of each culture. Each strain...
was serially diluted in 0.1% peptone and enumerated on TSA before compositing to ensure approximately equal numbers of each strain in the composite. All plates were counted after 48 h incubation at 30°C.

Hot dog batter preparation and inoculation. Roast beef and beef fat were purchased refrigerated at a local supermarket. To decrease the indigenous flora, the beef roast was wrapped in aluminum foil, steamed for 5 min at 121°C, and cooled quickly in an ice bath. The cooked outside layer was aseptically removed and discarded, and only the raw internal meat was used in the experiments. Big pieces of fat were not heated, but the outside layer was removed aseptically. The beef was ground with 30% fat and mixed to a homogenous appearance.

After the meat and fat were blended, the mix was separated into two stomacher bags containing 250 g each. A 25-g bag was also prepared and used for controls. One 250-g bag was inoculated with the pattern E strain (stationary-phase or starved cells) and the other bag with the composite of the six strains (stationary-phase or starved cells) to yield inoculum levels of $10^5$ cells per gram. Bags were stomached and massaged thoroughly to distribute the inoculum. The cure was then added and the bags were again stomached and massaged thoroughly.

The cure was formulated to yield the following concentrations in the hot dog batter: 156 ppm sodium nitrite (J. T. Baker, Phillipsburg, N.J.), 2% NaCl (Sigma Chemical Co., St. Louis, Mo.), 1.5% glucose (Sigma), 550 ppm erythorbate (Jiangxi Bexing, China) and 0.5% sodium tripolyphosphate (Nutrafos 088; Monsanto). The last two chemicals were supplied by a meat processor.

Microbial counts were determined on the uninoculated meat and fat mix, the mix after inoculation, and the mix after inoculation and addition of the cure. Duplicate 1-g samples from different areas of the bags were mixed with 9 ml of peptone water. After appropriate dilutions in peptone water, the samples were spread-plated on TSA (Difco) and on Oxford medium base with Oxford antimicrobial supplement (Difco) and incubated at 30°C for 24 h. The natural flora of the meat were identified by testing isolated colonies with a Vitek model 32 (bioMérieux, Vitek, Inc., Hazelwood, Mo.) following the manufacturer's procedures with GNI+ and GPI cards.

Heat resistance studies. Heat resistance studies in hot dog batter and in broth were conducted using an end-point procedure. For studies in hot dog batter, 3-g samples were distributed into individual polyster pouches (4 by 4 in.) (Kapax Corporation, Minneapolis, Minn.). Pouches were flattened to 0.4 to 0.6 mm to exclude air and to minimize come-up time. They were heat sealed and held at room temperature until testing (less than 20 min). One pouch per run was fitted with a type-T thermocouple, and temperature was monitored during heating using a datalogger (Kaye Instruments, Bedford, Mass.). Stationary-phase cells and starved cells were run on different days. Pouches were inserted in holders, suspended from a rod maintaining equal distances among them for water circulation, and fully immersed in a thermostatically controlled water bath (model DL 30; Haake, Saddle Brook, N.J.) preheated to the desired temperature. A weight added to the bottom of each pouch maintained the pouches vertically and flat in the water bath. Pouches were moved backward and forward in the water bath during the first minute to assure a rapid come-up time and homogeneous temperature distribution. Twenty pouches inoculated with the outbreak strain and an equal number inoculated with the L. monocytogenes composite were heated simultaneously. At designated times, five pouches of each inoculum were removed and cooled by immersing in ice water. Each pouch was opened aseptically, mixed with 10 ml of Listeria enrichment broth (Difco), rescaled, and incubated at 30°C. After 48 h, pouches were massaged to mix the contents, opened aseptically, and 0.1 ml was spread onto Oxford antimicrobial supplement and incubated at 30°C for 24 h. Triplicate experiments were performed at each test temperature (58, 62, and 66°C). Negative controls were prepared by filling five pouches with the uninoculated product and sealing them as above. Five pouches containing the inoculated product were designated as positive controls. Positive and negative controls were not heated but were enriched and incubated as for the test samples.

Heat resistance studies in broth were conducted in three-neck flasks (1,000 ml). The flasks were fitted with a thermocouple through a cotton plug in one of the openings, cotton plugs in the other two openings, and a stir-bar, and were autoclaved before use. TSB (150 ml) was dispensed aseptically into the flask. The flask was immersed in the water bath to a depth above the level of the broth, and allowed to equilibrate. The broth was agitated constantly with a submersible stirrer (model 230; VWR Scientific, Bridgeport, N.J.) and the temperature monitored. When thermal equilibrium was achieved, 1 ml of the inoculum was added to the flask to obtain a final concentration of approximately $10^5$ cells per ml. At five predetermined time intervals, 6-ml samples were removed with a single-use sterile pipette and dispensed into an empty test tube in an ice-water bath. The tube was shaken in the ice-water bath to cool the broth rapidly, and 1-ml aliquots were dispensed into five tubes containing 9 ml of TSB supplemented with 0.2% dextrose and 0.1% yeast extract and incubated at 30°C. Duplicate experiments were performed at each test temperature (56, 58, 60, 62, and 66°C). Negative controls were prepared by dispensing 1 ml of the unheated, uninoculated broth into each of five tubes containing 9 ml of TSB supplemented with 0.2% dextrose and 0.1% yeast extract. Positive controls were prepared similarly, using unheated, inoculated broth. Positive and negative controls were incubated along with the test samples.

To determine the initial number of cells, the inoculum was pour-plated with tempered TSA agar after appropriate decimal dilutions and incubated overnight at 30°C.

Calculation of $D$- and $z$-values. At each test temperature, decimal reduction times ($D$-values, in minutes) were determined by the formula:

$$D = \frac{t}{\log_{10}A - \log_{10}B},$$

where $t$ equals heating time in minutes; $A$ equals the initial number of L monocytogenes in the flask or pouch; and $B$ equals the final number of L monocytogenes in the flask or pouch at the last time point demonstrating L monocytogenes survivors. $B$ was estimated by the most probable number method of Halvorson and Zeigler (3), using the formula $B = \ln(N/1)$. The following example illustrates the methodology: Pouches with an initial concentration of $1.0 \times 10^5$ L monocytogenes cells/pouch were heated at 62°C, and five pouches were removed after 5, 10, 15, and 20 min. L monocytogenes was recovered from all pouches at 5 and 10 min, from only four pouches at 15 min, and from no pouches at 20 min. Therefore, $B$ at 15 min equals: $\ln(N/1) = 1.61; and D at 62°C equals: 15/\log(1.0 \times 10^5) - \log 1.61 = 3.13$ min. In experiments where all samples were L monocytogenes-positive at one time interval and all negative at the next time interval, D was estimated for the run by assuming one negative at the last all-positive time interval, and one positive at the first all-negative time interval, calculating separate $D$-values for each assumption, and averaging. The $z$-values were calculated as the negative in-
versus slope of the linear regression line for the log $D$-values over the range of heating temperatures tested.

$D$- and $z$-values for the outbreak strain were statistically compared to the respective values for the L. monocytogenes composite using Student’s $t$ test. Similar comparisons were made between stationary-phase and starvation-treated cells.

**RESULTS AND DISCUSSION**

The raw meat and fat, handled as described above, produced a ground product almost free of indigenous flora (<10 CFU/g). Microorganisms detected included Leclercia, Serratia, and Enterococcus species.

Two independent experiments were done in TSB and the averages are summarized in Table 1. Experiments in hot dog batter were done in triplicate and are presented in Table 2. Come-up times were instantaneous in TSB and negligible (<20 s) compared to heating times in hot dog batter.

$D$-values for the outbreak strain in broth and in hot dog batter were significantly lower ($P < 0.05$) than the $D$-values of the L. monocytogenes composite. Starvation of the cells resulted in an increased heat resistance when this was tested in broth, but only a slight increase in hot dog batter. This was true for the L. monocytogenes composite and the outbreak strain. For both the outbreak strain and the composite the heat resistance was higher in the cured meat than in TSB.

The $z$-value calculated for stationary-phase cells of the outbreak strain was approximately the same as the $z$-value calculated for stationary-phase cells of the composite. For both the composite and the outbreak strain the $z$-values for starved cells were also similar to the $z$-values for stationary cells (Tables 1 and 2).

The results obtained in broth, as well as in hot dog batter, clearly show that this new strain is not more heat resistant than the other L. monocytogenes strains tested. The similarity in $z$-values indicates that the strain is likewise not more heat resistant at temperatures other than those tested. The speculation that the L. monocytogenes pattern E strain has exceptional heat-resistance properties that may compromise commercial hot dog cooking processes is not supported by these results.

In developing critical limits for a cooking critical control point, processors need to consider the heat resistance values of the target pathogen (generally the most heat-resistant pathogen likely to occur) and the minimum number to be destroyed by the process. For hot dog processes where L. monocytogenes is the target organism, and where the number of L. monocytogenes coming into the process from the raw materials is not strictly controlled, a cooking process sufficient to destroy 4 to 5 logs of the organism; i.e., a $4D$ or $5D$ process, should be adequate. L. monocytogenes levels of 5 logs or higher are not reasonably likely to occur in hot dog batter in a facility operating under good manufacturing practices, even if the L. monocytogenes levels in the raw materials are not monitored.

A process to inactivate 5 logs of heat-shocked cells of L. monocytogenes Scott A in a brined ham containing 120 ppm nitrite was calculated from reported $D$-values to require 23.5 s at 71.1°C (160°F) (2). However, higher heat-resistance data have been reported in cured meats. A $5D$ processing time ranging from 71 to 174 s at 71.1°C (160°F), depending on the recovery medium used, can be calculated from data reported for stationary-phase L. monocytogenes in fermented beaker sausage (10). A process for raw meat was calculated at 62 s at 70°C (158°F), and addition of cure increased the heat resistance of L. monocytogenes (7).

For the hot dog formulation used in this study, based on the heat resistance of the starved L. monocytogenes composite in hot dog batter, a cooking process where the cold spot (i.e., slowest heating point) in the hot dog achieves 71.1°C (160°F) for 30 s, or its equivalent using a $z$-value.

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**TABLE 1. Heat resistance of L. monocytogenes composite and outbreak strain in TSB**

<table>
<thead>
<tr>
<th>Culture</th>
<th>Condition</th>
<th>56</th>
<th>58</th>
<th>60</th>
<th>62</th>
<th>66</th>
<th>$z$-value (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composite</td>
<td>Stationary</td>
<td>15</td>
<td>5.9</td>
<td>3.1</td>
<td>0.9</td>
<td>0.18</td>
<td>5.2</td>
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<td></td>
<td>Starvation</td>
<td>ND</td>
<td>11.2</td>
<td>ND</td>
<td>2.1</td>
<td>0.29</td>
<td>5.1</td>
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<tr>
<td>Outbreak strain</td>
<td>Stationary</td>
<td>8.2</td>
<td>2.9</td>
<td>1.0</td>
<td>0.3</td>
<td>0.08</td>
<td>4.9</td>
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<tr>
<td></td>
<td>Starvation</td>
<td>ND</td>
<td>4.4</td>
<td>ND</td>
<td>0.7</td>
<td>0.12</td>
<td>5.1</td>
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</tbody>
</table>

a Average of two independent experiments.
b ND, not determined.

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**TABLE 2. Heat resistance of L. monocytogenes composite and outbreak strain in hot dog batter**

<table>
<thead>
<tr>
<th>Culture</th>
<th>Condition</th>
<th>58</th>
<th>62</th>
<th>66</th>
<th>$z$-value (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composite</td>
<td>Stationary</td>
<td>14.6</td>
<td>3.2</td>
<td>0.65</td>
<td>5.9</td>
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<tr>
<td></td>
<td>Starvation</td>
<td>15.5</td>
<td>3.3</td>
<td>0.70</td>
<td>6.0</td>
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<tr>
<td>Outbreak strain</td>
<td>Stationary</td>
<td>11.0</td>
<td>1.8</td>
<td>0.40</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>Starvation</td>
<td>8.1</td>
<td>1.8</td>
<td>0.23</td>
<td>5.2</td>
</tr>
</tbody>
</table>

a Average of three independent experiments ± SD.
of 6°C (11°F), would be sufficient to inactivate 5 logs of *L. monocytogenes*. Processes designed to inactivate less than 5 logs of *L. monocytogenes* may also be adequate to assure product safety, depending on the maximum level of the target organism likely to occur in the raw materials.

REFERENCES