Pathogenicity of *Listeria monocytogenes* Grown on Crabmeat

ROBERT E. BRACKETT* AND LARRY R. BEUCHAT

Department of Food Science and Technology, Agricultural Experiment Station, University of Georgia, Griffin, Georgia 30223

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The pathogenicity of *Listeria monocytogenes* as influenced by growth on crabmeat at 5 and 10°C was studied. Crabmeat was inoculated with *L. monocytogenes* V7 (ca. 10⁴ CFU/g) and incubated for up to 14 days at 5 and 10°C. At selected incubation times, *L. monocytogenes* was removed from crabmeat by washing with 0.1 M potassium phosphate buffer (pH 7.0), and populations were determined by surface plating on LiCl-phenylethanol-moxalactam agar. Buffered suspensions were then centrifuged, and the resulting pellets were suspended in phosphate buffer containing 10% glycerol and stored at ~18°C. Thawed, diluted suspensions of cells were tested for pathogenicity by intraperitoneal injection into immunocompromised and nonimmunocompromised mice. *L. monocytogenes* cells recovered from crabmeat and then recultured in tryptose phosphate broth (TPB), as well as cells which had not been passed through crabmeat but had been cultured in TPB, were likewise harvested, suspended in buffered 10% glycerol, frozen, thawed, diluted, and tested for pathogenicity by intraperitoneal injection. Growth on crabmeat at 5 and 10°C did not have a significant effect on pathogenicity. The population of *L. monocytogenes* necessary to kill about 50% of the immunocompromised mice in each test set within 7 days was about 10⁵ CFU, and this result was not significantly affected by storage temperature of the crabmeat or type of substrate, i.e., crabmeat or TPB, on which it had grown.

*L. monocytogenes* is a gram-positive, nonsporulating bacterium which has recently become firmly established as a food-borne pathogen. Listeriosis outbreaks in North America have resulted from consumption of contaminated vegetables (12), pasteurized milk (5), soft cheese (2), and, most recently, turkey frankfurters (3). The organism is of particular concern to the food industry and public health regulatory agencies because it can grow at refrigeration temperatures (4°C) and is widespread in the environment (1). Moreover, *L. monocytogenes* has been detected in virtually every food group.

The role of seafoods in listeriosis is less clearly established than the roles of other types of foods. Weagant et al. (15) reported that at least 26% of frozen seafood products contain *L. monocytogenes*. The bacterium was detected in raw shrimp and lobster, fin fish, surimi-based seafoods, and cooked shrimp and crabmeat. Lennon et al. (11) suggested that raw seafoods may have played a part in some listeriosis cases in New Zealand. However, raw seafoods may actually pose less hazard than cooked seafoods and surimi, since they are usually subjected to thermal treatment sufficient to kill *L. monocytogenes* immediately before being consumed. Surimi and seafoods cooked and offered for sale at retail outlets are often stored refrigerated for extended periods (2 weeks or more), increasing the potential for growth of *L. monocytogenes* and subsequent consumption.

Despite the common occurrence of *L. monocytogenes* in various types of foods, little information describing changes in pathogenicity which might occur upon growth of the organism in foods has been reported. Schlech et al. (W. F. Schlech, D. Spence, S. Sperker, and A. Badley, Abstr. 10th Int. Symp. Listeriosis, p. 59, 1988) suggested that growth in dairy products may enhance the ability of *L. monocytogenes* to infect humans and thereby cause disease. Whether growth on other types of foods may also result in increased or decreased pathogenicity is not known.

Objectives of the work reported in this paper were to determine growth patterns of *L. monocytogenes* in crabmeat stored at 5 and 10°C and to quantify any resulting changes in pathogenicity.

**MATERIALS AND METHODS**

*Strain of *L. monocytogenes* used.* *L. monocytogenes* V7 (milk isolate; serotype 1), provided by the U.S. Food and Drug Administration, Washington, D.C., was used in all experiments. Stock cultures were grown (24 h, 30°C) and maintained (4°C) on tryptose phosphate agar (pH 7.3; TPA) slants. Cultures were activated in tryptose phosphate broth (pH 7.3; TPB) at 30°C with a minimum of three successive loop transfers at 24-h intervals before use as an inoculum for crabmeat and before analysis for morphological, biochemical, and pathogenicity characteristics.

**Preparation of crabmeat.** Fresh claw crabmeat was purchased at a local market and stored under refrigeration (4°C) until initiation of experiments.

Crabmeat was distributed in a shallow pan, mixed thoroughly, and then evenly spread in a layer approximately 2 cm thick. The crabmeat was then baked uncovered at 177°C to an internal temperature of 80°C and stored overnight (16 to 18 h) at 5°C before inoculation with *L. monocytogenes*.

**Inoculation of crabmeat.** Each of two (5°C storage) or three (10°C storage) 800-g lots of baked crabmeat was aseptically weighed into sterile 4-liter beakers. One (5°C storage) or two (10°C storage) lots were then inoculated with 16.0 ml of a 24-h culture of *L. monocytogenes* diluted in 0.1 M potassium phosphate buffer (pH 7.0) to yield about 10⁴ viable *L. monocytogenes* cells per g of crabmeat. Control lots were prepared by adding sterile buffer instead of *L. monocytogenes* suspension to crabmeat.

Thoroughly mixed 50-g portions of inoculated and control lots of crabmeat were dispensed into plastic zip-lock storage bags and incubated at either 5 or 10°C.

**Procedure for harvesting *L. monocytogenes* from crabmeat and TPB.** After 0, 2, 5, 8, 12, and 14 days of storage, duplicate bags of both inoculated and control crabmeat were

* Corresponding author.
analyzed for *L. monocytogenes* populations. Potassium phosphate buffer (0.1 M, pH 7.0, 100 ml) was combined with each 50-g sample, and the mixture was gently massaged for 1 min. The contents of each bag were filtered through sterile glass wool, and the filtrate was collected in 250-ml centrifuge bottles. Samples were centrifuged at 4,000 × g for 10 min at 25°C. The supernatant fluid was decanted, and the pellet was suspended in 5 ml of 0.1 M potassium phosphate buffer (pH 7.0) containing 10% glycerol. The population of *L. monocytogenes* in buffered glycerol was determined by surface plating duplicate serially diluted 0.1-ml quantities on LiCl-phenylethanol-moxalactam (LPM) agar (10). The remainder of the suspension was stored at −18°C until used in pathogenicity tests.

Cells recovered from crabmeat and recultured on TPB, as well as cells which had not originated from crabmeat but were cultured in TPB, were tested for pathogenicity. Duplicate 10-ml TPB cultures (24 h, 30°C) were centrifuged at 4,000 × g for 10 min at room temperature. The supernatant fluid was decanted, and the pellet was suspended in 10 ml of buffer. The suspension was again centrifuged, and the supernatant fluid was discarded. The pellet resulting from the second centrifugation was suspended in 5.0 ml of buffered 10% glycerol solution. The population of *L. monocytogenes* in this suspension was determined, and the remaining portion was stored at −18°C as described above for experiments using crabmeat. In experiments designed to confirm the pathogenicity of *L. monocytogenes*, dilutions (10^6 to 10^−5) of suspensions collected from TPB cultures were made before cells were suspended in buffered 10% glycerol and stored (10 days) at −18°C.

**Enumeration of *L. monocytogenes*.** Viable populations of *L. monocytogenes* in buffered glycerol suspensions were determined by surface plating duplicate serially diluted 0.1-ml quantities on LPM agar. Plates were incubated at 30°C for 44 to 48 h and examined for colonies of *L. monocytogenes*. Presumptive *L. monocytogenes* colonies were counted. Tests used for confirmation of *L. monocytogenes* were as described by Golden et al. (7).

**Procedure for testing pathogenicity.** Pathogenicity of cells was tested by the basic procedure described by Stelma et al. (14). Frozen *L. monocytogenes* cell suspensions from inoculated crabmeat and TPB were quickly thawed by immersing tubes in water at 20°C. Suspensions were appropriately diluted in 0.1 M potassium phosphate-buffered saline (pH 7.3; PBS) so that the final population of viable cells was about 10^3 CFU/ml. Control buffer washes from uninoculated crabmeat were diluted in PBS in the manner used to dilute cell suspensions harvested from crabmeat and TPB. Viable populations in PBS were determined by surface plating appropriate dilutions (0.1 ml) on LPM agar.

Pathogenicity was determined by intraperitoneal (i.p.) injection of 0.1 ml of PBS suspensions (including control washes) into both immunocompromised and nonimmunocompromised male Swiss mice (18 to 20 g). A portion of the mice had been immunocompromised by injection (i.p.) with carrageenan (Sigma type II, 200 mg/kg of body weight) 24 h before injection with test suspensions of *L. monocytogenes*. Sets of five immunocompromised and five nonimmunocompromised mice were used to test each suspension. Inoculated mice were observed over a 7-day period, and deaths were recorded daily. Fluid from spleens of all mice was streaked on LPM agar on day 7 to determine the presence of *L. monocytogenes*. Representative presumptive colonies of *L. monocytogenes* were screened for identity by noting cellular morphology, tumbling motility, and umbrella-shaped growth in motility medium.

**Statistical analysis.** Two replicates of each experiment were done for each storage temperature. However, we observed unusual levels of pathogenicity in *L. monocytogenes* isolated from crabmeat stored at 10°C. Therefore, two additional replicates of pathogenicity tests were done using these isolates to minimize effects of any experimental error. Analysis of variance was done to determine significant (P ≤ 0.05) differences in transformed proportions of dead mice. This transformation was done by taking the arcsine of the square root of the proportion of dead mice in each treatment group. Such a procedure is done to allow analysis of variance to be used for small sets of binomial data (8).

**RESULTS**

**Pathogenicity of *L. monocytogenes* V7.** Preliminary experiments using a limited number of mice confirmed that *L. monocytogenes* V7 was pathogenic to immunocompromised mice receiving an i.p. injection of 4.9 × 10^3 or more viable cells (Table 1). In contrast, only inocula containing 4.9 × 10^2 or more cells resulted in deaths of nonimmunocompromised mice.

Preliminary experiments also revealed that i.p. inoculation of mice with 0.1 ml of either buffered 10% glycerol or buffered saline did not result in death.

**Changes in pathogenicity as affected by growth on crab-**

![Graph](https://www.asm.org/content/56/4/1217.f1)

**FIG. 1.** Growth of *L. monocytogenes* V7 in crabmeat stored for up to 14 days at 5 and 10°C.

**TABLE 1. Pathogenicity of *L. monocytogenes* V7**

<table>
<thead>
<tr>
<th>Population of viable cells in inoculuma</th>
<th>Immunocompromisedb</th>
<th>Not immunocompromisedc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tested</td>
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</tr>
<tr>
<td>4.9 × 10^8</td>
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<td>2</td>
</tr>
<tr>
<td>4.9 × 10^7</td>
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</tr>
<tr>
<td>4.9 × 10^6</td>
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<td>4.9 × 10^5</td>
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</tr>
<tr>
<td>4.9 × 10^4</td>
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<td>4.9 × 10^3</td>
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<td>0b</td>
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* a 0.1 ml of test suspension was injected i.p.
  b Treated with carrageenan.
  c Number of deaths within 7 days; two mice were inoculated in each test.
  d Crabmeat was inoculated with buffered saline.
<table>
<thead>
<tr>
<th>Storage temp (°C)</th>
<th>Source of inoculum&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Storage time (days)</th>
<th>Inoculum population&lt;sup&gt;b&lt;/sup&gt;</th>
<th>No. of mice</th>
<th>Immunocompromised&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Not immunocompromised&lt;sup&gt;d&lt;/sup&gt;</th>
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<td>14</td>
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<sup>a</sup> 0.1 ml of buffer wash from inoculated and uninoculated crabmeat was injected i.p.
<sup>b</sup> Log<sub>10</sub> L. monocytogenes cells in inoculum.
<sup>c</sup> Treated with carrageenan.
<sup>d</sup> Number of deaths within 7 days.

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**TABLE 3. Pathogenicity of L. monocytogenes V7 recultured in TPB after isolation from crabmeat stored at 5 and 10°C**

<table>
<thead>
<tr>
<th>Source of inoculum&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Storage time (days)</th>
<th>Inoculum population&lt;sup&gt;b&lt;/sup&gt;</th>
<th>No. of mice</th>
<th>Immunocompromised&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Not immunocompromised&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Tested</td>
<td>Dead&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td>Recultured crabmeat isolates</td>
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<td>(5°C storage)</td>
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<td>(10°C storage)</td>
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<td>20&lt;sup&gt;f&lt;/sup&gt;</td>
<td>16</td>
<td>20&lt;sup&gt;f&lt;/sup&gt;</td>
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<sup>a</sup> 0.1 ml of test suspension injected i.p.
<sup>b</sup> Log<sub>10</sub> L. monocytogenes cells in inoculum.
<sup>c</sup> Treated with carrageenan.
<sup>d</sup> Number of deaths within 7 days.
<sup>e</sup> Four isolates tested, five mice per isolate.
meat. *L. monocytogenes* V7 grew on crabmeat stored at 5 and 10°C, ultimately reaching populations of 4.9 × 10^6 and 2.0 × 10^9, respectively (Fig. 1). Results of tests designed to determine pathogenicity of cells in buffer washes from inoculated and uninoculated crabmeat stored at 5 and 10°C are shown in Table 2. As expected, buffer washes from crabmeat inoculated with *L. monocytogenes* had significantly greater pathogenicity than uninoculated control washes.

(i) Effect of temperature. Pathogenicity of *L. monocytogenes* V7 cells isolated from crabmeat stored at 5 and 10°C did not differ significantly. Fewer deaths than expected were observed in the first two replicate experiments with mice injected with buffer washes from inoculated crabmeat stored at 10°C. However, additional pathogenicity tests using the same washes revealed that cells from crabmeat stored at 10°C were similar to those isolated from crabmeat stored at 5°C. Statistical analysis of combined pathogenicity data (four replicates) from 10°C compared with 5°C storage experiments indicated that temperature did not significantly affect pathogenicity.

(ii) Effect of time. Pathogenicity of *L. monocytogenes* V7 was not significantly affected by the length of time the organism was grown on crabmeat, regardless of incubation temperature or immune state of mice. The number of deaths was significantly higher in immunocompromised mice, regardless of temperature at which crabmeat had been stored. Although pathogenicity to immunocompromised mice appeared to be amplified on day 14 of storage at 10°C, differences were not significant.

(iii) Effect of reculturing *L. monocytogenes* isolates. Pathogenicity of recultured cells from crabmeat which had been stored at 5 or 10°C did not significantly differ from that of cells directly isolated from crabmeat or from that of cells which had not originated from crabmeat (Table 3).

*L. monocytogenes* was isolated from spleen fluids of mice inoculated with buffer washes from inoculated crabmeats. Although *L. monocytogenes* was not isolated from spleens of mice which had been inoculated with control washes, a large, spherical yeast-like organism was detected. This organism was prevalent in spleens of several immunocompromised mice inoculated with control washes from crabmeat stored at 10°C.

**DISCUSSION**

On the basis of statistical analysis of data, the hypothesis that growth of *L. monocytogenes* on crabmeat changes pathogenicity must be rejected. Moreover, the data indicate no trends that would suggest that storing crabmeat at 5 or 10°C for up to 14 days would result in even a slight negative effect on pathogenicity.

Some have speculated that refrigerated foods might constitute a greater hazard for listeriosis than nonrefrigerated foods. This possibility is based on reports (4, 6, 9, 13) indicating that production of hemolysin, often correlated with pathogenicity of *L. monocytogenes*, is greater at 4°C than at 37°C. Indeed, Wood and Woodbine (16) noted that one strain of *L. monocytogenes* killed more mice when it had been cultured at 4°C than when it had been cultured at 37°C. However, a second strain of *L. monocytogenes* did not show this effect.

Although statistical analysis of data indicates that there is no significant effect of test temperatures on pathogenicity, results do suggest that *L. monocytogenes* might have been approaching a more pathogenic state. This possibility is strengthened by the fact that fewer (0.5 to 1 log) *L. monocytogenes* cells recovered from crabmeat stored at 5°C were injected into mice compared with inocula from crabmeat stored at 10°C. However, a slightly higher proportion of test mice died when inoculated with *L. monocytogenes* grown on crabmeat stored at 5°C compared with mice injected with inoculum from crabmeat stored at 10°C.

In summary, results of this study indicate that growth of *L. monocytogenes* on crabmeat at 5 and 10°C does not result in reduced or increased pathogenicity. Moreover, the observation that *L. monocytogenes* grew on crabmeat during refrigerated storage suggests that the risk of consuming an infectious population increases with increased storage time.

**ACKNOWLEDGMENTS**

We thank Lynn Cheney and Brenda Nail for technical assistance and Glenn Ware for providing expertise in statistical analysis of the data.

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**LITERATURE CITED**

