Simultaneous Growth of *Listeria monocytogenes* and *Listeria innocua*

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**ABSTRACT**

*Listeria* spp. have been isolated from a wide variety of sources, and in many situations *Listeria innocua* is more commonly found than *Listeria monocytogenes*. Growth of three *L. monocytogenes* strains was studied when inoculated simultaneously with a rhamnose negative *L. innocua* strain into culture media and cheese sauce. Fraser broth (FB), Trypticase™ soy broth plus 0.6% yeast extract (TSB-YE), University of Vermont medium (UVM) modified *Listeria* enrichment broth, and cheese sauce were inoculated (ca. 10⁵ cells per ml) and incubated for 24 h; FB, TSB-YE, and cheese sauce at 35°C, UVM at 30°C. Growth of four rhamnose-positive, *L. innocua* strains was also studied in culture media. Growth of *L. monocytogenes* was similar to that for *L. innocua* in TSB-YE or cheese sauce. However, in FB and UVM, *L. innocua* populations were significantly higher than *L. monocytogenes*. This occurred when media were inoculated individually or simultaneously. This may explain in part why *L. innocua* is isolated more frequently than *L. monocytogenes* from foods and environmental samples.

Listeria spp. have been isolated from meat products (9,10), fish (6), raw eggs (11), poultry (8), fresh produce (10), and raw milk (5,13). In these studies, *Listeria innocua* was found more frequently than *Listeria monocytogenes*. This may be due to competition between the organisms.

Comparison between naturally occurring microorganisms and *L. monocytogenes* has been studied (15), and the type of organism was more important than the actual number of organisms present. Although many strains of bacteria may survive in *Listeria* enrichments, they do not necessarily compete (3) and *Listeria* spp. are still recovered by the standard Food and Drug Administration (FDA) isolation procedure (12). Duh et al. (4) found that *L. innocua* grew faster than *L. monocytogenes* at temperatures less than 40°C. This suggests that if both species were present in the same enrichment broth, *L. innocua* would grow to a higher population in a given time.

Since *L. innocua* has been isolated more frequently than *L. monocytogenes* from a variety of foods, it is of interest to determine how the two species respond when both are present in the same system. This work studied growth and population maxima of these two species when grown separately and simultaneously in *Listeria* culture media and in cheese sauce.

**MATERIALS AND METHODS**

**Strains**

Three *L. monocytogenes* and five *L. innocua* strains were used. *L. monocytogenes* Scott A and V37 were isolated from clinical and milk sources, respectively, and were obtained from the FDA Minneapolis District office. All other strains were isolated in this laboratory; *L. monocytogenes* strain 343, from a raw vegetable, and all *L. innocua* strains (#2, #3, #4, 90, and U7), from environmental samples. All strains except *L. innocua* #4 were rhamnose positive; thus *L. innocua* #4 was used as the reference strain for this work.

Cultures were maintained on tryptic soy agar (Difco) with added 0.6% yeast extract (Difco) slants at 4°C with monthly transfers. Portions of these cultures were also stored at -70°C in an 80% glycerin solution.

**Enumeration medium**

Purple agar base (10.0 g proteose peptone #3 [Difco], 1.0 g beef extract [Difco], 5.0 g NaCl [Mallinkrodt], 0.02 g brom cresol purple [Harleco], and 15 g purified agar [BBL]) in 900 ml distilled water) was sterilized at 121°C for 15 min. After cooling to 45°C, 100 ml of a filter-sterilized, 10% rhamnose (Sigma solution) was aseptically added (PABR) and plates were poured. Addition of LiCl (15 g/L, Sigma) and filter-sterilized moxalactam (2 ml/L of a 1% solution, BBL; PABR-LPM) was required for trials in cheese sauce to inhibit non-*Listeria*. The use of purified agar yielded clearer plates than commercially available dehydrated media, which aided in distinguishing rhamnose fermenters (yellow) from nonfermenters (white).

**Culture media trials**

Individual strains were grown overnight in Trypticase™ soy broth (BBL) plus 0.6% yeast extract TSB-YE, (Difco) at 35°C. Diluted cultures were inoculated (1 ml) into 100 ml TSB-YE, University of Vermont medium (UVM, BBL), or Fraser broth (FB, Difco) to achieve a population of ca. 10⁵ organisms per ml. TSB-YE and FB were incubated at 35°C, UVM at 30°C. Media were sampled periodically for up to 24 h and plated on PABR. Plates were incubated for 48 h at 35°C.
Cheese sauce trials
Six separate samples (100 g each) of cheese sauce were inoculated with ca. 100 \textit{L. monocytogenes} Scott A and \textit{L. innocua} \#4 per g. Immediately after inoculation, 25 g of each sample were cultured for the presence of \textit{Listeria} (7). Remaining portions of inoculated samples were then incubated at 35°C for 24 h, and Scott A and \#4 populations were monitored at intervals on PABR-LPM. Populations in the UVM at 30°C and in the FB at 35°C were also monitored periodically on PABR-LPM, to follow population dynamics of culture isolation procedure.

Statistical analysis
Multiple analysis of variance generated the 95% confidence intervals for means used to evaluate significant differences. Statgraphics version 5.0 (Statistical Graphics Corporation) performed these analyses on unbalanced data sets.

RESULTS

Growth in culture media
When grown individually in culture media, \textit{L. monocytogenes} and \textit{L. innocua} achieved stationary phase in 24 h. Because \textit{Listeria} isolation procedures use 24-h transfers of enrichment cultures, 24-h log\(_{10}\) populations of the two organisms were used for comparison purposes. When grown individually in TSB-YE, there was no significant difference between \textit{L. monocytogenes} Scott A and \textit{L. innocua} \#4, with mean log\(_{10}\) populations after 24 h of 9.36 and 9.59, respectively. In UVM and FB, \textit{L. innocua} \#4 reached significantly higher populations than \textit{L. monocytogenes} Scott A; 0.74 and 1.27 logs, respectively.

When \textit{L. monocytogenes} strains were grown together with \textit{L. innocua} \#4 in TSB-YE, UVM, and FB, similar results were observed (Table 1). In TSB-YE, there was no significant difference between \textit{L. monocytogenes} \#4 and two of the three \textit{L. monocytogenes} strains. However, in UVM, \textit{L. innocua} \#4 grew approximately 1 log higher than \textit{L. monocytogenes} strains. In FB, the average difference was 1.90 logs.

\textit{Listeria innocua} \#4 may not be a typical \textit{L. innocua} strain as it outgrew three of four strains studied in TSB-YE (Table 1). Conversely, in FB, growth of \#4 was not significantly different from three of the four other \textit{L. innocua} strains studied. It appears that in nonselective media such as TSB-YE, the rhamnose-negative strain may outgrow other \textit{L. innocua}, but in selective media such as FB, the growth patterns are very similar. Growth dynamics in selective media is key in isolation of organisms from food samples.

Growth in food systems
Similar growth patterns were observed for \textit{L. innocua} \#4 and \textit{L. monocytogenes} Scott A in cheese sauce. Representative data for cheese sauce sampled throughout a 24 h period with plating on PABR-LPM are presented (Fig. 1).

When samples of the inoculated cheese sauce were cultured for the presence of \textit{Listeria} using the method of Fraser and Sperber (7), only \textit{L. innocua} was isolated, even though \textit{L. monocytogenes} was also present. This occurred in six separate trials, even when the number of typical colonies picked for identification from LPM agar was increased from 4 to 10. Growth of \textit{L. innocua} \#4 and \textit{L. monocytogenes} in UVM and FB during this isolation procedure is presented (Fig 2). \textit{L. innocua} outgrew \textit{L. monocytogenes} by more than

<table>
<thead>
<tr>
<th>Medium</th>
<th>Species</th>
<th>Strain</th>
<th>n</th>
<th>Log diff.* 95% C.I.**</th>
</tr>
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<tbody>
<tr>
<td>TSB-YE</td>
<td>\textit{L. innocua}</td>
<td>#2</td>
<td>4</td>
<td>1.638 (1.138, 2.137)</td>
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<td></td>
<td></td>
<td>#3</td>
<td>4</td>
<td>0.095 (-0.404, 0.594)</td>
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<td></td>
<td></td>
<td>90</td>
<td>2</td>
<td>2.955 (2.249, 3.661)</td>
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<td></td>
<td></td>
<td>U7</td>
<td>2</td>
<td>3.200 (2.494, 3.906)</td>
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<td>4</td>
<td>0.803 (0.303, 1.302)</td>
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<td>2</td>
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<td>2</td>
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<tr>
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<td>-0.085 (-0.584, 0.414)</td>
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<td></td>
<td></td>
<td>90</td>
<td>2</td>
<td>-0.310 (-1.016, 0.396)</td>
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<tr>
<td></td>
<td></td>
<td>U7</td>
<td>2</td>
<td>0.110 (-0.596, 0.816)</td>
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<tr>
<td></td>
<td>\textit{L. monocytogenes}</td>
<td>Scott A</td>
<td>3</td>
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<td></td>
<td>#343</td>
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<td>1.363 (0.787, 1.940)</td>
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<td></td>
<td></td>
<td>V37</td>
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<td>1.027 (0.450, 1.603)</td>
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<tr>
<td>FB</td>
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<td>#2</td>
<td>4</td>
<td>0.988 (0.488, 1.487)</td>
</tr>
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<td>0.110 (-0.596, 0.816)</td>
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<tr>
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<td>\textit{L. monocytogenes}</td>
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<td>3</td>
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<tr>
<td></td>
<td></td>
<td>V37</td>
<td>3</td>
<td>2.570 (1.994, 3.146)</td>
</tr>
</tbody>
</table>

* (Log\(_{10}\) 24-h population of \textit{L. innocua} \#4) - (Log\(_{10}\) 24-h population of paired strain).

** 95% confidence intervals for means. Confidence intervals that include zero indicate no significant difference between the populations of the two strains studied after 24 h of growth.

Figure 1. Growth of \textit{L. innocua} \#4 (— — ) and \textit{L. monocytogenes} Scott A (—— ) in cheese sauce at 35°C. Points represent the average of six individual trials.

2 logs in UVM and by approximately 3 logs in FB. Similar results were observed in eight trials using sausage examined in the same way (data not shown).
Overall, *L. innocua* #4 grew to higher levels than *L. monocytogenes* in the more selective UVM and FB. In nonselective TSB-YE and food systems, there was little difference in population maxima.

*Listeria monocytogenes* was not isolated from inoculated food using conventional procedures, even with an inoculum of ca. 100 cells per g of food. Population levels of *L. innocua* were higher than those of *L. monocytogenes* when enrichment cultures were streaked on selective media for isolation. Because of this, colonies picked represented the more predominant *L. innocua* strain.

This research indicates that current cultural methods, that rely on the selective enrichment media UVM and FB for the isolation of *L. monocytogenes*, may not support recovery of the organism if significant levels of *L. innocua* are also present. Use of *L. monocytogenes* specific tests such as the Gen-Probe™ (1,14) or pathogenic *Listeria* tests such as Lister-Mac™ (2) may provide a more accurate assessment of the presence of this organism.

### DISCUSSION

Figure 2. Growth of *L. innocua* #4 (—O—) and *L. monocytogenes* Scott A (---●---) in UVM at 30°C (a) and FB at 35°C (b) during isolation procedures for inoculated cheese sauce. FB inoculated with 0.1 ml of 24-h UVM culture for six separate samples.

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### REFERENCES