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# Effect of *Listeria seeligeri* or *Listeria welshimeri* on *Listeria monocytogenes* detection in and recovery from buffered *Listeria* enrichment broth☆

Rachel C. Dailey [a], Lacinda J. Welch [a], Anthony D. Hitchins [b, 1], R. Derike Smiley [a, *]

[a] U.S. Food and Drug Administration, Arkansas Regional Laboratory, 3900 NCTR Road, Jefferson, AR 72079, USA
[b] U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, 5100 Paint Branch Parkway, College Park, MD 20705, USA

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ABSTRACT

The presence of multiple species of *Listeria* in regulated food products is not uncommon and can complicate the recovery of *Listeria monocytogenes* particularly on a non-differentiating medium. The potential complications of *Listeria seeligeri* and *Listeria welshimeri* on the recovery of *L. monocytogenes* from inoculated food test samples using the U.S. Food and Drug Administration's (FDA) selective enrichment procedure was investigated. Post-enrichment enumeration, in the absence of food product, indicates that some *L. seeligeri* and *L. monocytogenes* pairings may have population differentials as great as 2.7 ± 0.1 logs with *L. seeligeri* being the predominant species. A similar observation was noted for *L. welshimeri* and *L. monocytogenes* pairings which resulted in population differentials as large as 3.7 ± 0.2 logs with *L. welshimeri* being the predominant species. Select strain pairings were used to inoculate guacamole, crab meat, broccoli, and cheese with subsequent recovery by the FDA Bacteriological Analytical Manual (BAM) method with 10 colonies per sample selected for confirmation. The presence of *L. seeligeri* had little effect on the recovery of *L. monocytogenes*. The presence of *L. welshimeri* resulted in the failure to recover L. monocytogenes in three out of the four food matrices. This work extends the observation that non-pathogenic species of *Listeria* can complicate the recovery of *L. monocytogenes* and that competition during selective enrichment is not limited to the presence of just *Listeria innocua*.

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## 1. Introduction

The populations of *Listeria monocytogenes* in foods are typically low (<one CFU per gram) and require selective enrichment in order to achieve detectable or recoverable levels. Commonly used selective enrichment formulations do not display absolute species level specificity resulting in competition between *L. monocytogenes* and non-target background microorganisms (Dailey et al., 2014; Dallas et al., 1991; Tran et al., 1990) and between *L. monocytogenes* and the non-pathogenic species, *Listeria innocua* (Besse et al., 2005; Carvalheira et al., 2010; Curiale and Lewus, 1994; Keys et al., 2013; Petran and Swanson, 1993). Competition results in limited growth and ultimately a reduced final population of *L. monocytogenes* which lowers the overall sensitivity of subsequent detection platforms and hinders recovery. When *L. innocua* is present in the test sample, recovery of *L. monocytogenes* becomes especially problematic as both species have similar colony morphologies on isolation media such as Oxford agar and PALCAM agar. The use of species differentiating chromogenic media during streak plate isolation can help in the selection, but only if the population differential between *L. innocua* and *L. monocytogenes* is relatively small; if the population differential is large, then the resulting isolated colonies will all be the predominant species, which is typically *L. innocua* (Keys et al., 2013).

Multiple species of *Listeria* are routinely found together in foods and food processing environments and are not limited to *L. innocua* and *L. monocytogenes*. Of 100 food and food processing environmental samples that tested positive for the presence of *Listeria* (out of approximately 1600 samples tested), 54 samples resulted in the recovery of *L. monocytogenes* (unpublished data). Approximately 30% of those samples harboring *L. monocytogenes* ($n = 54$) also had one or more additional *Listeria* species recovered. Approximately 6% had two or more additional *Listeria* species recovered. Within

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* Corresponding author. Tel.: +1 870 543 4607; fax: +1 870 543 4031.
*E-mail address:* Ronald.Smiley@fda.hhs.gov (R.D. Smiley).

[1] Retired.

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**Table 1**
Generation times and 48 h selective BLEB enrichment populations for *Listeria* isolates used in this study.

| Strain[a] | Serogroup | GT[b] | 48 h Population[c] | Source[d] |
|---|---|---|---|---|
| Lm-050 | 1/2a | 1.7 ± 0.1 abc | 9.3 ± 0.1 c | RTE deli meat sandwich |
| Lm-057 | 1/2a | 1.4 ± 0.1 a | 9.1 ± 0.1 bc | Frozen blueberries |
| Lm-008 | 1/2b | 1.4 ± 0.2 a | 8.8 ± 0.2 ab | Butter |
| Lm-060 | 1/2b | 1.8 ± 0.2 bcd | 8.8 ± 0.2 ab | Frozen guacamole |
| Lm-006[e] | 4b | 1.9 ± 0.1 cde | 9.1 ± 0.2 bc | Smoked salmon |
| Lm-027[e] | 4b | 1.6 ± 0.1 ab | 9.2 ± 0.1 c | Mexican-style cheese |
| CFSAN-82[e] | 4b | 1.6 ± 0.1 ab | 8.7 ± 0.1 a | CFSAN culture collection |
| Ls-001 | – | 2.3 ± 0.1 f | 9.4 ± 0.1 c | RTE seafood sandwich manufacturer[f] |
| Ls-002 | – | 2.3 ± 0.2 f | 9.1 ± 0.1 bc | RTE seafood sandwich manufacturer[f] |
| Ls-005 | – | 2.1 ± 0.1 ef | 9.3 ± 0.1 c | Multi-product food manufacturer[f] |
| Ls-009 | – | 2.1 ± 0.1 ef | 9.3 ± 0.1 c | Multi-product food manufacturer[f] |
| Lw-001 | – | 2.0 ± 0.1 def | 9.4 ± 0.1 c | RTE deli-style sandwich manufacturer[f] |
| Lw-002 | – | 2.1 ± 0.1 ef | 9.4 ± 0.1 c | RTE tuna salad[f] |
| Lw-003 | – | 1.8 ± 0.1 bcd | 9.4 ± 0.1 c | RTE seafood sandwich manufacturer[f] |
| Lw-004 | – | 2.2 ± 0.1 ef | 9.3 ± 0.1 c | RTE seafood sandwich manufacturer[f] |

[a] Strain designations refer to ARL culture collection identification.
[b] Generation time in hours. Means within the same column followed by the same letter are not statistically different ($P < 0.05$).
[c] Log CFU/mL. Means within the same column followed by the same letter are not statistically different ($P < 0.05$).
[d] Isolates with the same description are from unrelated samples.
[e] Previously published values (Keys et al., 2013).
[f] Environmental isolate.

this same survey data, 46 samples had recoverable levels of one or more non-pathogenic species of *Listeria* only (unpublished data). Of those 46 samples, approximately 24% had two or more species of *Listeria* and 4% had three or more species of *Listeria*.

Because of the phenotypic similarities within this genus the presence of other species of *Listeria* could also complicate the recovery of *L. monocytogenes*. The focus of previous studies has been on the effects of competition between *L. innocua* and *L. monocytogenes* in various selective enrichment broth formulations with little consideration given to the other non-pathogenic species of *Listeria* especially in buffered *Listeria* enrichment broth (BLEB) (Carvalheira et al., 2010; Curiale and Lewus, 1994; Keys et al., 2013). Because *Listeria seeligeri* and *Listeria welshimeri* are periodically isolated from food products, it is of interest to determine how strains of *L. monocytogenes* respond to their presence during a 48 h selective enrichment in BLEB (Hitchins and Jinneman, 2011).

## 2. Materials and methods

### 2.1. Listeria strains

All strains (listed in Table 1) were obtained from unrelated food products or food processing environments following the procedures described in the United States Food and Drug Administration's (FDA) Bacteriological Analytical Manual (BAM) (Hitchins and Jinneman, 2011). The strain designations used in this study are culture collection identifiers specific to the Arkansas Regional Laboratory. The use of 16S rDNA sequence analysis was used to confirm the species of all isolates (Hellberg et al., 2013). Bacterial cultures were prepared and maintained as previously described (Keys et al., 2013; Dailey et al., 2014). Commercial bacterial identification kits (Vidas *Listeria*, bioMerieux; API *Listeria*, bioMerieux; MicroSeq *Listeria monocytogenes*, ABI) were used following manufacturer's instructions.

### 2.2. Generation times and selective enrichment populations

The generation times and populations following growth in buffered *Listeria* enrichment broth (BLEB) for 48 h at 30 °C were determined as previously described (Keys et al., 2013; Dailey et al., 2014). Briefly, growth was measured in 2.5 mL volumes of BLEB at 30 °C using a Synergy HT microplate reader (BioTek, Inc., Winooski, VT). Optical density measurements were collected every 0.5 h for 48 h and were then converted to log CFU per mL using a standard curve of known cell concentrations and their corresponding optical densities. The rate of growth was determined between optical densities of 0.2 and 0.4 ($\lambda$ = 600 nm) using the equation $\mu = 2.303(N_{0.4} - N_{0.2})/(t_2 - t_1)$, where $N$ is the cell population (log CFU/mL) at optical densities of 0.4 and 0.2 and $t$ is time (h). Generation times were derived from the growth rates. A one-way analysis of variance (ANOVA) with individual mean comparisons using the Tukey–Kramer multiple comparisons test was used to identify statistically significant differences between species and strains for both characteristics.

### 2.3. Inhibitory activity of L. seeligeri and L. welshimeri

A previously described, deferred antagonism plate assay was used to determine any inhibitory activity by all four strains of *L. seeligeri* and *L. welshimeri* against all six strains of *L. monocytogenes* (Bauernfeind and Burrows, 1978; Kalmokoff et al., 1999; Keys et al., 2013). Additionally, the ability of *L. monocytogenes* to inhibit the growth of *L. seeligeri* and *L. welshimeri* was assessed using the same assay.

### 2.4. Quantitative real-time PCR validation

Quantitative real-time PCR (qPCR) using the MicroSEQ® *Listeria monocytogenes* detection kit (Applied Biosystems, Inc.; Foster City, CA) was selected as the method to enumerate *L. monocytogenes* in this study. The reliability of the *L. monocytogenes* population estimates by qPCR when other species of *Listeria* are present was first determined by comparison with direct plating. *L. monocytogenes* strain CFSAN-82 was selected because it is naturally resistant to the antibiotic streptomycin sulfate; none of the other *Listeria* isolates used in this study were resistant to this antibiotic. *L. monocytogenes* CFSAN-82 was individually paired with all four strains of *L. seeligeri* and all four strains of *L. welshimeri*. Each pairing was inoculated in triplicate into 25 mL of pre-chilled ultra-high temperature (UHT) processed 2% milk and was then held for 24 h at 4 °C. The inoculation procedure has been previously described (Keys et al., 2013). The inoculated samples were blended with BLEB and incubated 48 h under selective conditions (Hitchins and Jinneman, 2011); this yielded a total of 12 observations per species pairing ($n$ = 12). The