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

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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*.

Keywords

Regulatory microbiology; Foodborne pathogen; Microbial competition; Real-time PCR; Immunofluorescence assay; Lateral flow device; Analytical microbiology; Selective enrichment; *Listeria*

☆This work is the opinion of the authors and not that of the U.S. FDA. The use of specific commercial technologies or test kits does not imply endorsement by the U. S. FDA nor does omission of similar technologies imply criticism.

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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 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 (λ = 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 total *Listeria* population was determined by surface plating onto Oxford agar. The population of *L. monocytogenes* was determined by surface plating onto Trypticase soy agar containing 0.6% yeast extract (TSAYE) which had been supplemented with 200 μg/mL streptomycin sulfate. Real-time PCR was

performed simultaneously on genomic DNA prepared from 1 mL aliquots of the 48 h enrichments using the MicroSEQ® *Listeria monocytogenes* detection kit (Applied Biosystems) on an ABI 7500 fast real-time PCR thermocycler (Applied Biosystems) following the manufacturer's recommendations. The resulting C_T values were converted to cell densities using a standard curve based on known concentrations of *L. monocytogenes* strain CFSAN-82 (determined by plate count). The mean values for the plate count data and the qPCR data were compared using a non-paired *t*-test to determine if there were statistically significant differences between the two enumeration methods.

2.5. Population differentials resulting from inter-species competition

Six strains of *L. monocytogenes* were individually paired with four strains of *L. seeligeri* and four strains of *L. welshimeri* resulting in 48 pairings total. Each pairing was used to inoculate 25 mL of pre-chilled 2% UHT processed milk; the inoculated milk was held at 4 °C for 24 h before analysis. The inoculation procedure has been previously described (Keys et al., 2013) and *L. monocytogenes* was typically added at a level that was approximately 1 log lower than *L. seeligeri* or *L. welshimeri*. Selective enrichments were prepared as described in the FDA BAM (Hitchins and Jinneman, 2011) and incubated for 48 h. A total *Listeria* species count was obtained by surface plating onto Oxford agar. The 48 h population of *L. monocytogenes* was estimated by qPCR as described in Section 2.3. The population of either *L. seeligeri* or *L. welshimeri* was determined by the difference between the total *Listeria* species count and the *L. monocytogenes* count. The population differential between *L. seeligeri* (and *L. welshimeri*) and *L. monocytogenes* is expressed in logs; a negative value indicates that *L. monocytogenes* was not the predominant species at the end of the enrichment.

2.6. Detection of L. monocytogenes from inoculated food matrices

L. monocytogenes strain Lm-008 and *L. seeligeri* strain Ls-001 were simultaneously inoculated into guacamole, crab meat, broccoli and cheese; *L. monocytogenes* strain Lm-060 and *L. welshimeri* strain Lw-004 were also used to inoculate the same matrices. These matrices were selected because they are known to routinely harbor *L. monocytogenes* or have been associated with foodborne outbreaks. These two particular *Listeria* species pairings were based on the population differentials from the experiments described in Section 2.4. Briefly, triplicate analytical portions (25 g) were inoculated with each of the two species and were then held at 4 °C for 24 h. Selective enrichments were then performed as described by the FDA BAM (Hitchins and Jinneman, 2011). Following the 48 h incubation, the presence of *Listeria* species was determined by lateral flow device (LFD) using the Reveal® 2.0 for *Listeria* (Neogen, Inc.; Lansing, MI) assay and by enzyme-linked immuno-fluorescence assay (ELFA) using the Vidas *Listeria* assay (BioMerieux, Inc.; Durham, NC). The presence of *L. monocytogenes* was determined by real-time PCR using the MicroSEQ® *Listeria monocytogenes* detection kit (Applied Biosystems) and by streak-plate analysis using Oxford agar plates. For streak-plate analysis, 10 well isolated colonies per enrichment were selected and confirmed by PCR (Keys et al., 2013) using primers originally described by Bubert et al. (1992) in place of traditional biochemical or immunological confirmation assays.

3. Results and discussion

3.1. Generation times and selective enrichment populations

The initial inoculum levels for *L. monocytogenes* ranged from 1.4 to 1.8 log CFU/mL with an overall mean of 1.6 ± 0.1. The initial inoculum levels for *L. seeligeri* ranged from 1.9 to 2.3 log CFU/mL with an overall mean of 2.2 ± 0.2. The initial inoculum levels for *L. welshimeri* ranged from 2.2 to 2.4 log CFU/mL with an overall mean of 2.3 ± 0.1. For *L. monocytogenes*, the average (3 replications) 48 h selective enrichment populations ranged from 8.8 ± 0.2 to 9.3 ± 0.1 log CFU/mL with some statistically significant strain differences being observed (Table 1). The serogroup 1/2b strains demonstrated the lowest 48 h cell densities of the *L. monocytogenes* isolates tested and were significantly ($P < 0.05$) lower than strain Lm-050 and Lm-027. The 48 h populations of the remaining four *L. monocytogenes* strains were all statistically similar ($P > 0.05$). For *L. seeligeri*, the average 48 h selective enrichment populations ranged from 9.1 ± 0.1 to 9.4 ± 0.1 log CFU/mL with all strains being statistically similar ($P > 0.05$) (Table 1). For *L. welshimeri*, the average 48 h selective enrichment populations ranged from 9.3 ± 0.1 to 9.4 ± 0.1 with all strains being statistically similar ($P > 0.05$) (Table 1). The overall mean 48 h selective enrichment populations were 9.0 ± 0.2, 9.3 ± 0.1, and 9.4 ± 0.1 log CFU/mL for *L. monocytogenes*, *L. seeligeri*, and *L. welshimeri* respectively; the final population of *L. monocytogenes* was statistically lower ($P < 0.05$) than that of *L. welshimeri* but was statistically similar ($P > 0.05$) to *L. seeligeri*.

The average (3 replications) generation times during exponential growth for *L. monocytogenes* ranged from 1.4 ± 0.2 to 1.9 ± 0.1 h with an overall mean of 1.6 ± 0.2 (Table 1). These values were similar to those previously reported for other foodborne strains of *L. monocytogenes* under similar experimental conditions (Keys et al., 2013; Dailey et al., 2014) and generally in agreement with values published by others under different experimental conditions (MacDonald and Sutherland, 1994; Gorski et al., 2006). The influence of experimental conditions on the generation times of *L. monocytogenes* has been previously discussed (Keys et al., 2013; Dailey et al., 2014). The average generation times for *L. seeligeri* ranged from 2.1 ± 0.1 to 2.3 ± 0.2 h with an overall mean of 2.2 ± 0.1 and for *L. welshimeri* the generation times ranged from 1.8 ± 0.1 to 2.2 ± 0.1 h with an overall mean of 2.0 ± 0.2 (Table 1). Statistically significant ($P < 0.05$) differences were observed between the various strains. The overall mean generation times for *L. seeligeri* and *L. welshimeri* were statistically similar ($P > 0.05$). The overall mean generation time for *L. monocytogenes* was significantly ($P < 0.05$) shorter than that of both *L. seeligeri* and *L. welshimeri*.

3.2. Quantitative real-time PCR validation

Analytical test portions of 2% UHT milk were spiked with *L. monocytogenes* strain CFSAN-82 at 2.0 ± 0.1 log CFU/mL and with *L. seeligeri* at levels ranging from 2.6 ± 0.1 to 2.7 ± 0.1 log CFU/mL. The average 48 h selective enrichment population for *L. monocytogenes* was 6.7 ± 0.2 log CFU/mL when determined by plate count and was 6.6 ± 0.2 log CFU/mL when determined by qPCR (Table 2). There was no statistically significant difference ($P > 0.05$) between the two methods. Additional milk samples were spiked with *L. monocytogenes* strain CFSAN-82 at 1.4 ± 0.1 log CFU/mL and with *L.*

welshimeri at levels ranging from 2.2 ± 0.1 to 2.9 ± 0.1 log CFU/mL. The average 48 h selective enrichment population for *L. monocytogenes* was 3.6 ± 0.4 log CFU/mL when determined by plate count and was 3.7 ± 0.5 log CFU/mL when determined by qPCR (Table 2). Again there was no statistically significant difference ($P > 0.05$) between the two methods. Real-time quantitative PCR was determined to be a suitable method for enumerating *L. monocytogenes* following competitive growth with either *L. seeligeri* or *L. welshimeri*.

3.3. Competition between L. monocytogenes and L. seeligeri

For determining population differentials, analytical test portions of 2% UHT milk were spiked with *L. monocytogenes* ranging from 1.6 ± 0.1 to 1.9 ± 0.1 log CFU/mL and with *L. seeligeri* ranging from 2.4 ± 0.1 to 3.2 ± 0.1 log CFU/mL. The population differentials were calculated as the difference between the 48 h populations, expressed in logs, between the two test species. A negative value indicates that the population of *L. seeligeri* was greater than that of *L. monocytogenes*. The population differentials ranged from 0 to −2.7 ± 0.1 (Table 3). *L. monocytogenes* strain Lm-008 was the most sensitive to the presence of *L. seeligeri* during the enrichment incubation period with population differentials that were greater than −2 logs. Strain Lm-050 was also highly sensitive to the presence of three of the four strains of *L. seeligeri* with population differentials of approximately −2 logs. The final population of *L. monocytogenes* strain Lm-050 was not affected by the growth of *L. seeligeri* strain Ls-005. *L. monocytogenes* strains Lm-027 and Lm-057 were the least sensitive to the presence of *L. seeligeri* during selective enrichment with final population differentials that were typically less than −1 log. The remaining strains of *L. monocytogenes* demonstrated intermediate levels of sensitivity that were dependent on the strain of *L. seeligeri* present during growth.

Many of the population differentials reported in Table 3 suggest that there is the potential for *L. monocytogenes* to be masked by the presence of *L. seeligeri* particularly when streak-plate isolation is being used as the detection method. To test this, four distinct food matrices were inoculated with *L. monocytogenes* strain Lm-008 and *L. seeligeri* strain Ls-001; these two strains were selected because they demonstrated the largest population differential (−2.7 logs) when simultaneously spiked into 2% UHT milk (Table 3). *Listeria* species were detected in all four food matrices by the ELFA method and in three of the four matrices by the LFD method (Table 4). It was unexpected that *Listeria* was not detected from any of the three enrichments of inoculated guacamole by the LFD method. It was subsequently established that the sensitivity of the method in pure culture was log 5 CFU/mL which was better than the manufacturer reported sensitivity of log 6 CFU/mL. Although the background microbial population was low (<2.0 log CFU/mL) for this matrix, it was determined that it was contaminated with non-*Listeria* species that were capable of growth under the selective conditions used. It has been shown that the presence of non-*Listeria* species in a food matrix can reduce the final enrichment population of *L. monocytogenes* by as much as 4 logs (Dailey et al., 2014). It is therefore likely that the presence of non-*Listeria* species prevented the levels of *Listeria* from reaching the minimum threshold required for this particular assay. *L. monocytogenes* was detected in all four matrices by both real-time PCR and streak plate

analysis using Oxford agar (Table 4). Detection by real-time PCR was expected as the sensitivity of this method was routinely log 2.0 CFU/mL or lower.

The detection of *L. monocytogenes* by streak-plate analysis was unexpected. The two strains of *Listeria* chosen for this experiment were selected based on the large population differential (−2.7 logs) that is reported in Table 3. Based on probability, a −2.7 log difference should require approximately 500 isolated colonies to be screened in order to find *L. monocytogenes*. In fact, such a differential would indicate that all of the well isolated colonies on a streak plate would likely be *L. seeligeri* and that any *L. monocytogenes* would be found in areas of the plate that demonstrate confluent growth. In this study only 10 colonies were selected for confirmation; therefore, it was expected that only *L. seeligeri* would be detected. Again, it is likely that the presence of non-*Listeria* species affected the growth patterns of both *L. monocytogenes* and *L. seeligeri* and that this was not observed with the 2% UHT milk as this product did not contain any organisms capable of growth under the selective conditions used.

3.4. Competition between L. monocytogenes and L. welshimeri

For determining population differentials, analytical test portions of 2% UHT milk were spiked with *L. monocytogenes* ranging from 1.2 ± 0.1 to 1.7 ± 0.1 log CFU/mL and with *L. welshimeri* ranging from 2.2 ± 0.1 to 2.8 ± 0.1 log CFU/mL. The 48 h selective enrichment population differentials ranged from −0.1 ± 0.2 to −3.7 ± 0.2 logs (Table 5). *L. monocytogenes* strain Lm-060 was the most sensitive to the presence of *L. welshimeri* with population differentials ranging from −1.5 ± 0.3 to −3.7 ± 0.2 logs. The sensitivity of the remaining strains of *L. monocytogenes* was highly variable and dependent on the strain of *L. welshimeri* present during the enrichment. Strain Lm-057 displayed minimal sensitivity to 3 of the 4 strains of *L. welshimeri*, however when paired with strain Lw-004 a population differential of −2.2 ± 0.2 logs was observed. *L. monocytogenes* strain Lm-008 had moderate population differentials of approximately −1 log with all 4 strains of *L. welshimeri*. Strain Lw-004 appeared to be the most influential strain of *L. welshimeri* with population differentials ranging from −0.7 ± 0.6 to −3.7 ± 0.2 logs when paired with the various strains of *L. monocytogenes*.

Eleven of the 24 *L. monocytogenes*/*L. welshimeri* pairings reported in Table 5 demonstrated population differentials of −1.0 log or greater. Such differences in the population between the two species indicate that the presence of *L. welshimeri* in a test sample might hinder the recovery of *L. monocytogenes* particularly when using a non-differentiating medium such as PALCAM agar or Oxford agar. Four particular pairings had population differentials of −2.2, −2.3, −2.5, and −3.7 logs. If streak plate analysis were being performed, these population differentials would require approximately 159, 200, 316, and 5012 colonies, respectively, to be screened to ensure that *L. monocytogenes* was recovered. *L. monocytogenes* recovery would be a difficult task even if using a *Listeria* specific agar with species differentiating ability. To test this, four distinct food matrices were inoculated with *L. monocytogenes* strain Lm-060 and *L. welshimeri* strain Lw-004; these two strains were selected because they demonstrated the largest population differential (−3.7 logs) when simultaneously spiked into 2% UHT milk (Table 5). *Listeria* species were detected in all four spiked food matrices by

ELFA which was expected as this is a genus specific assay (Table 6). However, the presence of *Listeria* species was detected in only 2 of 4 of the spiked food matrices using the genus specific LFD (Table 6). The two food matrices in which *Listeria* species were not detected were refrigerated crab meat and broccoli which had background microbial levels of 5.5 and 4.1 log CFU/g respectively. It was subsequently determined, by visual turbidity, that both food matrices contained non-*Listeria* species microorganisms capable of growth in BLEB under selective conditions. It is possible that the failure to detect *Listeria* species in these two products is because the strains of *L. monocytogenes* and *L. welshimeri* that were used for spiking did not compete well during enrichment with these background microorganisms. The reduction of the 48 h selective enrichment population of *Listeria* by non-*Listeria* species competitor microorganisms has been reported (Dailey et al., 2014). *L. monocytogenes* was detected in all four spiked food matrices by real-time PCR (Table 6). However, it was only detected in spiked cheese by spread plating when the number of colonies confirmed was limited to 10 per plate; only 2 of the 3 selective enrichments of spiked cheese were determined to be positive for *L. monocytogenes*.

3.5. Determinants of Listeria inter-species competition

Previous studies on the *Listeria* inter-species competition during selective enrichment have focused primarily on *L. monocytogenes* and *L. innocua* (Besse et al., 2005; Carvalheira et al., 2010; Cornu et al., 2002; Keys et al., 2013). In each of these studies data on growth rates and the ability to produce some type of inhibitory activity (e.g. bacteriocin or bacteriophage) by the competing strain against *L. monocytogenes* was evaluated in an effort to understand the cause and degree of competition. No clear dependence of growth rate or inhibitory activity on the post-enrichment predominant species could be established. Besse et al. (2005) noted that the predominant species at the beginning of the enrichment period was the predominant species at the end of the enrichment period.

In the present study, generation times, the ability to elicit an inhibitory response, and the 48 h population under selective enrichment conditions was compared to the level of inter-species competition between *L. seeligeri* and *L. monocytogenes* and *L. welshimeri* and *L. monocytogenes*. As in these earlier studies comparing *L. monocytogenes* and *L. innocua* competition (Besse et al., 2005; Carvalheira et al., 2010; Cornu et al., 2002; Keys et al., 2013), no clear correlation between these parameters and post-enrichment species predominance was evident and considerable strain variation was observed. However, unlike the above mentioned studies, in this study the predominant species at the start of the enrichment period was not necessarily the predominant species at the end of the enrichment period.

L. monocytogenes strain Lm-008 was the most sensitive to the presence of *L. seeligeri* during selective enrichment (Table 3); the population differentials were greater than −2 logs for all four pairings. It was not possible to attribute this to growth rate as this strain of *L. monocytogenes* had a shorter generation time than any of the four test strains of *L. seeligeri* (Table 1). Nor was it possible to attribute this to inhibitory activity by *L. seeligeri* against *L. monocytogenes* as none was observed between these particular pairings (Table 7). One possible explanation for these results can be speculated from the 48 h enrichment

populations that are presented in Table 1. The final enrichment population for strain Lm-008 was significantly ($P < 0.05$) less than 3 of the 4 strains of *L. seeligeri* and only reached 8.8 log CFU/mL. This might indicate that this strain is particularly sensitive to some parameter during the later stages of growth such as a decrease in the pH, a depletion of one or more specific nutrients, or the accumulation of a particular metabolic waste product.

Although sensitive to the presence of *L. seeligeri*, strain Lm-008 was only slightly affected by the presence of *L. welshimeri*; the population differentials were typically around −1 log or less (Table 5). It is not yet clear why strain Lm-008 was more sensitive to *L. seeligeri* than to *L. welshimeri* given the similarities of the measured experimental variables (i.e. generation time, inhibitory activity, and 48 h enrichment population) of the latter two organisms. The ability of *L. monocytogenes* to produce an inhibitory activity against *L. welshimeri* was subsequently ruled out (Table 8); only one of the four test strains of *L. welshimeri* was sensitive to the presence of *L. monocytogenes* as determined by the deferred antagonism plate assay.

L. monocytogenes strain Lm-060 was the most sensitive to competition by *L. welshimeri* during selective enrichment (Table 5). The population differentials could not be explained by differences in the generation times as *L. monocytogenes* had a significantly ($P < 0.05$) shorter generation time than two of *L. welshimeri* strains and a statistically similar generation time to the remaining two *L. welshimeri* strains. No obvious dependence of the population differentials on the ability of *L. welshimeri* to produce an inhibitory response against *L. monocytogenes* was observed (Table 7). The 48 h enrichment population of strain Lm-060, in pure culture, was however significantly ($P < 0.05$) less than the enrichment populations of all four *L. welshimeri* strains used in this study (Table 1). Again, this might indicate strain dependent sensitivities to certain growth parameters such as a decrease in pH or the depletion of particular nutrients.

3.6. Rapid screening versus isolate recovery

The ELFA-based and LFD-based methods used in this experiment were specific only to the genus level but were included to illustrate an important situation that frequently occurs in the microbiological examination of food products. The use of rapid genus-specific assays for screening analytical samples for the potential presence of *L. monocytogenes* can yield positive results, however additional testing, particularly streak-plate analysis, may only recover the non-pathogenic species. In such a situation the two results support one another (i.e. *Listeria* species were identified by both methods) with no evidence to indicate that additional *Listeria* species may be present. Under such a situation it might be concluded that the sample was free from *L. monocytogenes* when it was in fact present. The use of species-specific technologies for screening sample for the presence of *L. monocytogenes* can also result in a quagmire when subsequent streak plate analysis fails to recover the organism. This type of scenario might be interpreted as a “false positive” or as an indication of the presence of non-viable cells in the sample. Several examples in which *L. monocytogenes* was detected in a spiked matrix but was not subsequently recovered by streak plate analysis are shown in Table 6.

Although not as frequently isolated from foods as *L. innocua*, other species of non-pathogenic *Listeria* can also complicate recovery of *L. monocytogenes* through competition during the selective enrichment incubation. Several of the pairings evaluated in this study resulted in post-enrichment population differentials of greater than 2 logs and some greater than 2.5 logs. The detection of *Listeria* species by lateral flow device appeared to be hindered by the presence of non-*Listeria* microorganisms which likely affected the overall post-enrichment *Listeria* population. The recovery of *L. monocytogenes* on Oxford agar was also reduced in the presence *L. welshimeri*.

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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.

Table 2

The effects of non-pathogenic *Listeria* species on the 48 h BLEB selective enrichment population of *L. monocytogenes* CFSAN-82 as determined by plate count and quantitative real-time PCR.

	Pairing					
	L. monocytogenes/L. seeligeri			***L. monocytogenes/L. welshimeri***		
	Mean	**Range**	***n***	**Mean**	**Range**	***n***
Plate count[a,b]	6.7 ± 0.2	6.4–7.0	12	3.6 ± 0.4	2.8–4.4	12
PCR[a]	6.6 ± 0.2	6.3–6.8	12	3.7 ± 0.5	2.4–4.3	12

[a] Log CFU/mL.

[b] Determined using TSAYE supplemented with 200 μg/mL streptomycin sulfate.

Table 3

Effect[a] of *L. seeligeri* on the 48 h BLEB selective enrichment population of *L. monocytogenes* as determined by qPCR.

		L. monocytogenes					
	Serotype	**1/2a**		**1/2b**		**4b**	
	Strain	**Lm-050**	**Lm-057**	**Lm-008**	**Lm-060**	**Lm-006**	**Lm-027**
L. seeligeri	Ls-001	−2.1 ± 0.1	−0.7 ± 0.1	−2.7 ± 0.1	−1.0 ± 0.3	−0.4 ± 0.2	−0.4 ± 0.3
	Ls-002	−1.9 ± 0.1	−0.2 ± 0.2	−2.2 ± 0.1	−0.4 ± 0.1	−0.2 ± 0.1	−0.2 ± 0.1
	Ls-005	0	−0.3 ± 0.2	−2.3 ± 0.1	0	0	0
	Ls-009	−1.8 ± 0.1	−0.3 ± 0.2	−2.2 ± 0.7	−1.1 ± 0.2	−1.3 ± 0.1	−0.4 ± 0.2

[a]Post-enrichment population differentials were determined by [*L. monocytogenes*]$_{\text{Log CFU/mL}}$ − [*L. seeligeri*]$_{\text{Log CFU/mL}}$. Values reflect the mean and standard deviation of three individual enrichments.

Table 4

Incidences of *Listeria* species detection and the effect of *L. seeligeri* on the detection of *L. monocytogenes* in inoculated food matrices.

Product	Spike levels[c]		Background[c]	Incidences of detection			
	L. monocytogenes [a]	*L. seeligeri* [b]		*Listeria* species		*L. monocytogenes*	
				ELFA	LFD	Real-time PCR	Plating
Guacamole	1.7 ± 0.1	2.9 ± 0.1	<2.0	3/3	0/3	3/3	3/3
Crab meat	1.7 ± 0.1	2.9 ± 0.1	5.5 ± 0.1	3/3	3/3	3/3	3/3
Broccoli	1.4 ± 0.1	2.5 ± 0.1	4.1 ± 0.1	3/3	3/3	3/3	3/3
Cheese	1.4 ± 0.1	2.5 ± 0.1	4.2 ± 0.1	3/3	3/3	3/3	3/3

[a] Strain Lm-008.

[b] Strain Ls-001.

[c] Log CFU/mL.

Table 5

Effect[a] of *L. welshimeri* on the 48 h BLEB selective enrichment population of *L. monocytogenes* as determined by qPCR.

		L. monocytogenes					
	Serotype	**1/2a**		**1/2b**		**4b**	
	Strain	**Lm-050**	**Lm-057**	**Lm-008**	**Lm-060**	**Lm-006**	**Lm-027**
L. welshimeri	Lw-001	−0.5 ± 0.2	−0.1 ± 0.2	−0.2 ± 0.2	−1.7 ± 0.1	−1.8 ± 0.4	−0.7 ± 0.1
	Lw-002	−1.0 ± 0.2	−0.3 ± 0.3	−0.7 ± 0.1	−2.3 ± 0.1	−0.8 ± 0.3	−1.0 ± 0.1
	Lw-003	−0.9 ± 0.2	−0.2 ± 0.2	−0.7 ± 0.1	−1.5 ± 0.3	−2.5 ± 0.1	−0.9 ± 0.1
	Lw-004	−1.4 ± 0.2	−2.2 ± 0.2	−1.1 ± 0.1	−3.7 ± 0.2	−0.7 ± 0.6	−0.9 ± 0.2

[a]Post-enrichment population differentials were determined by [*L. monocytogenes*]$_{\text{Log CFU/mL}}$ − [*L. welshimeri*]$_{\text{Log CFU/mL}}$. Values reflect the mean and standard deviation of three individual enrichments.

Table 6

Incidences of *Listeria* species detection and the effect of *L. welshimeri* on the detection of *L. monocytogenes* in inoculated food matrices.

Product	Spike levels[c]		Background[c]	Incidences of detection			
	L. monocytogenes [a]	*L. welshimeri* [b]		*Listeria* species		*L. monocytogenes*	
				ELFA	LFD	Real-time PCR	Plating
Guacamole	1.0 ± 0.1	1.9 ± 0.1	<2.0	3/3	3/3	3/3	0/3
Crab meat	1.0 ± 0.1	1.9 ± 0.1	5.5 ± 0.1	3/3	0/3	3/3	0/3
Broccoli	1.0 ± 0.1	1.9 ± 0.1	4.1 ± 0.1	3/3	0/3	3/3	0/3
Cheese	1.0 ± 0.1	1.9 ± 0.1	4.2 ± 0.1	3/3	3/3	3/3	2/3

[a] Strain Lm-008.

[b] Strain Ls-001.

[c] Log CFU/mL.

Table 7

Inhibitory activity of *L. seeligeri* (*Ls*) and *L. welshimeri* (*Lw*) against *L. monocytogenes* (*Lm*) as determined by the deferred antagonism plate assay.

		Inhibition[a] of *Lm* by *Ls* and *Lw*					
	Serotype	**1/2a**		**1/2b**		**4b**	
	Strain	**Lm-050**	**Lm-057**	**Lm-008**	**Lm-060**	**Lm-006**	**Lm-027**
L. seeligeri	Ls-001	−	−	−	−	−	−
	Ls-002	−	−	−	−	−	−
	Ls-005	++	++	−	++	++	−
	Ls-009	−	−	−	−	−	−
L. welshimeri	Lw-001	−	−	−	−	−	−
	Lw-002	−	+	−	+	−	−
	Lw-003	−	−	−	−	−	−
	Lw-004	−	+	−	++	−	−

[a]Original colony size was 11 ± 1 mm; −, no zone observed; +, zone of clearing (13 ± 2 mm) approximately equal to the size of the original colony; ++, zone of clearing (29 ± 2 mm) extending beyond the original colony border.

Table 8

Inhibitory activity of *L. monocytogenes* (*Lm*) against *L. seeligeri* (*Ls*) and *L. welshimeri* (*Lw*) as determined by the deferred antagonism plate assay.

Species		Strain	Inhibition[a] of *Ls* and *Lw* by *Lm*							
			L. seeligeri				*L. welshimeri*			
			Ls-001	Ls-002	Ls-005	Ls-009	Lw-001	Lw-002	Lw-003	Lw-004
L. monocytogenes	1/2a	Lm-050	−	−	−	−	−	−	−	−
		Lm-057	−	−	−	−	−	−	−	−
	1/2b	Lm-008	−	−	−	−	+	−	−	−
		Lm-060	−	−	−	−	+	+	+	+
	4b	Lm-006	++	−	++	−	+	+	+	+
		Lm-027	++	−	++	−	+	−	+	+

[a]Original colony size was 12 ± 1 mm; −, no zone observed; +, zone of clearing (12 ± 1 mm) approximately equal to the size of the original colony; ++, zone of clearing (26 ± 1 mm) extending beyond the original colony border.