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The effects of competition from non-pathogenic foodborne bacteria during the selective enrichment of *Listeria monocytogenes* using buffered *Listeria* enrichment broth[☆]

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Abstract

The growth of *Listeria monocytogenes* during the pathogen specific enrichment of food samples can be limited by the presence of additional microorganisms that are resistant to the selective conditions being applied. If growth is severely limited and minimum post-enrichment threshold levels are not met then the presence of *L. monocytogenes* may go undetected. Several food products were screened for non-pathogenic commensal or spoilage microorganisms that are capable of growth under the conditions commonly used by regulatory testing laboratories to select for *Listeria* species. The effect of these potential competitor microorganisms on the ability to detect *L. monocytogenes* by several common molecular screening assays was then determined. Eight species of bacteria were isolated from foods that demonstrated the ability to grow in buffered *Listeria* enrichment broth under selective conditions. Growth of these competitor microorganisms during the enrichment incubation resulted in a decrease ranging from 1 to 4 logs in the 48 h population of *L. monocytogenes*. Three strains of *L. monocytogenes* representing serotypes 1/2a, 1/2b, and 4b were included in this study but no one serotype appeared to be most or least sensitive to the presence of competitor microorganisms. One additional strain of *L. monocytogenes* was identified as displaying minimal growth during the enrichment period in the presence of the *Citrobacter braakii* with the final population only reaching approximately 2.6 log CFU/ml after 48 h which was a 2 log increase over the initial population. This particular strain was subsequently shown to be difficult to detect following enrichment by an automated immunofluorescence assay and an antibody-based lateral flow device assay. In some enrichments, this strain was also difficult to detect by real-time PCR.

Keywords

Regulatory microbiology; Foodborne pathogen; Microbial competition; PCR; Real-time PCR; Lateral flow device; Automated immunofluorescence assay; Molecular screen; Analytical microbiology

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1. Introduction

The occurrences of *Listeria monocytogenes* in ready-to-eat (RTE) food products, particularly those that are preserved by refrigeration, pose a significant human health threat. Because this organism is a psychrotroph, cold storage may not be sufficient to control its growth even if the consumer properly refrigerates the contaminated product. For this reason and because of the severity of the illness caused by *L. monocytogenes*, there exists a “zero tolerance” level for this organism in RTE foods. The levels of *L. monocytogenes* in contaminated foods can be low (<1 cell/g product) and frequently selective enrichment is needed to ensure that levels of the organism reach the minimum threshold that is required by the particular recovery procedure or detection platform being used. A fundamental problem with selective enrichment procedures for *L. monocytogenes* is that an enrichment broth formulation that demonstrates absolute species-level specificity is not currently available. It has already been demonstrated that during the microbiological testing of food samples, *L. monocytogenes* can be masked by the simultaneous occurrence of the non-pathogenic bacterium *Listeria innocua* (Curiale and Lewus, 1994; Petran and Swanson, 1993).

Several studies (Curiale and Lewus, 1994; MacDonald and Sutherland, 1994; Petran and Swanson, 1993) have revealed important aspects with regards to *Listeria* intra-species competition during enrichment. First, there are strain differences with respect to the severity of the competition during growth that appear to be strongly determined by the relative resistance of the two species to the selective agents used in the enrichment broth formulation. Second, the population differentials reported suggest the potential for the failure to recover *L. monocytogenes* when *L. innocua* is present during the enrichment especially if using a non-*Listeria* species differentiating medium such as Oxford or PALCAM agars. Third, the increase in the population differentials between non-selective and selective conditions indicates that there is only a slight difference in the inherent growth rates between the two species and that the differences in the relative resistance between the two species to the selective agents used during the enrichment likely account for the population differences.

Although there appears to be a strong relationship between relative growth rates under selective conditions and competition between *L. monocytogenes* and *L. innocua* (Curiale and Lewus, 1994; MacDonald and Sutherland, 1994), at least two published studies downplay this hypothesis (Besse et al., 2005; Cornu et al., 2002).

When recovery is the ultimate goal of the sample analysis, then selective enrichment is usually followed by isolation plating (i.e. streak plating) onto a selective/differential solid medium. This step would help eliminate any non-*Listeria* species that might have survived or that demonstrated growth during the enrichment incubation. However, there has been a trend toward pre-screening enrichments using rapid molecular-based technologies such as PCR or labeled antibody detection before continuing with a full analysis. For accurate detection, the population of *L. monocytogenes* will have to meet some minimum threshold level that is dependent on the detection platform being utilized.

The aim of this study was to identify non-*Listeria* species foodborne microorganisms that are capable of growth in buffered *Listeria* enrichment broth (BLEB) during a 48 h incubation period; BLEB is a commonly used medium for *L. monocytogenes* enrichment. Additionally, this study reports the effects of the presence of non-*Listeria* species competitors on the final enrichment populations of several *L. monocytogenes* strains and determines if these effects are sufficient to limit the detection of this organism by an antibody-based lateral flow device, an automated immunofluorescence assay, and real-time PCR.

2. Materials and methods

2.1. *Listeria* and competitor isolates

The *L. monocytogenes* isolates used in this study were isolated from food products or food processing environments and confirmed to the species level as described in the United States Food & Drug Administration (US FDA) Bacteriological Analytical Manual (BAM) (Hitchins and Jinneman, 2011). Additionally, 16S rDNA sequence analysis was used to confirm all *Listeria* isolates (Hellberg et al., 2013). All commercial bacteria identification platforms were utilized per manufacturer's instructions. Foodborne competitor microorganisms were isolated by similar means except that colony isolation (i.e. streak plating) was performed with non-selective TSAYE agar plates. Pure isolates were identified by 16S rDNA sequencing. The ability of all competitor microorganisms to grow in BLEB under selective conditions was verified. Long term storage of all isolates was performed using BHI broth supplemented with 30% glycerol with storage at -80°C . Daily working stock cultures were maintained in motility agar tubes at room temperature and were replaced every six months.

2.2. Generation times and 48 h enrichment populations

The generation times for all isolates were determined as previously described (Keys et al., 2013). A one-way analysis of variance (ANOVA) with individual mean comparisons by the Tukey–Kramer multiple comparisons test was used to determine if any statistically significant differences existed between the 48 h enrichment populations of the competitor microorganisms and the three strains of *L. monocytogenes* used in this study. Statistically significant generation time means were also determined using a one-way ANOVA with individual mean comparisons being performed by the Tukey–Kramer multiple comparison test.

2.3. *L. monocytogenes* and competitor enrichments

The competitor microorganisms that demonstrated the ability to grow in BLEB under the conditions used for *L. monocytogenes* selection were each paired with three individual strains of *L. monocytogenes* representing serotypes 1/2a, 1/2b, and 4b; these were added to 24.5 ml of 2% UHT milk. Actual spike levels were estimated by plate count. This particular food matrix was selected because it represents the dairy class of food products and because it did not possess any background microorganisms capable of growth under the experimental conditions. The competitor microorganism was added at a slightly higher level (approximately 1 log) than *L. monocytogenes* since spoilage microorganisms are typically present at higher levels than foodborne pathogens in naturally contaminated foods. The

inoculated milk was tempered at 4 °C for 24 h. It was verified that the inoculation levels did not change during this short term storage. Selective enrichments were performed as described in the US FDA BAM beginning with the addition of 225 ml of BLEB (Hitchins and Jinneman, 2011). The levels of *L. monocytogenes* in the 48 h enrichments were determined by plate count using Oxford agar with incubation for 24 and 48 h at 35 °C. The inability of competitor microorganisms to form colonies on Oxford agar was verified. The combined levels of competitor microorganism and *L. monocytogenes* (i.e. aerobic plate count) was determined by surface plating onto TSAYE plates with incubation at 35 °C for 24 h. The levels of competitor microorganisms were determined from the difference between the aerobic plate count and Oxford agar plate count. The statistical significance of the enrichment population means between each competitor microorganism and *L. monocytogenes* pairing was determined using a one-way ANOVA with individual mean comparisons by the Tukey–Kramer multiple comparison test.

2.4. Effect of competitor levels on 48 h population of *L. monocytogenes*

Citrobacter braakii was paired with all three strains of *L. monocytogenes* that were previously used in the competitor enrichment experiments. Additionally, a fourth strain of *L. monocytogenes* serotype 1/2b, designated ARL-Lm-015 was included in this experiment. Several non-*Listeria* microorganisms demonstrated similar results when they were tested as competitors for *L. monocytogenes* and so only *C. braakii* was selected as the competitor for this portion of the study. Competition assays were performed and data were analyzed as described in Section 2.3.

2.5. Detection/recovery of *L. monocytogenes* from food matrices

The two strains of serotype 1/2b *L. monocytogenes* (ARL-Lm-015 and ARL-Lm-045) were each paired with *C. braakii* and used to inoculate three distinct food products (refrigerated guacamole, Mexican style soft cheese, and refrigerated crab meat). These products were selected because they have historically been associated with harboring *L. monocytogenes*. Triplicate 25 g test portions of product were spiked with *C. braakii* and either *L. monocytogenes* ARL-Lm-015 or ARL-Lm-045. The inoculated products were tempered 24 h at 4 °C. Selective enrichments were performed as described in the US FDA BAM (Hitchins and Jinneman, 2011). The 48 h enrichments were analyzed for the presence of *L. monocytogenes* using the MicroSEQ® *L. monocytogenes* real-time PCR detection kit (Applied Biosystems, Inc., Foster City, CA), the Reveal® 2.0 for *Listeria* (Neogen, Inc.; Lansing, MI) antibody-based lateral flow device (LFD) assay, the Vidas *Listeria* (BioMerieux, Inc.; Durham, NC) automated enzyme immunofluorescence assay (ELFA), and streak plate isolation using Oxford agar. The manufacturer's recommended protocols and procedures were followed for all commercial detection platforms.

3. Results and discussion

3.1. *Listeria* and competitor isolates

Eight different species of bacteria were isolated from various food products using the US FDA BAM procedure for *L. monocytogenes* (Hitchins and Jinneman, 2011) and were confirmed to grow in BLEB under selective conditions and thus were potential competitors

for *L. monocytogenes* during selective enrichment (Table 1). Five isolates belonged to the family Enterobacteriaceae and included *C. braakii*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Escherichia coli*, and *Klebsiella pneumoniae*. Three isolates belonged to the lactic acid bacteria group and included *Carnobacterium maltaromaticum*, *Enterococcus faecalis*, and *Enterococcus faecium*. Three strains of *L. monocytogenes* that included serotypes 1/2a, 1/2b, and 4b were also isolated from various products or food processing environments using the same procedure. Isolates with the same product description in Table 1 are from unrelated product samples.

3.2. Generation times and 48 hour enrichment populations

The initial inoculum levels for *L. monocytogenes* ranged from 1.5 to 2.2 log CFU/ml with an overall mean of 2.0 ± 0.4 (individual inoculum levels not shown). The average (3 replications) 48 h enrichment populations for *L. monocytogenes* ranged from 9.0 ± 0.1 to 9.1 ± 0.1 log CFU/ml (Table 1) and were not statistically different ($P > 0.05$) from one another. The initial inoculum levels of the competitor microorganisms ranged from 1.1 to 1.6 log CFU/ml with an overall mean of 1.4 ± 0.2 (individual inoculum levels not shown). The average (3 replications) 48 h enrichment populations for the competitor microorganisms in BLEB under selective conditions ranged from 8.8 ± 0.1 to 9.4 ± 0.1 log CFU/ml (Table 1). The 48 h selective enrichment populations of all of the competitor microorganisms within the family Enterobacteriaceae were significantly higher ($P < 0.05$) than the enrichment populations of *L. monocytogenes* strains ARL-Lm-003 and ARL-Lm-011 (Table 1). Four out of the five Enterobacteriaceae competitor microorganisms (*C. braakii* being the exception) also had 48 h selective enrichment populations significantly higher ($P < 0.05$) than *L. monocytogenes* strain ARL-Lm-045 (Table 1). All three lactic acid bacteria class of competitor microorganisms had 48 h selective enrichment populations that were statistically the same ($P > 0.05$) as those of *L. monocytogenes* strains ARL-Lm-003 and ARL-Lm-011 (Table 1). The selective enrichment population of *E. faecium* was lower than all three strains of *L. monocytogenes*, however this difference was only statistically significant ($P < 0.05$) with strain ARL-Lm-045.

The average (3 replications) generation times during exponential growth for the three primary strains of *L. monocytogenes* used in this study ranged from 93 ± 7 to 138 ± 6 min with an overall mean of 111 ± 24 min. These values are in good agreement with those previously reported for *L. monocytogenes* under the selective conditions prescribed by the U.S. FDA BAM (Keys et al., 2013) and are generally in agreement with the values published by MacDonald and Sutherland (1994) and Gorski et al. (2006). The generation times were determined in 2.5 ml volumes using 24-well microplates which have a large surface area relative to volume. Because of their microaerophilic nature, the generation times for *L. monocytogenes* are likely to be somewhat affected by the study conditions. This makes absolute comparisons with previous studies difficult as these earlier reports were performed using larger volumes and smaller surface area to volume ratios. In order to ensure a homogeneous suspension of cells, the microplates were agitated just prior to the optical density measurements. This likely further increased the aeration levels. The average (3 replications) generation times for the Enterobacteriaceae competitor microorganisms ranged from 24 ± 1 to 50 ± 2 min with an overall mean of 36 ± 10 min and were all significantly (P

< 0.05) shorter than the generation times for the three strains of *L. monocytogenes* (Table 1). The average (3 replications) generation times for the remaining competitor microorganisms, belonging to the lactic acid bacteria group, ranged from 30 ± 1 to 162 ± 5 min. The generation time for *C. maltaromaticum* was significantly ($P < 0.05$) longer than any of the strains of *L. monocytogenes*. The generation time for *E. faecalis* was significantly ($P < 0.05$) shorter than any of the strains of *L. monocytogenes*. The generation time for *E. faecium* was not significantly different ($P > 0.05$) from *L. monocytogenes* strains ARL-Lm-011 or ARL-Lm-045 but was significantly ($P < 0.05$) lower than that of strain ARL-Lm-003.

3.3. *L. monocytogenes* and competitor enrichments

Each test sample was spiked with both *L. monocytogenes* and one species of competitor microorganism. The average (3 replications) inoculum levels for *L. monocytogenes* ranged from 0.9 ± 0.1 to 1.8 ± 0.1 log CFU/ml and for the Enterobacteriaceae competitor microorganisms the average inoculum levels ranged from 2.1 ± 0.1 to 2.7 ± 0.1 log CFU/ml (Table 2). The 48 h enrichment populations for *C. braakii*, *E. aerogenes*, *E. cloacae*, and *K. pneumoniae* (Table 2) were similar to the 48 h populations obtained under the same enrichment conditions but in the absence of *L. monocytogenes* (Table 1). The 48 h enrichment population for *E. coli* was slightly reduced by the presence of *L. monocytogenes*. The 48 h selective enrichment populations for the three test strains of *L. monocytogenes* were all significantly ($P < 0.05$) reduced by the presence of the Enterobacteriaceae competitor microorganisms relative to growth under selective conditions without the addition of the competitors (Table 2). Some *L. monocytogenes* strain specific variation to the presence of competitor microorganisms was observed (Table 2 and Fig. 1). The presence of *C. braakii* during the enrichment incubation resulted in a 2.8 to 3.5 log decrease in the population of *L. monocytogenes* with serotype 4b being the most sensitive to this competitor (Fig. 1). Co-inoculation with *E. aerogenes* or *E. cloacae* resulted in a 1.7 to 3.4 log and a 2.5 to 4.1 log decrease respectively in the population of *L. monocytogenes* with serotype 1/2b being the most sensitive to these two competitors (Fig. 1). When *E. coli* was present during the enrichment, a 2.1 to 3.2 log decrease in the population of *L. monocytogenes* was observed with serotype 4b again demonstrating the largest population reduction (Fig. 1). Finally, the presence of *K. pneumoniae* during enrichment resulted in a 1.7 to 3.7 log decrease in the population of *L. monocytogenes* with serotype 4b being the most sensitive to this competitor (Fig. 1).

For enrichments that included the lactic acid bacteria class of competitor microorganisms, the average (3 replications) inoculum levels of *L. monocytogenes* ranged from 1.0 ± 0.1 to 1.6 ± 0.1 log CFU/ml and the average inoculum levels of the competitor microorganisms ranged from 2.1 ± 0.1 to 2.3 ± 0.1 log CFU/ml (Table 3). The 48 h enrichment populations for all three species of competitors were similar to those observed for growth under selective conditions but in the absence of *L. monocytogenes* (Tables 1 and 3). The 48 h populations of *L. monocytogenes* in enrichments that included competitor microorganisms were significantly lower ($P < 0.05$) when compared to the final *L. monocytogenes* populations under similar conditions but without the addition of competitors (Table 3). *C. maltaromaticum* had the smallest effect (0.9–1.6 log decrease) on the final population of *L. monocytogenes* compared to growth under selective conditions without the addition of

competitor; serotype 1/2b was most sensitive to the presence of this competitor (Fig. 2). When *E. faecalis* was present during the enrichment, a 2.3 to 3.7 log decrease in the 48 h population of *L. monocytogenes* was observed with serotype 4b being the most sensitive to this competitor (Fig. 2). Finally, the presence of *E. faecium* during enrichment resulted in a 1.3 to 2.2 log decrease in the population of *L. monocytogenes* with serotype 4b being the most sensitive to this competitor (Fig. 2).

3.4. Effect of competitor levels on 48 h population of *L. monocytogenes*

Test samples (25 ml) of 2% UHT processed milk received an average (3 replications) inoculum of *L. monocytogenes* that ranged between 0.8 ± 0.1 and 1.6 ± 0.1 log CFU/ml (Table 4). The milk samples were additionally spiked with varying levels of *C. braakii* covering a 4 log range. For two of the *L. monocytogenes* test strains (ARL-Lm-011 and ARL-Lm-015) no statistically significant differences ($P > 0.05$) in the 48 h *L. monocytogenes* populations were evident suggesting little dependence on the initial level of competitor (Table 4). For strain ARL-Lm-003, the 48 h *L. monocytogenes* population at the lowest initial competitor level was 5.1 log CFU/ml and was determined to be significantly ($P < 0.05$) less than the 48 h *L. monocytogenes* population at the 3.3 log CFU/ml initial competitor level which was 5.8 log CFU/ml. However, because the 48 h *L. monocytogenes* enrichment populations at the lowest and highest initial competitor levels (5.1 versus 5.3 log CFU/ml) were statistically similar there is probably little biological value in statistical differences involving the intermediate initial competitor levels. A similar observation was made for *L. monocytogenes* strain ARL-Lm-045 (Table 4). The 48 h *L. monocytogenes* enrichment population at the highest initial competitor level was significantly lower ($P < 0.05$) compared to that at the 2.5 log CFU/ml competitor spike level (6.0 versus 6.6 log CFU/ml). However, the 48 h *L. monocytogenes* enrichment population at the highest and lowest initial competitor levels were again statistically similar ($P > 0.05$) indicating that statistical significance among the intermediate initial competitor levels is likely due to experimental variation.

3.5. Detection/recovery of *L. monocytogenes* from food matrices

Analytical test portions (25 g) of three food matrices that included refrigerated guacamole, Mexican-style cheese, and refrigerated crab meat were spiked with *L. monocytogenes* strain ARL-Lm-045 or strain ARL-Lm-015 at average (3 replications) levels ranging from 1.4 ± 0.1 to 2.0 ± 0.2 log CFU/g; test samples were also spiked with *C. braakii* at approximately 2.6 ± 0.1 log CFU/g (Tables 5 and 6). After 48 h of selective enrichment, the ability to detect *L. monocytogenes* by real-time PCR, an antibody-based lateral flow device, and an automated immunofluorescence assay was determined. Detection of *L. monocytogenes* strain ARL-Lm-045 was more consistent among the three food products and by all three detection methods as compared to strain ARL-Lm-015. Strain ARL-Lm-045 was detected 100% of the time in all three spiked food products by real-time PCR and ELFA (Table 5). Strain ARL-Lm-045 was also detected 100% of the time in guacamole and crab meat using the antibody-based lateral flow device. Detection of strain ARL-Lm-045 in cheese was more difficult using the lateral flow device and it was only detected in 1 of 3 enrichments. On the other hand, *L. monocytogenes* strain ARL-Lm-015 was frequently missed following enrichment (Table 6). Real-time PCR was the most consistent molecular detection platform.

In spiked crab meat, strain ARL-Lm-015 was detected in all three enrichments by real-time PCR but detection dropped to 2 of 3 enrichments for both guacamole and cheese. Strain ARL-Lm-015 could not be detected by ELFA following enrichment of spiked guacamole or cheese but was detected in 2 of 3 enrichments of spiked crab meat. Strain ARL-Lm-015 also could not be detected with the antibody-based lateral flow device in any of the spiked food products following enrichment. Strain ARL-Lm-015 was isolated from all enrichments by the streak plate method using Oxford agar. It should be noted however, that on the area of the plate that normally would have displayed confluent microbial growth (the first quadrant of a four quadrant streak plate) there were typically only 2–3 total colonies with the remainder of the plate having no growth. This was observed for the majority of the enrichments regardless of the food product being tested.

3.6. Significance of microbial competition during enrichment

The microbiological analysis of food and food processing environmental samples typically follow one of two paths; pathogen recovery or pathogen detection. Pathogen detection methods probe for the presence of specific biomolecular markers that must be present at minimum threshold levels for a signal to be produced. Enrichment incubations are still frequently used to increase the levels of pathogens to detectable levels. A food matrix dependent variation in the recovery of *L. monocytogenes* following enrichment was noted early on during the development of the current *Listeria* isolation method (Hitchins and Tran, 1990). This recovery variation was initially thought to be related to the overall level of microbial contamination of the food but it was later shown that little correlation existed between total microbial load and *L. monocytogenes* recovery sensitivity (Tran et al., 1990). It was speculated that the variability in *L. monocytogenes* recovery was due to a subset of the total food product microbial load particularly those that could grow and compete under conditions of selectivity (Tran et al., 1990). It was subsequently confirmed that several species of bacteria could compete with *L. monocytogenes* and affect the recovery of this organism under certain circumstances (Dallas et al., 1991). However, this was primarily an issue when the competing microorganism was present at initial levels of 5–7 logs higher than *L. monocytogenes*. These studies were performed prior to the increased use of molecular-based screening technologies and so competition was measured as the failure to isolate *L. monocytogenes* by the streak plate method following enrichment and not necessarily by the failure to detect the organism.

The recovery of *L. monocytogenes* from foods by culturing-based methods can be a time consuming process; 1–2 days for selective enrichment, 1–2 days for isolation, and 2–3 days for confirmation. Because of the time, space requirements, and cost associated with foodborne pathogen testing there has been a trend toward the application of molecular-based technologies for pre-screening analytical samples. This would eliminate samples that are likely negative and allow isolation efforts to focus on those samples that tested positive during screening. All molecular testing platforms have a minimum target threshold level that must be met in order to elicit a detectable signal. Because foodborne pathogens such as *L. monocytogenes* can frequently be present at low levels (<1 cell/g) selective enrichment is still needed as the initial step of a molecular-based screening method. Buffered *Listeria* enrichment broth is a commonly used enrichment formulation for the isolation of *L.*

monocytogenes; however, BLEB does not demonstrate absolute species-level selectivity. The growth of non-target microorganisms during the selective enrichment incubation can have undesirable consequences such as nutrient depletion, waste product accumulation, pH reduction, changes in redox potential, and production of inhibitory molecules/compounds; all of these factors can have a negative impact on *L. monocytogenes* growth. The three primary strains used in this study (ARL-Lm-003, ARL-Lm-011, and ARL-Lm-045) demonstrated varying sensitivities to the presence of non-*Listeria* competitor microorganisms during the enrichment incubation. The competitor microorganisms identified and subsequently used in this study were all common foodborne background or spoilage microorganisms and could be expected to be present on any food sample undergoing *Listeria* testing. Although reductions in the final enrichment population of *L. monocytogenes* as high as 4 logs was observed, these three strains were still routinely detected using common molecular-based detection platforms. It should be noted that the *L. monocytogenes* spike levels used in this study may not reflect actual levels found in naturally contaminated foods which might be considerably lower; a greater severity of competition is expected in naturally contaminated foods. The use of molecular detection platforms with a moderate sensitivity, such as the antibody-based lateral flow device, uncovered some food matrix associated detection issues most notably with cheese. Overall, the data suggests that molecular screening is generally effective for determining the presence of *L. monocytogenes* in foods with a relatively high microbial background including species capable of growth under selective conditions. This study did reveal however, that there are food associated strains of *L. monocytogenes* such as ARL-Lm-015 that are highly sensitive to the presence of competitor microorganisms during enrichment and that these strains may be difficult to detect using molecular screening platforms.

3.7. Significance of strain ARL-Lm-015

Strain ARL-Lm-015 is a serotype 1/2b *L. monocytogenes* that was isolated from deli style sandwich manufacturing facility. It was identified early on in this study as being extremely sensitive to the presence of microbial competitors during enrichment. This strain is likely not representative of the majority of naturally occurring foodborne strains of *L. monocytogenes* but rather it likely represents a rare phenotype; for these reasons this strain was not used for many of the experiments in this study. It was included in some of the later experiments in order to show that there are foodborne strains of *L. monocytogenes* that have difficulty competing during enrichment and that these strains can be difficult to detect in foods using molecular-based detection platforms. The true prevalence of strains of *L. monocytogenes* in foods that are extremely sensitive to microbial competition during enrichment is not known and will likely be difficult to determine. However, it is clear that a better understanding of microbial competition during *L. monocytogenes* selective enrichment is needed for reliable screening of analytical samples by molecular-based methods. As an expansion of the food safety surveillance, both the food industry and regulatory agencies are increasingly relying on environmental microbial testing; environmental monitoring can result in large numbers of samples increasing the reliance on molecular-based screening.

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Fig. 1. Effect of Enterobacteriaceae competitors on the 48 h BLEB enrichment populations of *L. monocytogenes*. Enrichment was performed on artificially inoculated 2% UHT milk with initial and final cell levels specified in Table 2.

Fig. 2. Effect of lactic acid bacteria competitors on the 48 h BLEB enrichment populations of *L. monocytogenes*. Enrichment was performed on inoculated 2% UHT processed milk with initial and final cell levels specified in Table 3.

Table 1

Generation time, 48 h population following growth in BLEB, and source for competitor and *L. monocytogenes* isolates used in this study.

Species	GT ^a	Population ^b	Isolate source
<i>C. braakii</i>	31 ± 2a	9.3 ± 0.1ab	Papaya
<i>E. aerogenes</i>	29 ± 2a	9.4 ± 0.1a	Poultry feed
<i>E. cloacae</i>	50 ± 2b	9.4 ± 0.1a	Potato chips
<i>E. coli</i>	43 ± 2b	9.4 ± 0.1a	Black pepper
<i>K. pneumoniae</i>	24 ± 1a	9.4 ± 0.1a	Tarragon
<i>C. maltaromaticum</i>	162 ± 5e	9.1 ± 0.1bc	Smoked salmon
<i>E. faecalis</i>	30 ± 1a	9.0 ± 0.1cd	Mexican-style cheese
<i>E. faecium</i>	92 ± 10c	8.8 ± 0.1d	Vegetable seasoning mix
<i>L. monocytogenes</i> 4b ^c	138 ± 6d	9.0 ± 0.1cd	Mexican-style cheese
<i>L. monocytogenes</i> 1/2a ^d	93 ± 7c	9.0 ± 0.1cd	Avocado pulp
<i>L. monocytogenes</i> 1/2b ^e	101 ± 8c	9.1 ± 0.1bc	Deli meat ^f

^aGeneration time in minutes. Means followed by the same letter are not statistically significant.

^bLog CFU/ml. Means followed by the same letter are not statistically significant.

^cLaboratory isolate designation ARL-Lm-003.

^dLaboratory isolate designation ARL-Lm-011.

^eLaboratory isolate designation ARL-Lm-045.

^fFood Processing Environmental Isolate.

Effect of acriflavin and nalidixic acid resistant Enterobacteriaceae on 48 h enrichment populations of *L. monocytogenes* from inoculated 2% UHT processed milk.

Table 2

Pairing		Pre-enrichment spike ^a		48 h enrichment population ^a	
Competitor	Target	Competitor	Target	Competitor	Target
<i>C. braakii</i>	<i>L. monocytogenes</i> (4b)		1.6 ± 0.1	9.3 ± 0.1	5.5 ± 0.1bc
	<i>L. monocytogenes</i> (1/2a)	2.4 ± 0.1	1.5 ± 0.1	9.3 ± 0.1	6.2 ± 0.1d
	<i>L. monocytogenes</i> (1/2b)		1.4 ± 0.1	9.3 ± 0.1	6.3 ± 0.1d
<i>E. aerogenes</i>	<i>L. monocytogenes</i> (4b)		1.5 ± 0.1	9.4 ± 0.1	7.3 ± 0.1f
	<i>L. monocytogenes</i> (1/2a)	2.3 ± 0.1	1.7 ± 0.1	9.4 ± 0.1	7.0 ± 0.1ef
	<i>L. monocytogenes</i> (1/2b)		1.0 ± 0.1	9.4 ± 0.1	5.7 ± 0.1c
<i>E. cloacae</i>	<i>L. monocytogenes</i> (4b)		1.0 ± 0.1	9.4 ± 0.01	6.5 ± 0.1d
	<i>L. monocytogenes</i> (1/2a)	2.1 ± 0.1	1.3 ± 0.1	9.4 ± 0.01	6.2 ± 0.1d
	<i>L. monocytogenes</i> (1/2b)		0.9 ± 0.1	9.4 ± 0.01	5.0 ± 0.1a
<i>E. coli</i>	<i>L. monocytogenes</i> (4b)		1.4 ± 0.1	9.1 ± 0.1	5.8 ± 0.1c
	<i>L. monocytogenes</i> (1/2a)	2.3 ± 0.1	1.5 ± 0.1	9.1 ± 0.1	6.9 ± 0.1e
	<i>L. monocytogenes</i> (1/2b)		1.6 ± 0.1	9.1 ± 0.1	6.5 ± 0.1d
<i>K. pneumoniae</i>	<i>L. monocytogenes</i> (4b)		1.5 ± 0.1	9.5 ± 0.1	5.3 ± 0.1ab
	<i>L. monocytogenes</i> (1/2a)	2.7 ± 0.1	1.6 ± 0.1	9.4 ± 0.1	7.3 ± 0.1f
	<i>L. monocytogenes</i> (1/2b)		1.8 ± 0.1	9.4 ± 0.1	7.2 ± 0.1ef
None	<i>L. monocytogenes</i> (4b)		1.1 ± 0.1	–	9.0 ± 0.1g
	<i>L. monocytogenes</i> (1/2a)	–	1.0 ± 0.1	–	9.0 ± 0.1g
	<i>L. monocytogenes</i> (1/2b)		1.2 ± 0.1	–	9.1 ± 0.1g

^aPopulations expressed as log CFU/ml. For the 48 h populations the reported error is the standard error associated with the mean of three individual replications (i.e. enrichments). A single competitor and target stock suspension was used to inoculate all three replications therefore the reported error for the initial inoculum is the standard error associated with the mean of three individual measurements (i.e. triplicate plating). 48 h enrichment population means followed by the same letter are not statistically significant.

Table 3

Effects of acriflavin and nalidixic acid resistant lactic acid bacteria on 48 h enrichment populations of *L. monocytogenes* from inoculated 2% UHT processed milk.

Pairing		Pre-enrichment spike ^a		48 h enrichment population ^a	
Competitor	Target	Competitor	Target	Competitor	Target
<i>C. maltaromaticum</i>	<i>L. monocytogenes</i> (4b)		1.2 ± 0.1	9.0 ± 0.1	8.0 ± 0.1d
	<i>L. monocytogenes</i> (1/2a)	2.1 ± 0.1	1.3 ± 0.1	9.0 ± 0.2	8.1 ± 0.1d
	<i>L. monocytogenes</i> (1/2b)		1.1 ± 0.1	9.0 ± 0.1	7.5 ± 0.1c
<i>E. faecalis</i>	<i>L. monocytogenes</i> (4b)		1.3 ± 0.1	9.2 ± 0.1	5.3 ± 0.1a
	<i>L. monocytogenes</i> (1/2a)	2.3 ± 0.1	1.3 ± 0.1	9.1 ± 0.1	6.6 ± 0.1b
	<i>L. monocytogenes</i> (1/2b)		1.6 ± 0.1	9.0 ± 0.1	6.8 ± 0.1b
<i>E. faecium</i>	<i>L. monocytogenes</i> (4b)		1.5 ± 0.1	9.2 ± 0.1	6.8 ± 0.1b
	<i>L. monocytogenes</i> (1/2a)	2.1 ± 0.1	1.6 ± 0.1	9.2 ± 0.1	7.7 ± 0.1c
	<i>L. monocytogenes</i> (1/2b)		1.6 ± 0.1	9.1 ± 0.1	7.6 ± 0.1c
None	<i>L. monocytogenes</i> (4b)		1.1 ± 0.1	–	9.0 ± 0.1e
	<i>L. monocytogenes</i> (1/2a)	–	1.0 ± 0.1	–	9.0 ± 0.1e
	<i>L. monocytogenes</i> (1/2b)		1.2 ± 0.1	–	9.1 ± 0.1e

^aPopulations expressed as log CFU/ml. For the 48 h populations the reported error is the standard error associated with the mean of three individual replications (i.e. enrichments). A single competitor and target stock suspension was used to inoculate all three replications therefore the reported error for the initial inoculum is the standard error associated with the mean of three individual measurements (i.e. triplicate plating). 48 h enrichment population means that are followed by the same letter are not statistically significant.

Table 4

Effect of pre-enrichment competitor levels on the final enrichment populations of *L. monocytogenes* from inoculated 2% UHT processed milk.

Isolate	Pre-enrichment spike ^a		48 h enrichment population ^a	
	<i>L. monocytogenes</i>	<i>C. braakii</i>	<i>L. monocytogenes</i>	<i>C. braakii</i>
ARL-Lm-003	1.3 ± 0.1	4.3 ± 0.1	5.3 ± 0.3ab	9.1 ± 0.2
		3.3 ± 0.1	5.8 ± 0.3b	9.1 ± 0.1
		2.3 ± 0.1	5.6 ± 0.2ab	9.1 ± 0.1
		1.3 ± 0.1	5.1 ± 0.1a	9.1 ± 0.1
ARL-Lm-011	1.3 ± 0.1	4.4 ± 0.1	6.7 ± 0.1a	9.0 ± 0.1
		3.4 ± 0.1	6.4 ± 0.2a	9.1 ± 0.1
		2.4 ± 0.1	6.6 ± 0.2a	9.1 ± 0.1
		1.4 ± 0.1	6.5 ± 0.1a	9.1 ± 0.1
ARL-Lm-015	0.8 ± 0.1	3.8 ± 0.1	1.5 ± 0.1a	9.1 ± 0.1
		2.8 ± 0.1	2.7 ± 0.7a	9.2 ± 0.1
		1.8 ± 0.1	2.6 ± 0.7a	9.3 ± 0.1
		0.8 ± 0.1	2.6 ± 0.2a	9.2 ± 0.1
ARL-Lm-045	1.6 ± 0.1	4.5 ± 0.1	6.0 ± 0.2a	9.3 ± 0.1
		3.5 ± 0.1	6.2 ± 0.1ab	9.2 ± 0.1
		2.5 ± 0.1	6.6 ± 0.3b	9.4 ± 0.1
		1.5 ± 0.1	6.2 ± 0.1ab	9.3 ± 0.1

^aLog CFU/ml. Within each isolate pairing the 48 h enrichment population means that are followed by the same letter are not statistically significant.

Table 5

Effect of the presence of *C. braakii* on post-enrichment detection of *L. monocytogenes* serotype 1/2b strain ARL-Lm-045 from inoculated food matrices using multiple platforms.

Test sample	Spike ^a		Background ^a	Platform ^b	Incidence of detection
	<i>L. monocytogenes</i>	<i>C. braakii</i>			
Guacamole	1.4 ± 0.1	2.6 ± 0.1	<2.0 est	Real-time PCR	3/3
				LFD	3/3
				ELFA	3/3
Cheese				Streak Plate	3/3
	1.5 ± 0.1	2.7 ± 0.1	3.9 ± 0.1	Real-time PCR	3/3
				LFD	1/3
				ELFA	3/3
Crab Meat				Streak Plate	3/3
	1.7 ± 0.1	2.6 ± 0.1	4.1 ± 0.1	Real-time PCR	3/3
				LFD	3/3
				ELFA	3/3
				Streak Plate	3/3

^aLog CFU/g.

^bLFD = antibody-based lateral flow device; ELFA = enzyme linked immunofluorescence assay.

Effect of the presence of *C. braakii* on post-enrichment detection of *L. monocytogenes* serotype 1/2b strain ARL-Lm-015 from inoculated food matrices using multiple platforms.

Table 6

Test sample	Spike ^a		Background ^a	Platform ^b	Incidences of detection
	<i>L. monocytogenes</i>	<i>C. braakii</i>			
Guacamole	1.5 ± 0.1	2.6 ± 0.1	<2.0 est	Real-time PCR	2/3
				LFD	0/3
				ELFA	0/3
				Streak plate	3/3
Cheese	2.0 ± 0.2	2.6 ± 0.1	3.9 ± 0.1	Real-time PCR	2/3
				LFD	0/3
				ELFA	0/3
				Streak plate	3/3
Crab meat	2.0 ± 0.2	2.6 ± 0.1	4.1 ± 0.1	Real-time PCR	3/3
				LFD	0/3
				ELFA	2/3
				Streak plate	3/3

^aLog CFU/g.

^bLFD = antibody-based lateral flow device; ELFA = enzyme linked immunofluorescence assay.