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Contribution of Selective Conditions to Microbial Competition in Four *Listeria* Selective Enrichment Formulations

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Abstract

Microbial competition during selective enrichment negatively affects *Listeria monocytogenes* populations and may hinder the subsequent detection or recovery of this organism. Competition assays among 10 selected strains of *Listeria* and *Citrobacter braakii* were performed in buffered *Listeria* enrichment broth, 3-(*N*-morpholino)propanesulfonic acid–buffered *Listeria* enrichment broth, University of Vermont medium–modified *Listeria* enrichment broth, and Fraser broth. The individual contributions of each selective agent in these media were also assessed, as well as the contribution of incubation temperature. Acriflavine hydrochloride and sodium nalidixate were ineffective at preventing the overgrowth of *C. braakii*; this resulted in substantially lower populations of *Listeria* than when the competitor was absent. At the higher levels, both of these selective agents were detrimental to *Listeria* populations. The highest enrichment populations of *Listeria* were observed when either NaCl or LiCl was present. In the absence of selective agents, the final populations of *Listeria* following competitive growth with *C. braakii* were not substantially affected by temperature; however, in the presence of selective agents, the *Listeria* populations were statistically higher at the higher incubation temperature. There are a limited number of selective agents available for use in *Listeria*-specific enrichment media, resulting in formulations that are only somewhat selective for this species. The optimization of current formulations may help researchers to improve *Listeria* recovery, particularly from products with a high microbial load. The understanding of the behavior and interactions between target and nontarget microorganisms in the presence of these available selective agents is a necessary step in the optimization of *Listeria* selective enrichment formulations.

Keywords

Foodborne pathogen; *Listeria*; *Listeria monocytogenes*; Microbial competition; Regulatory microbiology; Selective enrichment

Listeria monocytogenes is a recognized foodborne pathogen capable of persisting in a wide range of food products that are designed to be consumed without further processing,

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generally referred to as ready-to-eat foods. Because *L. monocytogenes* is a psychrotroph and because of the seriousness of the illness that it causes, particularly in certain high-risk populations, regulatory agencies in the United States have imposed a zero tolerance level for this organism in ready-to-eat foods. The current method used by the U.S. Food and Drug Administration (FDA) specifies a detection and recovery sensitivity of 1 CFU/25 g of analytical test portion. To achieve this level of sensitivity, the FDA uses a 48-h two-stage selective enrichment procedure (13) with buffered *Listeria* enrichment broth (BLEB). BLEB is buffered Trypticase soy broth with 0.6% yeast extract (TSBYE; Difco, BD Diagnostics, Inc., Sparks, MD), or the equivalent from other manufacturers, to which 10 mg/liter acriflavine hydrochloride (acriflavine-HCL), 40 mg/liter sodium nalidixate, and 50 mg/liter cycloheximide are added after an initial 4-h incubation period; this initial nonselective incubation period is intended to resuscitate injured cells. In addition to BLEB, other *Listeria*-selective enrichment formulations are used by U.S. regulatory agencies, including University of Vermont medium–modified *Listeria* enrichment (UVM-1) and Fraser broth. For selectivity, UVM-1 broth relies on 12 mg/liter acriflavine-HCl and 20 mg/liter sodium nalidixate, and Fraser broth relies on 24 mg/liter acriflavine-HCl and 20 mg/liter sodium nalidixate. In addition, both UVM-1 and Fraser broths have high levels (20 g/liter) of NaCl for increased selectivity, and Fraser broth has 3 g/liter LiCl. These two formulations are commonly used sequentially in a two-stage enrichment procedure for the examination of ready-to-eat deli meats (2), and either can be used individually for single-stage enrichments.

Despite the inclusion of selective agents, the growth of non-*Listeria* species can occur and may reduce the sensitivity of *L. monocytogenes* detection through population suppression (1, 7, 9, 14, 17, 20). When multiple species of *Listeria* are present in the test sample, competitive growth during selective enrichment will frequently result in an intraspecies population differential that is sufficiently large to prevent the recovery of *L. monocytogenes* during subsequent selective plating steps. This has been shown for the FDA *Listeria*-selective enrichment method (8, 16), the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS)–recommended method (6, 19), and the European Union International Organization for Standardization (EN ISO 11290-1) method (5, 22). The added presence of non-*Listeria* species competitors also contributes to large postenrichment population differentials (17).

Even with all the aforementioned studies, it is difficult to evaluate the contributions of individual selective components of each formulation or to assess which selective components are most influential on microbial competition during enrichment. The purpose of this study was to compare the effects of microbial competition on final *Listeria* species populations in four selective media used by U.S. regulatory agencies: BLEB, UVM-1 broth, Fraser broth, and 3-(*N*-morpholino)propanesulfonic acid–buffered BLEB (MOPS-BLEB). The goal of the study was not to determine which enrichment is best but to exploit the differences among the enrichments to determine which selective components are most influential on the final populations of *Listeria* species in the presence of a non-*Listeria* species competitor. To that end, we determined the final populations of 10 strains of *Listeria* species, in the presence of the competitor *C. braakii*, in all four selective media at both 30 and 35°C. In addition, we evaluated the concentration effects of the selective components

(sodium nalidixate, acriflavine-HCl, cycloheximide, LiCl, and NaCl) on final *Listeria* species population sizes.

Materials and Methods

Bacterial strains

Seven strains of *L. monocytogenes* were included in the study, comprising serotypes 1/2a (strains ARL-Lm-031, ARL-Lm-054, and ARL-Lm-087) and 1/2b (strains ARL-Lm-007, ARL-Lm-015, ARL-Lm-062, and ARL-Lm-065) as determined by the PCR method proposed by Doumith et al. (10). The serotypes of the two strains of *Listeria innocua* (ARL-Ln-011 and ARL-Ln-017) and the one strain of *Listeria seeligeri* (ARL-Ls-003 [Ls003]) were not determined because the PCR method is specific for *L. monocytogenes* only. A single foodborne strain of *C. braakii* (ARL-Cb-01) was used as the competitor microorganism and was selected because it was the most competitive *Enterobacteriaceae* strain identified in a previous study (17). We selected the 10 strains of *Listeria* species used in this study because we determined that they were the least competitive of the 200 strains that we had initially screened against this particular strain of *C. braakii*. The original competition screening included 100 strains of *L. monocytogenes*, with serotypes 1/2a ($n = 55$), 1/2b ($n = 31$), and 4b ($n = 14$) represented. The full results from that competitive screening are detailed in a previous article (17). All *Listeria* strains and *C. braakii* were isolated from foods or food processing environments and identified using the FDA *Listeria* isolation method (13) and 16S rRNA gene sequencing (12). All strains were maintained cryoprotectively at -80°C from their initial time of isolation, and the working stocks were maintained in motility agar medium at room temperature.

Assessment of selective enrichment media

The BLEB was prepared with TSBYE (Difco, BD Diagnostics, Inc.), buffering salts, sodium pyruvate, sodium nalidixate, acriflavine-HCl, and cycloheximide according to FDA guidelines (13). Sodium pyruvate, the buffering salts, and the selective agents were obtained from Sigma-Aldrich (St. Louis, MO). The MOPS-BLEB was prepared from TSBYE buffered with MOPS according to USDA-FSIS guidelines (2). Commercially prepared dehydrated Fraser broth (Neogen, Inc., Lansing, MI) and UVM-1 broth (Neogen, Inc.) were prepared following manufacturer's instructions.

All strains of *Listeria* and *C. braakii* were prepared by inoculating 9 ml of nonselective BLEB directly from the motility agar stocks using disposable 10- μl calibrated inoculating loops. The cultures were statically grown at 35°C for 18 to 24 h. Target inoculum levels of 1 to 5 CFU/ml for the *Listeria* strains and 10 to 50 CFU/ml for *C. braakii* were prepared using Butterfield's phosphate buffer formulation (4). We verified the target inoculum levels by surface plating onto Trypticase soy agar supplemented with 0.6% yeast extract (Difco, BD Diagnostics, Inc.) with an incubation time of 24 h at 35°C . All assays were performed using 25 ml of selective enrichment broth in 50-ml conical centrifuge tubes. Each tube was inoculated with *C. braakii* and with 1 of the 10 strains of *Listeria*. The inoculated tubes were statically incubated for 48 h at 30°C for the BLEB and UVM-1 broth enrichments, and at 35°C for the MOPS-BLEB and Fraser broth enrichments. We estimated the 48-h *Listeria*

species enrichment populations by surface plating onto Oxford agar (Difco, BD Diagnostics, Inc.) and incubating the plates for 24 and 48 h at 35°C. The strain of *C. braakii* used in this study is not capable of forming colonies on Oxford agar; we determined the postenrichment populations of *C. braakii* using MacConkey agar (Difco, BD Diagnostics, Inc.). None of the strains of *Listeria* species used in this study is capable of forming colonies on MacConkey agar. We performed the experiments in triplicate.

We analyzed the data using a one-way analysis of variance (ANOVA) with the enrichment broth type as the fixed variable and the 48-h *Listeria* species population as the response variable. Mean comparisons were performed using Tukey's test because this test is the most conservative (i.e., the least likely to result in a significant difference). We verified a normal distribution for the residual terms using the Shapiro-Wilkes normality test ($P > 0.05$). We determined the equal variance of the residual terms by visually inspecting a scatterplot of the residuals for the absence of discernible patterns. All statistical analyses were performed using SigmaPlot version 12.0 (Systat Software, Inc., San Jose, CA).

Effect of temperature on *Listeria* species enrichment populations

To assess the effect of incubation temperature on final *Listeria* selective enrichment populations, we tested the enrichment formulation and temperature pairings that were not included in the aforementioned experiment: BLEB, 35°C; MOPS-BLEB, 30°C; UVM-1 broth, 35°C; and Fraser broth, 30°C. Three experimental replications were performed for each broth-temperature combination. Each replication was performed as previously described.

The data were analyzed using a two-way ANOVA with the enrichment broth type and temperature as the fixed variables and the 48-h *Listeria* species population as the response variable. We performed the mean comparisons using Tukey's test, and we verified the statistical assumptions of normal distribution and equal variance for the residual terms.

Effects of selective additives on *Listeria* selective enrichment levels

The BLEB was prepared with TSBYE, sodium pyruvate, and appropriate buffering salts. We then supplemented it with either 10 mg/liter acriflavine-HCl (final concentration), 40 mg/liter sodium nalidixate (final concentration), or 50 mg/liter cycloheximide (final concentration).

We prepared the UVM-1 broth using tryptone (enzymatic digest of casein), Bacto Peptone (enzymatic digest of animal tissue), and beef extract (all three from Difco, BD Diagnostics, Inc.); yeast extract; esculin; and appropriate buffering salts. The formulation (sans selective agents) that we used was the one specified by Difco for its commercial preparations of UVM-1 broth (Difco catalog no. 22230). We supplemented the UVM-1 basal medium with 20 g/liter NaCl, 12 mg/liter acriflavine-HCl (final concentration), or 20 mg/liter sodium nalidixate.

The basal formulation of Fraser broth is the same as UVM-1 broth. We supplemented the Fraser broth basal medium with 20 g/liter NaCl, 3 g/liter LiCl, 24 mg/liter acriflavine-HCl (final concentration), or 20 mg/liter sodium nalidixate (final concentration).

Each 25 ml of test broth was inoculated with both *C. braakii* and 1 of the 10 *Listeria* test strains. We performed three experimental replications for each test broth, and each replication was performed as previously described.

The data were analyzed using a one-way ANOVA with the test broth condition as the fixed variable and the 48-h *Listeria* species population as the response variable. We performed the mean comparisons using the Tukey test and we checked the statistical assumptions of normal distribution and equal variance for the residual terms.

Results and Discussion

Competitor strain selection

Microbial competition during the selective enrichment of *Listeria* species has been studied since the initial work by Petran and Swanson (19) and has since that time received a modest level of attention. Two proposed primary modes of action have emerged from these studies. The first is based on the ability of a competitor microorganism to produce or harbor a specific anti-*Listeria* biological molecule, such as a bacteriocin or a bacteriophage, and is supported by several published studies (15, 16, 18, 21). The second proposed mode of action is that the rapid growth of a competitor microorganism and resulting depletion of one or more essential nutrients in the enrichment broth causes *Listeria* to enter stationary phase at a population much lower than the generally accepted maximum of approximately 9 log CFU/ml. This is also supported by several published studies (5, 8, 16). In actuality, both of these modes of action probably contribute to varying degrees, depending on the matrix and the competitors present in that matrix, to the suppression of the *Listeria* population.

In this study, we used the gram-negative *Enterobacteriaceae*, *C. braakii*, as a model competitor to evaluate the effects of rapid growth and nutrient depletion on *Listeria* species populations during selective and nonselective growth. This organism was selected because it does not demonstrate the ability to produce or harbor any specific anti-*Listeria* biological molecules (7) and because it demonstrates rapid growth under the test conditions, resulting in *Listeria* population suppression (7, 17). In addition, *C. braakii* is a common foodborne contaminant, typically associated with spoilage, that can be isolated from a wide variety of products that are routinely screened for the presence of *Listeria* by regulatory agencies. Because *Listeria* population suppression is attributable only to the difference in growth rate between it and the competitor, we anticipate that similar results will be achieved using any competitor organism demonstrating a similar growth rate under the test conditions. Indeed, Dailey et al. (7) showed similar levels of *Listeria* population suppression with multiple competitor species, using both gram-negative and gram-positive microorganisms during competitive growth in BLEB.

Assessment of selective enrichment media

L. seeligeri strain Ls003 could not be recovered from either the UVM-1 or Fraser broths. Although we could not determine the reason for our inability to recovery Ls003, we speculate that the combined stress of the high levels of NaCl and the selective agents was inhibitory because we also could not recover this strain from UVM-1 or Fraser broths in the

absence of *C. braakii*. We therefore omitted Ls003 from all further statistical analyses. The highest overall mean *Listeria* population ($n = 27$; 9 strains $\times$ 3 replications) following competitive growth with *C. braakii* was 8.6 ± 0.4 log CFU/ml and occurred in Fraser broth incubated at 35°C for 48 h (data not shown). Overall mean *Listeria* populations ($n = 27$) of 6.6 ± 1.1 , 5.7 ± 1.3 , and 4.0 ± 0.5 log CFU/ml were observed for UVM-1 broth, MOPS-BLEB, and BLEB, respectively (data not shown). The overall mean *Listeria* populations were all statistically different ($P < 0.05$). Considerable strain variation was observed for each selective enrichment condition we tested (Fig. 1); however, for each strain the lowest population was usually observed with BLEB, followed by MOPS-BLEB and UVM-1 broth. Fraser broth generally showed the highest *Listeria* populations for each *Listeria* strain in this study.

Although Fraser broth consistently had the highest *Listeria* populations, there are not enough data to support a conclusion that this selective enrichment formulation is superior to the others. This study was performed using a single competitor strain, and that strain was selected because it previously had performed well in BLEB (17); the 10 strains of *Listeria* were selected because of their poor performance in BLEB (17). The observed populations of *C. braakii* following competitive growth with *Listeria* were 8.0 ± 0.1 , 7.9 ± 0.5 , 6.9 ± 0.3 , and 5.1 ± 1.6 log CFU/ml for UVM-1 broth, BLEB, MOPS-BLEB, and Fraser broth, respectively (data not shown). The two lowest mean *C. braakii* populations were observed in selective enrichment broths that were incubated at 35°C, suggesting that this strain may demonstrate a temperature-dependent sensitivity to one or more of the selective agents. It is possible that competitor microbial strains selected based on their performance in UVM-1 and Fraser broths would result in *Listeria* population suppression similar to that observed with *C. braakii* in BLEB.

Effect of temperature on *Listeria* species enrichment populations

The mean *Listeria* populations ($n = 108$; 9 strains $\times$ 4 media $\times$ 3 replications), for all strains and all media, were 5.8 ± 1.9 and 6.7 ± 1.8 log CFU/ml at 30 and 35°C, respectively (data not shown). The mean *Listeria* populations ($n = 54$; 9 strains $\times$ 2 temperatures $\times$ 3 replications), for all strains at both temperatures, were 4.5 ± 1.2 , 5.1 ± 1.3 , 6.9 ± 1.1 , and 8.5 ± 0.6 log CFU/ml for BLEB, MOPS-BLEB, UVM-1 broth, and Fraser broth, respectively (data not shown). Both the incubation temperature and selective enrichment medium were statistically significant ($P < 0.05$) effects in the two-way ANOVA model; in addition, the interaction between incubation temperature and selective enrichment medium was also significant ($P < 0.05$). The means, the standard deviations, and the results from the Tukey's test of significance for each medium and temperature combination are shown in Figure 2.

Considerable strain variation was observed at both temperatures and for all four selective enrichment formulations (data not shown). In BLEB, the mean populations for the individual *Listeria* strains were generally higher at 35°C than at 30°C and were significantly different ($P < 0.05$) for all but two strains (ARL-Lm-015 and ARL-Lm-087). In MOPS-BLEB, the mean populations of each *Listeria* strain were statistically higher ($P < 0.05$) at 35°C than at 30°C, with the exception of strain ARL-Lm-015, for which the two mean populations were statistically equivalent ($P > 0.05$). In UVM-1 broth, the mean populations of each *Listeria*

strain were statistically higher ($P < 0.05$) at 35°C than at 30°C, with the exceptions of strains ARL-Lm-007, ARL-Lm-065, and ARL-Ln-017, which were statistically equivalent ($P > 0.05$). In Fraser broth, the mean populations of *Listeria* strains ARL-Lm-015, ARL-Lm-031, ARL-Lm-062, ARL-Lm-087, and ARL-Ln-017 were statistically higher ($P < 0.05$) at 35°C than at 30°C; the mean populations of strains ARL-Lm-007, ARL-Lm-054, ARL-Lm-065, and ARL-Ln-011 at 35 and 30°C were statistically equivalent ($P > 0.05$).

Effects of selective additives on *Listeria* enrichment populations

We assessed the effects of the individual selective agents on the final population of *Listeria* during competitive growth with *C. braakii* for each medium except for MOPS-BLEB, which uses the same selective agents and concentrations as BLEB. The mean *Listeria* populations in basal BLEB, basal UVM-1 broth, and basal Fraser broth and their selective derivatives are shown in Figure 3.

The mean *Listeria* populations during competitive growth with *C. braakii*, in the absence of selective agents, were statistically equivalent ($P > 0.05$) despite a 0.7-log difference between the highest and lowest mean populations (Fig. 3A). In the absence of selective agents, the remaining components of each medium are primarily for nutrition and buffering. The lack of statistical differences indicates that the basal formulations of the three media are functionally equivalent despite their different individual components. All the *Listeria* strains included in this study were capable of reaching 9 log CFU/ml in all three basal enrichment mediums in the absence of the competitor *C. braakii* (data not shown). The population retardation exerted by *C. braakii* under nonselective conditions is consistent with the simple hypothesis that the faster-growing competitor has the higher population when exponential growth ceases, which is usually because of nutrient depletion (7, 11). The basal formulations of UVM-1 and Fraser broths, sans selective agents, are identical. The lack of statistical significance between the mean *Listeria* populations in these two broths indicates that the incubation temperature, 35°C for Fraser broth and 30°C for UVM-1 broth, is not influential under nonselective conditions when a 48-h incubation period is used. It is likely that any growth rate enhancement of *Listeria* at the higher temperature is offset by the growth rate enhancement of *C. braakii*.

However, this study does show that the incubation temperature was significantly influential on the *Listeria* populations when multiple selective agents were present, as indicated in Figure 1, which shows that the *Listeria* populations in selective MOPS-BLEB at an incubation temperature of 35°C were consistently higher than in selective BLEB at an incubation temperature of 30°C. In addition, the *Listeria* populations in selective Fraser broth at an incubation temperature of 35°C were consistently higher than in selective UVM-1 broth at an incubation temperature of 30°C (Fig. 1). As previously mentioned, the temperature effect may be the result of *C. braakii* sensitivity at 35°C to one or more of the selective agents and not necessarily of a growth rate enhancement of *Listeria*.

The mean *Listeria* populations following competitive growth with *C. braakii* with sodium nalidixate as the sole selective agent were statistically equivalent ($P > 0.05$) for the UVM-1 and Fraser broths (Fig. 3B). This is not surprising because, in the absence of the other selective agents, these two formulations are identical and differ only in their incubation

temperatures. The lack of any statistical difference between sodium nalidixate-supplemented basal UVM-1 and Fraser broths also suggests that temperature is not an important contributing factor to the final *Listeria* populations when a 48-h incubation is used; this is similar to what we observed under nonselective conditions. The mean *Listeria* population in basal BLEB supplemented with only sodium nalidixate was statistically lower ($P < 0.05$) than in the basal UVM-1 and Fraser broths supplemented with sodium nalidixate (Fig. 3B). The final levels of sodium nalidixate were 20 mg/liter in the UVM-1 and Fraser broths and 40 mg/liter in BLEB. These results suggest that the strains used in this study demonstrate some level of sensitivity to sodium nalidixate that manifests itself more at the higher level of this agent in BLEB. These results may at first seem to contradict the earlier report of Beumer et al. (3), which indicated that sodium nalidixate up to a final level of 40 mg/liter did not significantly affect the growth of *L. monocytogenes*. However, recall that the strains used in our study were specifically selected, out of 200 strains tested, based on their poor competitive performance in BLEB (17). It is possible that sensitivity to sodium nalidixate contributed to their poor performance in the test study (17) and that the strains used in the Beumer et al. study (3) were simply not sensitive at these levels.

In acriflavine-HCl-supplemented basal BLEB and UVM-1 broth, the mean *Listeria* populations were not statistically different ($P > 0.05$) (Fig. 3C). None of the strains of *Listeria* included in this study could be recovered from acriflavine-HCl-supplemented basal Fraser broth. The levels of acriflavine-HCl were 10, 12, and 25 mg/liter for BLEB, UVM-1 broth, and Fraser broth, respectively. These results suggest that the 10 strains of *Listeria* in our study demonstrate some level of sensitivity to acriflavine-HCl that manifests itself more at the highest level we tested. *L. monocytogenes* sensitivity to acriflavine-HCl has been previously reported and was attributed to an increase in lag time and a decrease in the specific growth rate of the test strains (3). To confirm this for our test strains, we performed a growth rate analysis, as described in previous studies (7, 8, 16). The mean generation times were 150 ± 23 min ($n = 9$), 160 ± 18 min ($n = 9$), and 204 ± 43 min ($n = 9$) in nonselective basal BLEB, acriflavine-HCl-supplemented basal BLEB, and fully selective BLEB, respectively. The mean *Listeria* generation times in nonselective and acriflavine-HCl-supplemented basal BLEB were statistically similar ($P > 0.05$), and both were statistically lower ($P < 0.05$) than in fully selective BLEB. Acriflavine-HCl, when used alone, did not appear to appreciably slow the growth of the *Listeria* strains in this study. For comparison, the mean generation times of the *C. braakii* strain ARL-Cb-01 were approximately 65, 70, and 70 min in nonselective basal BLEB, acriflavine-HCl-supplemented basal BLEB, and fully selective BLEB, respectively. Finally, note that the inability to recover *Listeria* from acriflavine-HCl-supplemented basal Fraser broth does not appear to be because of overgrowth by *C. braakii*. Rather, it appears that acriflavine-HCl has a suppressive effect that is not present in fully selective Fraser broth (compare Figs. 3C and 1). Acriflavine-HCl-supplemented basal Fraser broth and fully selective Fraser broth differ in that the latter has 20 g/liter NaCl, 3 g/liter LiCl, and 20 mg/liter sodium nalidixate. It is possible that one of these other additives, perhaps NaCl, negates the suppressive effect of acriflavine-HCl that we see in Figure 3C; however, additional studies are needed to fully resolve this issue.

The level of NaCl in BLEB is 5 g/liter; it has a nutritional role and a role in maintaining osmotic balance. The level of NaCl in UVM-1 and Fraser broths is 20 g/liter, and at this

level it functions to aid in selectivity. The mean *Listeria* populations in NaCl-supplemented basal UVM-1 and Fraser broths were statistically the same ($P > 0.05$) (Fig. 3D). NaCl-supplemented basal UVM-1 and Fraser broths are identical except for the incubation temperatures, and the results indicate that incubation temperature is not important when NaCl is the sole selective agent and a 48-h incubation period is used. This is consistent with what we observed for the nonselective broths and the sodium nalidixate-supplemented derivative (Fig. 3A and 3B).

Of the selective media in this study, LiCl was a supplement in only the Fraser broth. The mean *Listeria* population in LiCl-supplemented basal Fraser broth (Fig. 3E) was statistically similar to that observed in NaCl-supplemented basal Fraser broth and was statistically higher than in NaCl-supplemented basal UVM broth (Fig. 3D). This may be an indication that incubation temperature is an important contributing factor for *Listeria* enrichment populations when LiCl is present; however, additional work is necessary to fully resolve the relationship between LiCl and temperature.

In this study, cycloheximide was a supplement in only the BLEB enrichment formulation. The mean *Listeria* population when cycloheximide was present as the sole selective agent (Fig. 3F) was statistically similar to that in nonselective BLEB (Fig. 3A). This is to be expected because cycloheximide is added as an antimycotic and should not negatively impact bacterial growth.

In summary, the recovery of *Listeria* from contaminated foods is complicated by its slow rate of growth relative to other foodborne microorganisms that might be present and a general lack of resistance to potential selective agents that could be used to suppress background microbial competitors. We compared four common selective enrichment formulations used by U.S. regulatory authorities for *Listeria* surveillance using 10 strains of *Listeria* and a single *Enterobacteriaceae* species competitor. The goal of the study was not to determine which enrichment is best; data using only one competitor species would not support such a conclusion. Rather, our goal was use the media comparison to identify the selective components that appear most influential on microbial competition during enrichment. Acriflavine-HCl and sodium nalidixate are present at various levels in all four media we tested; however, their presence did little to prevent the overgrowth of the competitor and the subsequent population suppression of *Listeria*, regardless of level. At higher levels, both of these selective agents were shown to be detrimental to *Listeria* populations. We determined that the salts NaCl and LiCl were most influential at enhancing the populations of *Listeria* in the presence of the competitor. Further investigation into these salt effects appears warranted. We chose as the competitor a strain of *C. braakii* that had been identified as the most competitive organism in an earlier study using BLEB (17), and BLEB does not have salt levels high enough to be considered selective. Studies using competitor microorganisms selected based on their performance in UVM-1 and Fraser broths are needed to fully evaluate the significance of the effects of salt levels on the final enrichment populations of *Listeria* species.

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Figure 1. Mean populations for 10 strains of *Listeria* following competitive growth with *C. braakii* in four selective enrichment media. Error bars reflect the standard error associated with three replications.

Figure 2. Incubation temperature effects on the mean *Listeria* population following competitive growth with *C. braakii* in four selective enrichment media. Lowercase letters at the base of the bars denote statistical significance at the 95% probability level as determined by Tukey's mean comparison test. Error bars reflect the standard deviation associated with 10 *Listeria* strains and three replications ($n = 30$). Means are presented in ascending order.

Figure 3.

Comparison of selective conditions on the mean populations of *Listeria following competitive growth with C. braakii*. Nonselective basal formulations (A); basal formulations supplemented with sodium nalidixate only (B), acriflavine-HCl only (C), NaCl only (D), LiCl only (E), and cycloheximide only (F); and fully selective enrichment formulations (G). Incubation times were all 48 h. Incubation temperatures were 30°C for BLEB and its selective derivatives, and for basal UVM-1 broth and its selective derivatives. An incubation temperature of 35°C was used for basal Fraser broth and its selective derivatives. Lowercase letters at the base of the bars denote statistical significance at the 95% probability level as determined by Tukey's mean comparison test. Error bars reflect the standard deviation of nine *Listeria* strains and three replications ($n = 27$). One strain, Ls003, was omitted from the analysis because it could not be recovered from the UVM-1 or Fraser broths. Open bars, BLEB and BLEB selective derivatives; gray bars, UVM-1 broth and UVM-1 broth selective derivatives; dotted bars, Fraser broth and Fraser broth derivatives.