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SEL, a Selective Enrichment Broth for Simultaneous Growth of *Salmonella enterica*, *Escherichia coli* O157:H7, and *Listeria monocytogenes*▽†

Hyochin Kim and Arun K. Bhunia*

Molecular Food Microbiology Laboratory, Department of Food Science, Purdue University, West Lafayette, Indiana

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Multipathogen detection on a single-assay platform not only reduces the cost for testing but also provides data on the presence of pathogens in a single experiment. To achieve this detection, a multipathogen selective enrichment medium is essential to allow the concurrent growth of pathogens. SEL broth was formulated to allow the simultaneous growth of *Salmonella enterica*, *Escherichia coli* O157:H7, and *Listeria monocytogenes*. The results were compared to those obtained with the respective individual selective enrichment broths, Rappaport-Vassiliadis (RV) for *S. enterica*, modified *E. coli* broth with 20 mg of novobiocin/liter for *E. coli* O157:H7, and Fraser broth for *L. monocytogenes*, and a currently used universal preenrichment broth (UPB). The growth of each pathogen in SEL inoculated at 10^1 or 10^3 CFU/ml was superior to that in the respective individual enrichment broth, except in the case of RV, in which *Salmonella* cells inoculated at both concentrations grew equally well. In mixed-culture experiments with cells of the three species present in equal concentrations or at a 1:10:1,000 ratio, the overall growth was proportional to the initial inoculation levels; however, the growth of *L. monocytogenes* was markedly suppressed when cells of this species were present at lower concentrations than those of the other two species. Further, SEL was able to resuscitate acid- and cold-stressed cells, and recovery was comparable to that in nonselective tryptic soy broth containing 6% yeast extract but superior to that in the respective individual selective broths. SEL promoted the growth of all three pathogens in a mixture in ready-to-eat salami and in turkey meat samples. Moreover, each pathogen was readily detected by a pathogen-specific immunochromatographic lateral-flow or multiplex PCR assay. Even though the growth of each pathogen in SEL was comparable to that in UPB, SEL inhibited greater numbers of nontarget organisms than did UPB. In summary, SEL was demonstrated to be a promising new multiplex selective enrichment broth for the detection of the three most prominent food-borne pathogens by antibody- or nucleic acid-based methods.

Every year, up to 81 million people in the United States suffer from food-borne diseases, and food-borne pathogens continue to be a major public health concern (37, 40). Among the known food-borne pathogens, *Salmonella enterica*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 are of major concern because of their continued association with highly popular foods such as poultry products, ready-to-eat meats, dairy products, and fruits and vegetables. Above all, these pathogens have very high incidence and mortality rates and have been involved in several recent outbreaks (37). Therefore, the control and prevention of these pathogens are of high priority to improve the safety of the U.S. food supply. The accurate and rapid detection of these three pathogens is essential, and testing is sometimes mandatory before certain food items can be distributed for retail sale for human consumption.

Though the sensitivities of many of the modern detection methods, such as antibody-, nucleic acid-, and biosensor-based methods, have improved significantly (6, 23, 38, 46), an enrichment step is still needed. This step is required not only to increase the target-pathogen concentration in a sample but also to resuscitate physiologically stressed or injured cells. Selective enrichment is also necessary to suppress the natural background microorganisms so as to improve detection efficiency and to avoid false results. However, the drawbacks of some of the selective enrichment broths are that the selective agents can be inhibitory or can delay the recovery and growth of healthy or stressed target pathogens (26) and may also down regulate antigen expression, thus affecting the detection of pathogens (21, 24, 34).

Current research trends emphasize the development of multipathogen platforms in a single-assay format. For example, multiplex PCR assays (5, 20, 30, 42), protein/antibody microarray biosensors (35, 50), array-based immunosorbent assays (14), and DNA microarray methods (15) continue to be developed. The multipathogen detection approach is attractive and economically favorable since it can reduce the total space requirement for handling a large number of samples, as well as the bench space, supplies, reagents, and labor needed, thus reducing the overall cost of testing per pathogen. Furthermore, multiplex detection is a rational approach since many foods, such as milk and dairy products (1), meat and poultry (16, 45), and fruits and vegetables (4, 10), are common carriers of *S. enterica*, *E. coli* O157:H7, and *L. monocytogenes*. Moreover, multipathogen detection can mitigate the industry and regulatory needs for

* Corresponding author. Mailing address: Department of Food Science, 745 Agriculture Mall Dr., Purdue University, West Lafayette, IN 47907-2009. Phone: (765) 494-5443. Fax: (765) 494-7953. E-mail: bhunia@purdue.edu.

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the testing of foods that have a high risk of contamination with these pathogens.

To facilitate multipathogen detection in a single-assay format, a suitable enrichment medium is urgently needed. A universal preenrichment broth (UPB) for multipathogen enrichment (2) is commercially available from Difco Lab, Sparks, MD; however, this medium lacks inhibitory agents to provide selectivity for target pathogens and, thus, may not be suitable for samples with high levels of background microflora, such as raw or unprocessed samples from animal and plant origins. Thus, the objectives of this study were to formulate a single medium that can support the simultaneous growth primarily of three food-borne pathogens, *S. enterica*, *E. coli* O157:H7, and *L. monocytogenes*, if present in a single sample and to demonstrate the performance of the medium by employing an antibody-based immunochromatographic lateral-flow assay (ICLFA) and a multiplex PCR assay. The multipathogen medium, designated SEL (for *Salmonella*, *Escherichia*, and *Listeria*), was developed in this study, and its performance as an enrichment broth was verified by growing three pathogens in various proportions and detecting the bacteria by using ICLFA and multiplex PCR. The spectra of growth of target and nontarget bacteria obtained from our collection, as well as natural isolates from food, in SEL were also determined. Next, the ability of SEL to resuscitate acid- or cold-stressed bacteria was investigated. Finally, the performance of SEL was examined and compared with that of UPB by testing pathogen-inoculated meat samples.

MATERIALS AND METHODS

Bacterial cultures and growth conditions. *E. coli* O157:H7 EDL 933, *L. monocytogenes* V7 (serotype 1/2a), and *S. enterica* serovar Enteritidis phage type 1 (PT1) cultures were used as standard reference cultures in all the studies and were maintained on brain heart infusion (BHI; Accumedia, Lansing, MI) agar plates at 4°C. Fresh cultures were prepared by inoculating tryptic soy broth containing 0.6% yeast extract (TSBYE; Difco Lab, Sparks, MD) at 37°C. The other organisms used in this study are listed in Table 1 and were maintained similarly, except for lactic acid bacteria, which were grown and maintained in deMann Rogosa Sharpe (MRS) broth and on MRS agar (both from Difco).

The individual selective enrichment broths and plating agars (all purchased from Difco) used in this study included modified *E. coli* broth with 20 mg of novobiocin/liter (mEC+n) and sorbitol MacConkey agar with cefixime-tellurite (CT-SMAC) for *E. coli* O157:H7, Rappaport-Vassiliadis (RV) broth and xylose-lysine-deoxycholate (XLD) agar for *Salmonella* serovars Enteritidis and Typhimurium, and Fraser broth (FB) and modified Oxford agar (MOX) for *L. monocytogenes*. The cefixime-tellurite supplement was purchased from BioMerieux (Hazelwood, MO), and UPB was purchased from Difco Lab.

Formulation of multipathogen selective enrichment broth SEL. Commercially available buffered *Listeria* enrichment broth base (BLEB; Difco Laboratories) without an antibiotic supplement was used as a base medium for the development of the multipathogen enrichment broth SEL. Four antimicrobial agents, acriflavine (ICN Biomedical Inc., Aurora, OH) and cycloheximide, fosfomycin, and nalidixic acid (all purchased from Sigma, St. Louis, MO), were used as selective agents. The concentration of each to be used for SEL formulation was optimized by growing all three pathogens separately in a series of growth curve experiments (31). The final composition of the SEL medium is presented in Table 2.

Antibody-based ICFLA. Widely used antibody-based ICLFA kits were employed to verify the antibody-based detection of target pathogens following enrichment in SEL. Reveal kits (Neogen Corp., Lansing, MI) for *Salmonella*, *E. coli* O157:H7, and *Listeria* were used for verification. The Reveal kits for *E. coli* O157:H7 and *Salmonella* allow the testing of samples without heat treatments, while the Reveal kit for *Listeria* recommends a heat treatment (80°C for 20 min) prior to testing. Briefly, following the growth of test organisms in SEL, 120-μl aliquots of *E. coli* and *Salmonella* samples and 135-μl heat-inactivated *L. monocytogenes* samples were dispensed into the sample ports of the ICLFA strips, and the strips were incubated at room temperature for 15 to 20 min. Positive antibody reactions (indicated by the appearance of a dark band in the viewing window) were recorded by capturing digital images, and the reaction intensities were quantified by using a densitometer software program (Scion Corp., Frederick, MD). As controls, the procedures recommended by the manufacturer (Neogen Corp.) for each pathogen were used.

Multiplex PCR. Multiplex PCR assays were employed to verify whether SEL could be used as an enrichment broth for PCR-based detection of pathogens. DNA was extracted from 1 ml of each culture by using DNA extraction kits (DNeasy tissue kits; catalog no. 69506) per the instructions of the manufacturer (Qiagen, Valencia, CA). The primer sequences and the putative product sizes for each amplicon are listed in Table 3. The PuReTaq ready-to-go PCR beads (GE Healthcare, Piscataway, NJ) were used for PCR amplification (39). PCR mixtures (25 μl) each contained 1 μg of each DNA template, 15 pmol of each primer, and one PuReTaq PCR bead containing 2.5 U of PuReTaq polymerase, 200 μmol of each deoxynucleoside triphosphate, 10 mM Tris-HCl, 50 mM KCl, and 1.5 nM $MgCl_2$. After the initial DNA denaturation at 94°C for 3 min, 40 amplification cycles consisting of 1 min of denaturation at 94°C, 1.5 min of annealing at 60°C, and 1.5 min of elongation at 72°C were done in a thermal cycler (MJ Research, Watertown, MA). Amplified DNA products were detected in agarose gel (1.5%, wt/vol) containing 1 μg of ethidium bromide/ml.

Growth kinetics of individual target pathogens in SEL. To examine the growth of target pathogens in SEL, two inoculation levels, 10^1 and 10^3 CFU/ml, were chosen. Volumes of 100 ml of SEL were inoculated with freshly grown *Salmonella* serovar Enteritidis, *E. coli* O157:H7, and *L. monocytogenes* and incubated at 37°C in a shaking incubator (Edison, New Brunswick, NJ) set at 150 rpm. The growth rates were determined by enumerating bacterial cells at every 2-h interval by plating the cells onto BHI agar plates. At the same time, the growth of target pathogens in the respective specific selective enrichment broths, RV broth for *Salmonella* serovar Enteritidis, mEC+n for *E. coli* O157:H7, and FB for *L. monocytogenes*, was also evaluated. The Gompertz equation (47) was used to compare the growth kinetics of the different pathogens in SEL. ICLFA and PCR assays were used to evaluate the medium performance. These experiments were repeated twice.

Growth kinetics of target pathogens in a mixture. Four different combinations of initial cell numbers were used to examine the growth kinetics of each pathogen in SEL. In experiment I, equal concentrations of *Salmonella* serovar Enteritidis, *E. coli* O157:H7, and *L. monocytogenes* cultures (ca. 3×10^2 CFU of each pathogen/ml) were inoculated into 100 ml of SEL. In experiments II through IV, the ratio of the cultures used as inocula was set at 1:10:1,000, with the proportion of each culture varying in order throughout the different experiments. In experiment II, the inocula contained *Salmonella* serovar Enteritidis cells at a mean concentration ± a standard deviation (SD) of 13.5 ± 1.1 CFU/ml, *E. coli* at 1,327 ± 166 CFU/ml, and *L. monocytogenes* at 1.3 ± 0.6 CFU/ml. In experiment III, the mixture consisted of *Salmonella* serovar Enteritidis at 1.4 ± 0.1 CFU/ml, *E. coli* at 14.6 ± 1.6 CFU/ml, and *L. monocytogenes* at 1,180 ± 125 CFU/ml, and in experiment IV, *Salmonella* serovar Enteritidis at 1,178 ± 124 CFU/ml, *E. coli* at 1.3 ± 0.1 CFU/ml, and *L. monocytogenes* at 11.5 ± 1.9 CFU/ml were used. The inoculated SEL broths (100 ml each) were incubated at 37°C for 24 h in a shaking incubator, and samples were withdrawn every 2 h. The cell counts for each pathogen were determined by plating the cells onto plates with the appropriate selective agar: XLD agar for *Salmonella* serovar Enteritidis, CT-SMAC for *E. coli* O157:H7, and MOX for *L. monocytogenes*. The lateral-flow immunoassay and multiplex PCR assays were performed with culture samples taken at 16 to 18 h of growth to determine if a pathogen-specific detection assay could be employed for the detection of individual pathogens from a mixed culture. These experiments were repeated three times with two replicates per trial.

In a separate experiment, several samples of ready-to-eat sliced turkey meat (25 g each; see below for details on meat sample procurement and the preparation procedure) were inoculated with bacterial mixtures as listed above for experiments I to IV, enriched with 225 ml of SEL for 24 h, and analyzed by multiplex PCR as described above.

Isolation of resident bacteria from food. Bacterial isolates were obtained from ready-to-eat meats. A total of two pieces of roasted turkey breast and three Genoa salamis (218 g each) were procured from several different local grocery stores (West Lafayette, IN). Each meat sample (25 g) was homogenized in 225 ml of 20 mM phosphate-buffered saline (pH 7.0), dilutions were plated onto BHI or MRS agar plates, and the plates were incubated at 37°C. Colonies were randomly picked and identified by metabolic fingerprinting using the BioLog culture identification system (Hayward, CA) or by ribotyping (27) employing an

TABLE 1. Growth spectra of food-borne bacteria in multipathogen enrichment broth SEL

Organism	Source[a]	Ribotyping result	Growth in SEL (mean OD_{595} ± SD) at:			Growth in UPB (mean OD_{595} ± SD) at:		
			12 h	16 h[c]	24 h	12 h	16 h[c]	24 h
Target pathogens								
S. enterica								
Serovar Enteritidis PT1	Our collection	DUP-2035	1.05 ± 0.02	1.09 ± 0.03 A	1.09 ± 0.03	0.62 ± 0.02	0.80 ± 0.07 B	0.93 ± 0.07
Serovar Kentucky 1271-94	Our collection	NT[b]	0.95 ± 0.04	1.05 ± 0.02 A	1.07 ± 0.01	0.50 ± 0.04	0.70 ± 0.07 B	0.85 ± 0.11
Serovar Tennessee 825-94	Our collection	NT	0.99 ± 0.01	1.12 ± 0.01 A	1.19 ± 0.03	0.47 ± 0.05	0.73 ± 0.05 B	0.88 ± 0.05
Serovar Typhimurium	Our collection	DUP-1167	1.01 ± 0.03	1.10 ± 0.01 A	1.15 ± 0.01	0.72 ± 0.02	0.84 ± 0.04 B	1.00 ± 0.06
E. coli								
O157:H7 G5303 (EHEC)	CDC	NT	0.98 ± 0.04	1.01 ± 0.04 A	0.96 ± 0.03	0.61 ± 0.00	0.80 ± 0.02 B	0.86 ± 0.06
O157:H7 G5324 (EHEC)	CDC	NT	0.98 ± 0.09	1.08 ± 0.04 A	0.97 ± 0.09	0.65 ± 0.05	0.77 ± 0.05 B	0.95 ± 0.02
O157:H7 C7927 (EHEC)	Apple cider	NT	1.03 ± 0.04	1.04 ± 0.06 A	1.03 ± 0.06	0.63 ± 0.01	0.76 ± 0.06 B	0.89 ± 0.02
O25:K98:NM (ETEC)	M. Donnenberg	DUP-18656	0.04 ± 0.02	0.37 ± 0.04 B	0.52 ± 0.06	0.62 ± 0.06	0.77 ± 0.08 A	0.88 ± 0.13
O78:H11 (ETEC)	M. Donnenberg	DUP-19199	0	0 B	0	0.63 ± 0.06	0.82 ± 0.00 A	0.94 ± 0.12
O127:H6 ATCC 35401 (EPEC)	ATCC	DUP-3017	0.96 ± 0.09	1.03 ± 0.06 A	1.00 ± 0.10	0.67 ± 0.06	0.75 ± 0.09 B	0.87 ± 0.06
O142:H6 ATCC 43886 (EPEC)	ATCC	NT	0.09 ± 0.02	0.29 ± 0.13 B	0.40 ± 0.06	0.63 ± 0.00	0.71 ± 0.00 A	0.87 ± 0.00
K-12 (nonpathogenic)	Our collection	NT	1.05 ± 0.07	1.13 ± 0.05 A	1.11 ± 0.09	0.63 ± 0.10	0.80 ± 0.08 B	0.86 ± 0.09
L. monocytogenes								
V7 (1/2a)	FDA (dairy)	DUP-1039	0.09 ± 0.02	0.69 ± 0.05 A	0.91 ± 0.01	0.13 ± 0.01	0.47 ± 0.01 B	0.42 ± 0.01
Scott A (4b)	FDA (human)	DUP-1042	0.07 ± 0.00	0.73 ± 0.01 A	1.03 ± 0.00	0.40 ± 0.02	0.46 ± 0.01 B	0.44 ± 0.01
F4244 (4b)	CDC (human)	DUP-1044	0.03 ± 0.00	0.59 ± 0.01 A	0.94 ± 0.05	0.36 ± 0.08	0.36 ± 0.05 B	0.31 ± 0.05
F4260 (1/2b)	CDC (human)	DUP-1042	0.13 ± 0.00	0.94 ± 0.01 A	1.09 ± 0.02	0.43 ± 0.00	0.42 ± 0.01 B	0.41 ± 0.00
Listeria innocua F4248	CDC	DUP-1006	0.19 ± 0.02	0.97 ± 0.02 A	1.19 ± 0.02	0.43 ± 0.00	0.43 ± 0.00 B	0.42 ± 0.00
Nontarget bacteria								
Bacillus cereus MS1-9	J. Handelsman	DUP-12561	0	0	0	0.40 ± 0.01	0.43 ± 0.00 A	0.48 ± 0.00
Bacillus megaterium ATCC 6633	ATCC	DUP-12551	0	0	0	0.39 ± 0..00	0.47 ± 0.00 A	0.57 ± 0.01
Bacillus subtilis ATCC 9885	ATCC	DUP-16973	0	0	0	0.21 ± 0.02	0.18 ± 0.00 A	0.18 ± 0.00
Enterobacter aerogenes	Our collection	DUP-14591	1.11 ± 0.04	1.17 ± 0.03 A	1.23 ± 0.01	0.82 ± 0.03	0.93 ± 0.01 B	1.14 ± 0.10
Enterococcus faecalis ATCC 344	ATCC	NT	0	0.03 ± 0.01 B	0.03 ± 0.01	0.45 ± 0.01	0.54 ± 0.01 A	0.48 ± 0.00
Hafnia alvei	Our collection	DUP-18066	0.57 ± 0.11	0.09 ± 0.06 B	0.80 ± 0.07	0.62 ± 0.06	0.74 ± 0.06 A	0.86 ± 0.08
Streptococcus mutans ATCC 25175	ATCC	NT	0.32 ± 0.01	0.89 ± 0.10 A	0.84 ± 0.07	0.51 ± 0.01	0.66 ± 0.01 B	0.80 ± 0.01
Pseudomonas aeruginosa ATCC 10145	ATCC	DUP-11042	0.15 ± 0.04	0.29 ± 0.02 A	0.37 ± 0.06	0.09 ± 0.05	0.28 ± 0.01 A	0.56 ± 0.01
Proteus vulgaris	Our collection	DUP-10074	0	0 B	0	0.34 ± 0.00	0.54 ± 0.04 A	0.65 ± 0.03
Brochothrix thermosphacta	Our collection	NT	0	0	0	0	0	0
Serratia marcescens	Our collection	NT	0.03 ± 0.02	0.21 ± 0.06 B	0.83 ± 0.06	0.51 ± 0.01	0.70 ± 0.07 A	0.71 ± 0.05
Lactobacillus acidophilus ATCC 4356	ATCC	NT	NT	NT	0	NT	NT	0
Lactobacillus casei KCTC 3109	KCTC	NT	NT	NT	0	NT	NT	0.068 ± 0.00
Lactobacillus rhamnosus GG ATCC 53103	ATCC	NT	NT	NT	0	NT	NT	0.07 ± 0.00
Lactobacillus plantarum NCDO955	NCDO	NT	NT	NT	0	NT	NT	0.07 ± 0.00
Lactococcus lactis subsp. *lactis* ATCC 11454	ATCC	NT	NT	NT	0	NT	NT	0.14 ± 0.00
Pediococcus sp.	Our collection	NT	NT	NT	0	NT	NT	0.00 ± 0.00
Leuconostoc mesenteroides	Our collection	NT	NT	NT	0	NT	NT	0.03 ± 0.00
Natural food isolates								
Bacillus megaterium HK1	This study	DUP-6058	0	0 B	0	0.40 ± 0.00	0.50 ± 0.04 A	0.59 ± 0.06
Staphylococcus epidermidis HK7	This study	DUP-4123	0.21 ± 0.04	0.74 ± 0.04 A	0.65 ± 0.00	0.48 ± 0.09	0.69 ± 0.00 A	0.82 ± 0.02
Enterobacter cloacae HK8	This study	DUP-15301	1.06 ± 0.04	1.15 ± 0.05 A	1.13 ± 0.07	0.75 ± 0.01	0.85 ± 0.00 B	0.98 ± 0.00
Lactococcus lactis subsp. *lactis* HK21	This study	DUP-12773	0	0 B	0	0.36 ± 0.00	0.33 ± 0.00 A	0.32 ± 0.00
Pediococcus acidilactici HK32	This study	DUP-5600	0	0 B	0	0.11 ± 0.01	0.10 ± 0.00 A	0.21 ± 0.00

[a] FDA, Food and Drug Administration; CDC, Centers for Disease Control and Prevention; ATCC, American Type Culture Collection; KCTC, Korean Culture Type Collection; NCDO, National Collection of Dairy Organisms; J. Handelsman, University of Wisconsin, Madison; M. Donnenberg, University of Maryland, Baltimore.

[b] NT, not tested.

[c] OD_{595} readings for growth in SEL and UPB at the 16-h time point that are in the same row and labeled with the same letter (A or B) are not significantly different at P of <0.05.

automated RiboPrinter (Qualicon, Wilmington, DE). Five selected isolates were used in this study (Table 1).

Growth profiles of food-borne microorganisms in SEL. To investigate the spectra of bacterial growth in SEL, several found food-borne pathogens and spoilage and resident bacterial isolates (Table 1) were inoculated (ca. 10^3 CFU/ml) into 10 ml of SEL and incubated at 30 or 37°C with agitation (100 rpm). Aliquots of 1.0 ml of each culture were withdrawn at 12, 16, and 24 h into polystyrene disposable cuvettes, and the growth was monitored by measuring the absorbance at 595 nm in a DU-640 spectrophotometer (Beckman-Coulter). This experiment was performed three times with six replicates per trial. At the same time, bacterial growth in UPB and the respective specific selective enrichment broths, RV broth, mEC+n, and FB, under similar conditions was examined.

Recovery of cold- or acid-stressed bacteria in SEL. The abilities of SEL to resuscitate stress-exposed bacterial cells and enrich samples were investigated. The two most common stress conditions, exposure to acid (pH 4.5 and 5.5) and cold (4°C) (24), were examined. Each freshly grown culture of *Salmonella* serovar Enteritidis, *E. coli* O157:H7, and *L. monocytogenes* was inoculated into 30 ml of

TABLE 2. Composition of SEL (*Salmonella*, *Escherichia*, *Listeria*) broth

Ingredient	Amt (g per liter)	Comment
Pancreatic digest of casein	17	Same as in BLEB
Yeast extract	6	Same as in BLEB
Dextrose	2.5	Same as in BLEB
Soytone	3	Same as in BLEB
Sodium chloride	5	Same as in BLEB
Monopotassium phosphate	1.35	Same as in BLEB
Dipotassium phosphate	2.5	Same as in BLEB
Disodium phosphate	9.6	Same as in BLEB
Sodium pyruvate	1.1	Same as in BLEB
Acriflavine	0.01	Modification of BLEB recipe
Cycloheximide	0.05	Modification of BLEB recipe
Fosfomycin	0.05	Newly added (not in BLEB)
Nalidixic acid	0.002	Modification of BLEB recipe

TSBYE (1%, vol/vol) and then incubated at 37°C in a shaker incubator (150 rpm) to mid-log phase (see Fig. 1): 2 h for *E. coli* O157:H7, 4 h for *L. monocytogenes*, and 2.5 h for *Salmonella* serovar Enteritidis. Aliquots (5 ml each) were centrifuged (5,000 × *g* for 10 min) and washed once with 30 ml of phosphate-buffered saline, and the cell pellets were resuspended and held for 3 h in 5 ml of TSBYE with the appropriate stressors: (i) TSBYE adjusted to pH 4.5 and (ii) TSBYE adjusted to pH 5.5 by using 1 M lactic acid and (iii) TSBYE at 4°C (precooled TSBYE was used). The cells exposed to acid stress were incubated at 37°C, and cold-stressed cells were incubated at 4°C. Each stress-exposed culture (1%, vol/vol) was transferred into SEL, TSBYE, or the corresponding individual selective enrichment broth and incubated for 3 h (short recovery) and 6 h (long recovery) at 37°C in a shaking incubator. Bacterial cell counts immediately after the exposure to stress and after 3 and 6 h of recovery in different media were determined by surface plating of cells onto BHI agar plates (1, 29).

Comparative enrichment of artificially inoculated meat samples with pathogens in SEL and UPB broth and subsequent detection by ICLFA and PCR. Several 218-g portions of ready-to-eat deli meats (roasted turkey breast and Genoa salami) were purchased from local grocery stores in West Lafayette, IN. The turkey breast samples had 5% fat and 15 g of protein per 56-g serving, and the salami samples had 28% fat and 21 g of protein per 56-g serving. The absence of *Salmonella* serovar Enteritidis, *E. coli* O157:H7, and *L. monocytogenes* in each meat sample was confirmed by using standard procedures as outlined in the *Bacteriological Analytical Manual* (18) before the initiation of the challenge study. Twenty-five grams of each meat sample was placed into a stomacher bag containing an inner filter lining (Whirl-Pak [catalog no. B01318; Nasco Fort Atkinson, WI]). The meat samples were inoculated with approximately 3×10^2 CFU of each culture/g and held at room temperature for 15 min to allow bacterial adsorption. Then, a 225-ml volume of SEL or UPB was added to each bag, and the samples were blended for 2 min by using a Stomacher 400 (Seward, Norfolk, United Kingdom). The homogenized meat samples were incubated at 37°C for 24 h. Uninoculated meat samples (25 g of meat in 225 ml of SEL) served as negative controls. After 8, 10, 12, 16, and 24 h of incubation, 5-ml aliquots were collected from each bag, serially diluted in 0.1% sterile peptone water, and analyzed for microbial counts by being plated onto the corresponding selective agar plates. Samples were also tested by PCR and lateral-flow immunoassay as described above. In a separate experiment, the influence of different meat samples (two different brands of salami and two brands of turkey) on pathogen enrichment in SEL was evaluated as described above.

Gompertz equation and statistical analyses. To determine the exponential growth rate (EGR), generation time (GT), lag-phase duration (LPD), and maximum population density (MPD), the growth of each bacterium in SEL was modeled with the Gompertz equation (47) by using a nonlinear mixed model with PROC NLMIXED in software version 9.1 for Windows (SAS Institute Inc., Cary, NC). To test for differences among the broths in the comparison experiments, the statistical significance was assessed by a *t* test; a P value of <0.05 was considered significant.

RESULTS

Growth kinetics of individual target pathogens in SEL. (i) *Salmonella* serovar Enteritidis. The growth of *Salmonella* serovar Enteritidis in SEL was compared with that in RV broth. Both media were inoculated with 10^1 and 10^3 CFU/ml. Data extrapolated from the fitted Gompertz curves indicated that the average EGRs, GTs, LPDs, and MPDs for the two broths at the two inoculation levels were comparable (Fig. 1A; see Table S1 in the supplemental material), suggesting that the performance of SEL was equivalent to that of RV broth.

(ii) *E. coli* O157:H7. The *E. coli* O157:H7 growth rate in SEL was also examined and compared with that in mEC+n after both media were inoculated with 10^1 and 10^3 CFU/ml. The first distinguishable result was that no growth of *E. coli* O157:H7 inoculated at 10^1 CFU/ml into mEC+n was observed, whereas SEL supported growth at that inoculation level (Fig. 1B). Data extrapolated from the fitted Gompertz curves indicated that the average EGR in SEL inoculated with 10^3 CFU/ml (0.89 $\log_{10}$ CFU/ml/h) was significantly ($P < 0.05$) higher than that in mEC+n inoculated with the same concentration (0.73 $\log_{10}$

TABLE 3. Oligonucleotide primers used in the multiplex PCR

Pathogen	Target virulence factor	Target gene	Primer[b]	Sequence (5′ to 3′)	Product size (bp)	Reference
Salmonella serovar Enteritidis	*Salmonella* serovar Enteritidis fimbrial antigen	*sefA*	F	GCAGCGGTTACTATTGCAGC	310	53
			R	TGTGACAGGGACATTTAGCG		
	Salmonella plasmid virulence factor	*spv*[a]	F	GCCGTACACGAGCTTATAGA	250	48
			R	ACCTACAGGGGCACAATAAC		
E. coli O157:H7	Attachment and effacement	*eaeA*	F	TCAATGCAGTTCCGTTATCAGTT	482	52
			R	GTAAAGTCCGTTACCCCAACCTG		
	Shiga-like toxin 1	stx_1	F	CAGTTAATGTGGTGGCGAAGG	348	13
			R	CACCAGACAATGTAACCGCTG		
	Shiga-like toxin 2	stx_2	F	ATCCTATTCCCGGGAGTTTACG	584	13
			R	GCGTCATCGTATACACAGGAGC		
L. monocytogenes	Actin polymerization protein	*actA*	F	GACGAAAATCCCGAAGTGAA	385	27
			R	CTAGCGAAGGTGCTGTTTCC		
	Internalin B	*inlB*	F	AAAGCACGATTTCATGGGAG	146	17
			R	ACATAGCCTTGTTTGGTCGG		

[a] *spv*-specific primer sets are designated S1 and S4 in reference 50.
[b] F, forward; R, reverse.

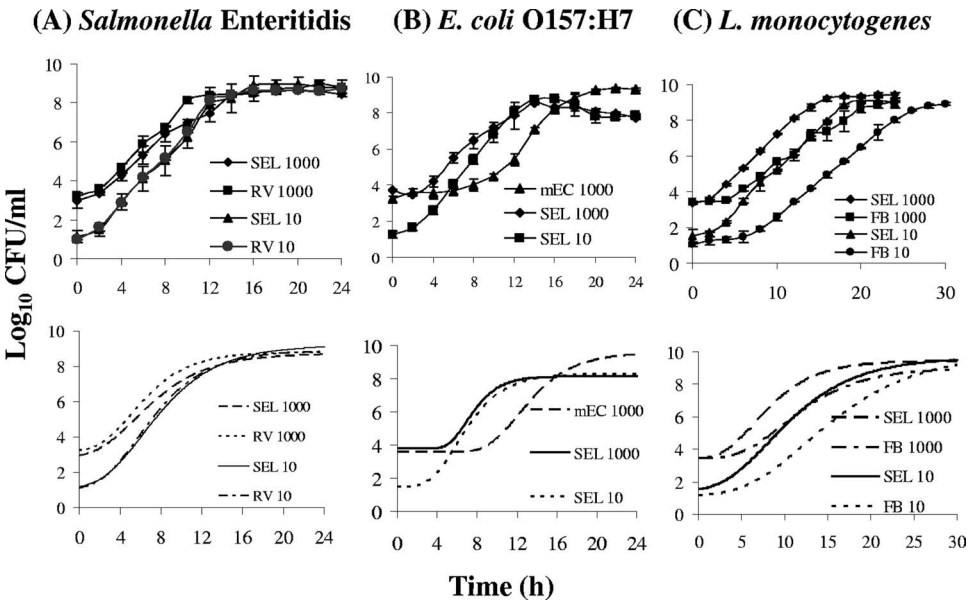

FIG. 1. Growth curves for the individual pathogens *Salmonella* serovar Enteritidis (A), *E. coli* O157:H7 (B), and *L. monocytogenes* (C) in SEL inoculated at two different concentrations (10 and 1,000 CFU/ml). The growth of each pathogen in SEL was compared with that in the respective individual selective enrichment broth: RV broth for *Salmonella*, mEC+n for *E. coli*, and FB for *Listeria*. The broths were inoculated at the indicated concentrations, and the cultures were incubated at 37°C in a shaker incubator. The top panels show the actual growth curves, and the plots in the bottom panels are corresponding Gompertz fitted curves.

CFU/ml/h) and that the GT and LPD in SEL were shorter than those in mEC+n. However, the MPD in mEC+n was greater than that in SEL (see Table S1 in the supplemental material). Overall, these data indicate that *E. coli* O157:H7 had a higher growth rate but reached a lower maximum cell population in SEL than in mEC+n.

(iii) *L. monocytogenes*. At both inoculation levels (10^1 and 10^3 CFU/ml), *L. monocytogenes* growth in SEL was significantly better than that in FB (Fig. 1C). Though the EGRs and MPDs in the two media were comparable, the GT and LPD in SEL were significantly shorter than those in FB (see Table S1 in the supplemental material).

Growth of the three target pathogens in a mixture in SEL. (i) Experiment I: *Salmonella* serovar Enteritidis/*E. coli* O157:H7/*L. monocytogenes* culture ratio, 1:1:1. In a mixture (containing ca. 3×10^2 CFU of each pathogen/ml), the three pathogens grew well and showed similar growth patterns (Fig. 2A). The values extrapolated from Gompertz fitted curves indicated that the EGR of *L. monocytogenes* (0.72 CFU/ml/h) was the lowest, followed by those of *Salmonella* serovar Enteritidis (0.82 CFU/ml/h) and *E. coli* O157:H7 (1.10 CFU/ml/h). Of the three pathogens, *E. coli* O157:H7 exhibited the shortest GT and LPD, 0.68 and 3.21 h, compared to 0.84 and 3.64 h for *Salmonella* serovar Enteritidis and 0.96 and 3.48 h for *L. monocytogenes*, respectively (Table 4). Furthermore, *E. coli* cells had a higher MPD than *Salmonella* serovar Enteritidis and *L. monocytogenes* cells (Table 4). In summary, these data indicate that SEL is capable of supporting the concurrent growth of *Salmonella* serovar Enteritidis, *E. coli* O157:H7, and *L. monocytogenes* but with a lower growth rate for *L. monocytogenes* than for the other species when cells of the three species are present at equal initial concentrations.

(ii) Experiment II: *Salmonella* serovar Enteritidis/*E. coli* O157:H7/*L. monocytogenes* culture ratio, 10:1,000:1. The overall growth profiles of the three cultures were highly proportional to their initial inoculation levels (*Salmonella* serovar Enteritidis at 13.5 ± 1.1 CFU/ml, *E. coli* at 1,327 ± 166 CFU/ml, and *L. monocytogenes* at 1.3 ± 0.6 CFU/ml). The growth rate and the MPD of *Salmonella* serovar Enteritidis cells were the highest of those of the pathogens in SEL (Fig. 2B). In this mixture, *L. monocytogenes*, inoculated at the lowest concentration (1 CFU/ml), grew in the presence of two other bacterial species inoculated at higher initial cell concentrations. The GT of *L. monocytogenes* was the longest of those of the three pathogens, and the EGR of *L. monocytogenes* was the lowest. Furthermore, the MPD of *L. monocytogenes* cells was 4.28 $\log_{10}$ CFU/ml, while *E. coli* O157:H7 and *Salmonella* serovar Enteritidis reached 8.58 and 7.11 $\log_{10}$ CFU/ml, respectively (Table 4). This result indicates that the fast-growing *Salmonella* and *E. coli* possibly utilized the most nutrients and that, thus, the depleted nutrient levels probably resulted in a lower growth rate for *Listeria*, normally a slow-growing bacterium.

(iii) Experiment III: *Salmonella* serovar Enteritidis/*E. coli* O157:H7/*L. monocytogenes* culture ratio, 1:10:1,000. When the inoculation level of *L. monocytogenes* cells (1,180 ± 125 CFU/ml) was greater than those of *Salmonella* serovar Enteritidis (1.4 ± 0.1 CFU/ml) and *E. coli* (14.6 ± 1.6 CFU/ml) cells, *L. monocytogenes* showed better growth than the other two pathogens (Fig. 2C). Interestingly, *Salmonella* serovar Enteritidis cells exhibited a shorter LPD and a higher MPD than *E. coli* O157:H7 cells, although the initial number of *Salmonella* serovar Enteritidis cells was lower than that of *E. coli* O157:H7 cells (Table 4). Additionally, the MPDs of *Salmonella* serovar Enteritidis and *E. coli* O157:H7 cells approached barely 5 to 6

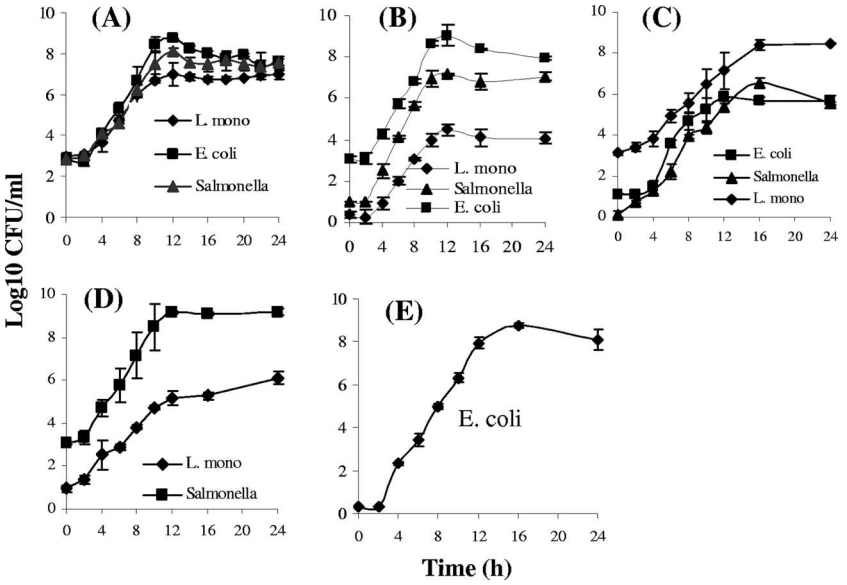

FIG. 2. (A to D) Growth curves for the three pathogens *Salmonella* serovar Enteritidis, *E. coli* O157:H7, and *L. monocytogenes* (L. mono) mixed at various ratios in SEL: *Salmonella* CFU/*E. coli* CFU/*L. monocytogenes* culture ratios, 1:1:1 (A), 10:1,000:1 (B), and 1:10:1,000 (C), and *Salmonella* CFU/*L. monocytogenes* culture ratio, 1,000:10 (D). (E) Growth curve for *E. coli* O157:H7 alone after inoculation at ~1 CFU/ml.

$\log_{10}$ CFU/ml, while that of *L. monocytogenes* cells reached about 8.5 $\log_{10}$ CFU/ml.

(iv) Experiment IV: *Salmonella* serovar Enteritidis/*E. coli* O157:H7/*L. monocytogenes* culture ratio, 1,000:1:10. In experiment IV, the inoculation level for *Salmonella* serovar Enteritidis was 1,178 ± 124 CFU/ml, that for *E. coli* was 1.3 ± 0.1 CFU/ml, and that for *L. monocytogenes* was 11.5 ± 1.9 CFU/ml. We were able to determine the growth curves for *Salmonella* and *Listeria* but not for *E. coli* (Fig. 2D). As expected, *Salmonella* cells had a significantly higher growth rate than *Listeria* cells, with a shorter GT (0.88 versus 1.46 h) and a higher MPD (9.33 versus 6.0 $\log_{10}$ CFU/ml) (Table 4). *E. coli* O157:H7 cells could not be enumerated after inoculation at 1 CFU/ml because of the overgrowth of *Salmonella* (for which the initial inoculation level was 1,000 times higher than that for *E. coli*) on the CT-SMAC plate, which hindered the *E. coli* colonies. *E. coli* growth in this mixture was further confirmed by obtaining positive reactions in the ICLFA (data not shown) and PCR assay (see Fig. 4C). In a separate experiment, we demonstrated that *E. coli* inoculated at 1 CFU/ml was indeed capable of growing in SEL (Fig. 2E). Though CT-SMAC is a selective medium for *E. coli* O157:H7, *Salmonella* was able to grow on CT-SMAC, producing opaque pink colonies, while *E. coli* O157:H7 produced colorless gray-white colonies because of its inability to ferment sorbitol. This result indicates that improvement in differential plating media is necessary for separations of *E. coli* O157:H7 and *Salmonella* serovar Enteritidis from the same sample during the plating procedure.

Growth patterns of food-borne bacteria in SEL. The growth patterns of different microorganisms that are commonly as-

TABLE 4. Growth kinetics values[a] for *Salmonella* serovar Enteritidis, *E. coli* O157:H7, and *L. monocytogenes* in mixed cultures set up in SEL

Expt	Organism (CFU/ml)[b]	EGR ($\log_{10}$ CFU/ml/h)	GT (h)	LPD (h)	MPD ($\log_{10}$ CFU/ml)
I	*Salmonella* serovar Enteritidis (1)	0.82 ± 0.10	0.84 ± 0.10	3.64 ± 0.58	7.66 ± 0.08
	E. coli O157:H7 (1)	1.10 ± 0.11	0.68 ± 0.07	3.21 ± 0.42	8.01 ± 0.10
	L. monocytogenes (1)	0.72 ± 0.06	0.96 ± 0.08	3.48 ± 0.33	6.89 ± 0.05
II	*Salmonella* serovar Enteritidis (10)	1.04 ± 0.06	0.67 ± 0.04	2.70 ± 0.24	7.11 ± 0.09
	E. coli O157:H7 (1,000)	0.96 ± 0.09	0.72 ± 0.07	3.02 ± 0.41	8.58 ± 0.14
	L. monocytogenes (1)	0.71 ± 0.07	0.98 ± 0.10	3.70 ± 0.38	4.28 ± 0.09
III	*Salmonella* serovar Enteritidis (1)	0.65 ± 0.05	1.07 ± 0.08	2.67 ± 0.53	6.25 ± 0.19
	E. coli O157:H7 (10)	1.00 ± 0.09	0.69 ± 0.06	3.56 ± 0.24	5.71 ± 0.09
	L. monocytogenes (1,000)	0.52 ± 0.04	1.33 ± 0.10	3.01 ± 0.61	8.44 ± 0.09
IV	*Salmonella* serovar Enteritidis (1,000)	0.79 ± 0.07	0.88 ± 0.08	2.17 ± 0.54	9.33 ± 0.18
	E. coli O157:H7 (1)	ND[c]	NA[d]	NA	NA
	L. monocytogenes (10)	0.47 ± 0.02	1.46 ± 0.08	1.75 ± 0.49	6.00 ± 0.06

[a] The growth kinetics values for the three pathogens in mixtures were extrapolated from data from Fig. 2 by using the Gompertz equation (47). Values are expressed as means ± SDs.

[b] The numbers in parentheses indicate the initial inoculation levels for the given experiment.

[c] ND, not determined. Counts of *E. coli* CFU in the mixture could not be determined because of the overgrowth of *Salmonella* on *E. coli*-selective CT-SMAC plates (see the text for a detailed explanation).

[d] NA, not applicable.

TABLE 5. Comparisons of recovery rates for stress-exposed *Salmonella* serovar Enteritidis, *E. coli* O157:H7, and *L. monocytogenes* cells in TSBYE, SEL, and the respective individual selective enrichment broths[a]

Stress or condition	Organism	Log_{10} CFU/ml (mean ± SD) at 0 h	Log_{10} CFU/ml (mean ± SD) at 3 h in:			Log_{10} CFU/ml (mean ± SD) at 6 h in:		
			TSBYE	Selective enrichment broth[b]	SEL	TSBYE	Selective enrichment broth[b]	SEL
Temp (°C)	*Salmonella* serovar							
37	Enteritidis	7.27 ± 0.24	8.81 ± 0.20*	8.35 ± 0.15	8.29 ± 0.28	9.23 ± 0.10*	8.41 ± 0.29	9.27 ± 0.14
4		6.25 ± 0.14	8.31 ± 0.34*	7.35 ± 0.48*	7.66 ± 0.22*	9.10 ± 0.05*	8.40 ± 0.05*	9.21 ± 0.14*
pH								
4.5		5.20 ± 0.54	7.22 ± 0.02*	5.39 ± 0.68	5.07 ± 0.00	7.33 ± 0.07*	5.83 ± 1.05	4.89 ± 1.06
5.5		6.69 ± 0.02	8.71 ± 0.26*	8.25 ± 0.36*	8.28 ± 0.28*	9.05 ± 0.06*	8.53 ± 0.09*	9.35 ± 0.17*
Temp (°C)	*E. coli* O157:H7							
37		7.16. ± 0.11	8.63 ± 0.11*	7.53 ± 0.10	7.84 ± 0.07	9.02 ± 0.11*	8.08 ± 0.18	8.73 ± 0.17
4		5.57 ± 0.09	8.02 ± 0.14*	5.02 ± 0.73	5.77 ± 0.91	8.86 ± 0.07*	4.90 ± 0.49	7.80 ± 0.23*
pH								
4.5		5.08 ± 0.36	6.43 ± 0.11*	3.21 ± 0.28	3.54 ± 0.28	8.56 ± 0.49*	3.66 ± 0.68	5.24 ± 0.37
5.5		6.69 ± 0.15	8.43 ± 0.11*	6.89 ± 0.13	7.43 ± 0.21	9.02 ± 0.13*	6.99 ± 0.60	8.66 ± 0.20*
Temp (°C)	*L. monocytogenes*							
37		7.56 ± 0.04	8.72 ± 0.03*	8.20 ± 0.10	8.38 ± 0.12	9.60 ± 0.10*	8.78 ± 0.10	9.17 ± 0.16
4		6.57 ± 0.08	8.04 ± 0.08*	7.28 ± 0.10	7.67 ± 0.08*	9.06 ± 0.10*	8.36 ± 0.07*	8.88 ± 0.09*
pH								
4.5		6.42 ± 0.17	7.60 ± 0.18*	6.74 ± 0.08	7.33 ± 0.11*	9.22 ± 0.49*	7.75 ± 0.09*	8.06 ± 0.64*
5.5		6.71 ± 0.04	8.23 ± 0.10*	7.46 ± 0.10	7.91 ± 0.16*	9.56 ± 0.03*	8.41 ± 0.06*	8.99 ± 0.09*

[a] Each culture was inoculated at 3×10^2 CFU/ml. Values marked with * indicate the recovery of stressed cells as defined by an increase in cell numbers of ≥1 log in the given medium compared to the initial numbers after stress.
[b] The selective enrichment broths were RV broth for *Salmonella* serovar Enteritidis, mEC+n for *E. coli*, and FB for *L. monocytogenes*.

sociated with food, along with those of additional species and other strains or serovars of the three target pathogens, in SEL were investigated and compared with those in UPB (Table 1) and mEC+n, RV broth, and FB (see Table S2 in the supplemental material) by measuring optical densities at 595 nm (OD_{595}) at 12, 16, and 24 h. Seven enterohemorrhagic *E. coli* (EHEC) strains, two enteropathogenic *E. coli* (EPEC) strains, and one enterotoxigenic *E. coli* (ETEC) strain showed significantly higher levels of growth ($P < 0.05$; 16 h of growth) in SEL than in UPB; another strain of ETEC (O78:H11) failed to grow in SEL but showed good growth in UPB. Four strains of *L. monocytogenes* belonging to serovars 1/2a, 1/2b, and 4b and a strain of *Listeria innocua* exhibited better growth in SEL than in UPB. Likewise, four serovars of *S. enterica* showed improved growth in SEL compared to that in UPB. Among the nontarget bacteria, three *Bacillus* species, three *Lactobacillus* species, and one strain each of *Enterococcus faecalis*, *Proteus vulgaris*, *Lactococcus lactis*, and *Leuconostoc mesenteroides* did not show any growth in SEL but did grow in UPB. Among the five natural food isolates (obtained in this study), *Bacillus megaterium* HK1, *Lactococcus lactis* HK21, and *Pediococcus acidilactici* HK32 did not grow in SEL but showed some growth in UPB (Table 1). Among the test organisms, only three (*Brochothrix thermosphacta*, *Lactobacillus acidophilus*, and *Pediococcus* sp.) did not show any detectable growth in either medium. We also observed that several nontarget bacteria, including *Streptococcus mutans*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Enterobacter aerogenes*, *Enterobacter cloacae*, and *Hafnia alvei*, showed good growth in SEL and that these organisms also grew well in UPB and certain selective media (Table 1; see also Table S2 in the supplemental material). Overall, these data indicate that the levels of growth of most desirable target pathogens in SEL were higher than those in UPB and that SEL was more inhibitory to several food-borne organisms than UPB (Table 1).

Furthermore, bacterial growth in SEL was superior to that in individual selective enrichment broths for the respective target pathogens, such as RV broth for *Salmonella*, mEC+n for *E. coli*, and FB for *Listeria*, when analyzed after 24 h of enrichment (see Table S2 in the supplemental material).

Recovery of acid- and cold-stressed cells in SEL. The ability of SEL to resuscitate acid- and cold-injured cells was evaluated and compared with the recovery ability of TSBYE, a commonly used nonselective enrichment broth, as well as those of the respective individual selective enrichment broths. As expected, the stress conditions caused the inhibition of cell growth (Table 5), resulting in 0.5- to ~2.1-log reductions in cell counts for target pathogens compared with those in the control (incubated at 37°C). The pH 4.5 stress caused the highest numbers of cell deaths among all three pathogens, reducing populations by more than 2 logs (2.07 and 2.08 logs) for *Salmonella* serovar Enteritidis and *E. coli* O157:H7, respectively, and 1.15 logs for *L. monocytogenes*. Cold stress resulted in moderate cell injury: a re-

duction in the bacterial population of about 1.6 logs for *E. coli* O157:H7, 1 log for *Salmonella* serovar Enteritidis, and 0.99 log for *L. monocytogenes*. Finally, cultures under the pH 5.5 stress condition showed the least cell injury (population reductions of less than 1 log for all three pathogens) among those exposed to the three stress conditions (Table 5). Stress-exposed cells were allowed to recover in TSBYE, SEL, and their respective selective enrichment broths for 3 and 6 h. Data for each pathogen are presented below. An increase in bacterial cell counts of $\geq$1 log was considered to indicate significant recovery in the corresponding medium (Table 5).

(i) *Salmonella* serovar Enteritidis. All three media (TSBYE, SEL, and RV broth) resuscitated cold (4°C)- and pH 5.5-stressed *Salmonella* serovar Enteritidis; however, RV broth and SEL failed to resuscitate pH 4.5-stressed cells after 3 and 6 h of recovery (Table 5). Overall, TSBYE yielded the best recovery at 3 h, while SEL supported the best recovery at 6 h. RV broth showed the lowest recovery rates for all stress conditions at both time points.

(ii) *E. coli* O157:H7. SEL and TSBYE allowed injured *E. coli* O157:H7 cells to recover; however, mEC+n failed to allow recovery (Table 5). Stress-exposed *E. coli* O157:H7 grown in TSBYE showed recovery at both time points (3 and 6 h); however, SEL helped to resuscitate cold (4°C)- and pH 5.5-stressed cells only after 6 h. SEL was unable to show any resuscitation of pH 4.5-stressed cells.

(iii) *L. monocytogenes*. SEL and TSBYE resuscitated injured *L. monocytogenes* after 3 and 6 h of recovery, while FB did so only after 6 h (Table 5). The recovery rates in order from highest to lowest were those for TSBYE, SEL, and FB.

In summary, the stress recovery studies indicate that SEL supported the recovery of stress-exposed cells and that its performance was equivalent to that of TSBYE when a 6-h recovery period was allowed, with the exception of pH 4.5-induced stress for *E. coli* O157:H7 and *Salmonella* serovar Enteritidis. As a selective enrichment broth, SEL demonstrated performance superior to that of the respective individual selective enrichment broths, RV broth, mEC+n, and FB.

Detection of pathogens by antibody-based ICLFA and multiplex PCR. (i) ICLFA. *Salmonella* serovar Enteritidis, *E. coli* O157:H7, and *L. monocytogenes* grown in SEL gave positive reactions in the ICFLA, and the reaction intensities for *Salmonella* serovar Enteritidis and *L. monocytogenes* grown in SEL were significantly higher ($P < 0.05$) than those for the same pathogens grown in their respective individual selective enrichment broths, RV broth and FB (Fig. 3A and C). The antibody reaction intensity for *E. coli* grown in SEL was slightly higher than but comparable to that for mEC+n-grown cells (Fig. 3B). When all three pathogens grown in SEL were inoculated at equal cell concentrations as a mixture, they all gave positive reactions with their respective Reveal kits; however, the band intensity for *L. monocytogenes* was the strongest, followed by those for *E. coli* O157:H7 and *Salmonella* serovar Enteritidis (Fig. 3D). The overall reaction of *Salmonella* serovar Enteritidis grown either in SEL or RV broth with the *Salmonella* Reveal kit was relatively weaker than those of the other two pathogens with their kits. It was later confirmed that the ICLFA kit (Neogen Corp.) is intended primarily for the detection of *Salmonella* serovar Typhimurium; however, we used *Salmonella* serovar Enteritidis as the test organism, thus obtaining a weaker reaction. Altogether, ICLFA data demonstrated that SEL is suitable for enrichment with the three pathogens individually or in a mixture for subsequent detection by the antibody-based ICLFA method.

(ii) Multiplex PCR. *Salmonella* serovar Enteritidis, *E. coli* O157:H7, and *L. monocytogenes* grown individually or in mixtures (experiments I to IV) were also tested in a multiplex PCR assay. As expected, *E. coli* grown individually or in a mixture in SEL alone or in the presence of meat (ready-to-eat turkey) showed three amplified products, those for stx_2 (584 bp), *eaeA* (482 bp), and stx_1 (348 bp) (Fig. 4). Similarly, *Salmonella* serovar Enteritidis gave amplified products for *sefA* (310 bp) and/or *spv* (250 bp) and *L. monocytogenes* gave products for *actA* (385 bp) and *inlB* (146 bp) without any nonspecific amplification when grown individually or in a mixture (Fig. 4). PCR analysis of uninoculated control meat did not yield any DNA amplification with species-specific primers, thus confirming the absence of background target pathogens in the product (Fig. 4B). When *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* cells were inoculated in equal (Fig. 4B) or variable (Fig. 4C) proportions into turkey meat, all gave positive amplified PCR products, except in experiment II, in which *L. monocytogenes* was undetectable. In experiment II, *L. monocytogenes* cells were inoculated at about 1.5 CFU/g together with *Salmonella* (~13 CFU/g) and *E. coli* (~1,300 CFU/g) cells (Fig. 4C). The lack of amplification is attributed to the poor growth of *L. monocytogenes* in the mixture compared to that of the other two organisms (Fig. 2B). Nevertheless, these data demonstrate that all three pathogens could be readily detected when grown in SEL individually or in a mixture in a meat sample by using species-specific PCR primer sets, suggesting that SEL is a suitable enrichment broth for PCR-based detection. However, some situations in which *L. monocytogenes* cells are present in low numbers (~1 CFU/g) along with a large number of other microbes, as described above, may yield negative PCR results.

Selective enrichment of artificially inoculated meat samples with pathogens in SEL broth and subsequent detection using viable-cell counting lateral-flow and PCR assays. In artificially inoculated ready-to-eat turkey and salami samples, the detection of three target pathogens grown in SEL after 12 and 24 h of enrichment was demonstrated. The growth patterns of *Salmonella* serovar Enteritidis and *E. coli* O157:H7 were similar, and cell numbers in all meat samples reached 8 to 9 $\log_{10}$ CFU/ml at 12 and 24 h (see Table S3 in the supplemental material). The level of growth of *L. monocytogenes* (which reached 5 to 7 $\log_{10}$ CFU/ml) was lower than those of the other two target pathogens. Numbers of *Salmonella* serovar Enteritidis and *L. monocytogenes* cells in turkey samples were higher than those in salami samples. The growth of *E. coli* O157:H7 was not affected by the food type. Although growth behaviors varied among the target pathogens, SEL supported the growth of these pathogens concurrently in the artificially inoculated meat samples.

In the lateral-flow immunoassay, *E. coli* O157:H7 gave strong positive reactions after both 12 and 24 h of enrichment (see Fig. S1 in the supplemental material). After 12 h of enrichment in SEL, *Salmonella* serovar Enteritidis showed a weak positive reaction, but the reaction was slightly improved

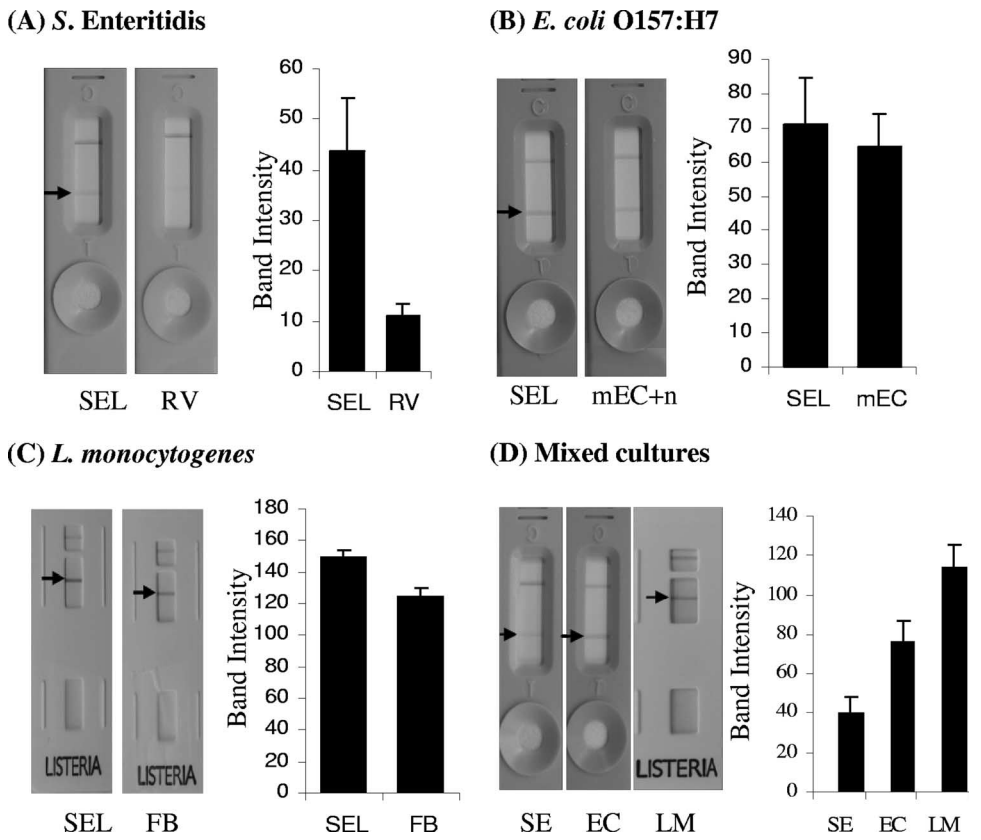

FIG. 3. Results from ICLFA showing the reaction patterns of cells of the pathogens *Salmonella* serovar Enteritidis (SE) (A and D), *E. coli* O157:H7 (EC) (B and D), and *L. monocytogenes* (LM) (C and D) grown individually (with each pathogen inoculated at 10^3 CFU/ml) (A to C) or in mixed cultures (with each pathogen inoculated at ca. 3×10^2 CFU/ml) set up in SEL (D). The ICLFA reaction patterns were also compared with those of cells grown in the respective selective enrichment broths (RV broth, mEC+n, and FB). Cultures were incubated at 37°C for 16 to 18 h in a shaker incubator. The lateral-flow strips were loaded with 120-μl samples of *Salmonella* serovar Enteritidis and *E. coli* O157:H7 live cultures or 135 μl of heat-killed *L. monocytogenes* cells, and the antibody reaction intensities (band densities in pixels) were quantified by using software (Scion Crop., Frederick, MD) and presented as bar graphs.

after 24 h of enrichment in the salami sample. No reaction was observed for *L. monocytogenes* in salami after 12 h of enrichment, but the reaction intensities were high after 24 h of incubation. These data indicate that SEL could be used as an enrichment broth for antibody-based detection by ICLFA; however, the duration of enrichment time is critical to obtain a strong reaction. PCR assays of the same inoculated turkey and salami samples after 12 and 24 h of enrichment showed positive PCR-amplified products for the target pathogens (Fig. 5), confirming that SEL could potentially be used as an enrichment broth for PCR-based detection.

Comparison of SEL performance with UPB performance for sample enrichment and detection of three pathogens in artificially inoculated meat samples. Turkey and salami samples were inoculated with three pathogens at equal cell concentrations and subjected to enrichment in SEL and UPB for up to 24 h. The performance of SEL was compared with that of UPB by determining bacterial cell counts in each medium and by performing ICLFA and PCR assays.

In turkey samples, the overall growth of *E. coli* O157:H7 in UPB was better than that in SEL, while the growth of *Salmonella* serovar Enteritidis and that of *L. monocytogenes* were better in SEL (Table 6). During the early periods of meat enrichment (8 to 10 h), the numbers of cells of the three pathogens grown in UPB were higher than those grown in SEL. However, 12 h postenrichment, sharply accelerated growth in SEL was observed. In the lateral-flow immunoassay, there were no differences between the two broths in the reaction intensities for *Salmonella* serovar Enteritidis or *E. coli* O157:H7; however, *L. monocytogenes* was detected after as little as 8 h of growth in SEL compared to 10 h of growth in UPB (see Table S3 in the supplemental material). PCR showed positive amplifications for the three pathogens at all incubation time points and in both media (see Fig. S2 in the supplemental material).

In salami samples, generally, bacterial counts were lower than those in turkey samples, due possibly to the presence of preservatives and bacterial inhibitors. The growth of *L. monocytogenes* in salami in SEL, in particular, was reduced by almost 2 logs compared to that in turkey in SEL (Table 6). Similarly, the growth of *Salmonella* serovar Enteritidis in salami in UPB showed a l-log reduction compared to that in turkey in UPB. Overall, the levels of growth of all three pathogens in salami in SEL were comparable to those in salami in UPB. Lateral-flow immunoassay results for each pathogen in SEL and UPB were similar; however, *L. monocytogenes* was detected after as little

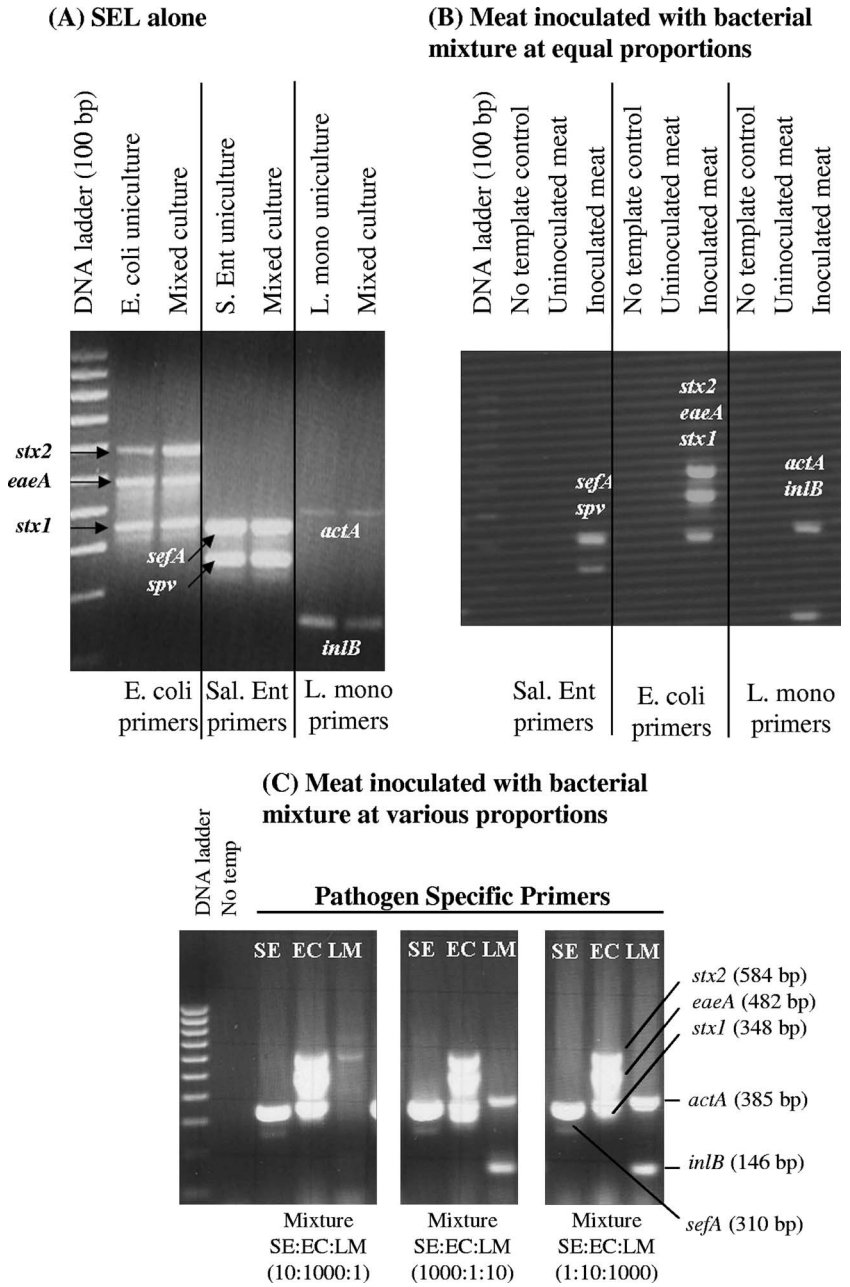

FIG. 4. Results from multiplex PCR assays for the detection of *Salmonella* serovar Enteritidis, *E. coli* O157:H7, and *L. monocytogenes* bacteria grown individually (ca. 3×10^2 CFU/ml) in SEL broth (A) or in mixtures in meat (B and C). (A) Cultures were incubated at 37°C for 16 to 18 h in a shaker incubator and analyzed by PCR assays using species-specific primer sets: primers targeting *sefA* (310 bp) and *spv* (250 bp) for *Salmonella* serovar Enteritidis (S. Ent, or Sal. Ent), *actA* (395 bp) and *inlB* (146 bp) for *L. monocytogenes* (L. mono), and stx_2 (584 bp), *eaeA* (482 bp), and stx_1 (348 bp) for *E. coli* O157:H7. (B) Ready-to-eat sliced turkey meat samples were inoculated with *Salmonella* serovar Enteritidis (SE), *E. coli* O157:H7 (EC), and *L. monocytogenes* (LM) cultures at equal concentrations (ca. 3×10^2 CFU/ml) and analyzed by PCR after 18 h of enrichment in SEL broth. (C) Meat samples were inoculated with three mixtures of *Salmonella* serovar Enteritidis, *E. coli*, and *L. monocytogenes* CFU prepared with the bacteria at various ratios as indicated, enriched for 18 h, and assayed by multiplex PCR using *Salmonella* serovar Enteritidis-, *E. coli*-, or *L. monocytogenes*-specific primers. No temp, no-template DNA control.

as 12 h when subjected to enrichment in SEL, compared to the 16 h of incubation needed in UPB. PCR analysis showed unambiguous positive amplified bands for the three target pathogens at all time points, except for *L. monocytogenes*, which produced very faint amplified bands in SEL after 8 h of enrichment (see Fig. S2 in the supplemental material).

DISCUSSION

Current industry trends emphasize the need for the detection of multiple pathogens on a single-assay platform to reduce the cost of testing per pathogen. Moreover, a multipathogen detection scheme could mitigate the industry and regulatory

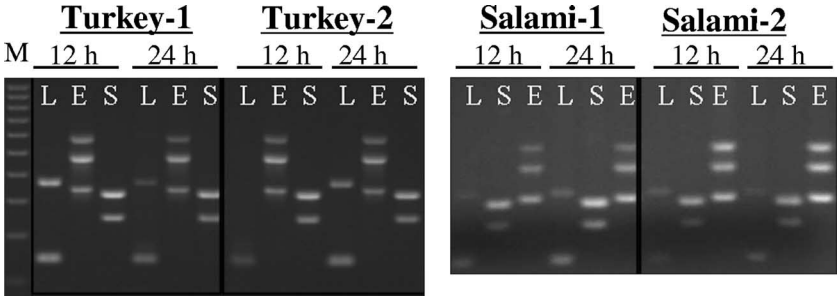

FIG. 5. Results from PCR assays of *Salmonella* serovar Enteritidis-, *L. monocytogenes*-, and *E. coli* O157:H7-inoculated ready-to-eat turkey and salami samples. The meat samples (25 g each) were inoculated (ca. 3×10^2 CFU of each pathogen/g), mixed with 225 ml of SEL, and incubated for 12 and 24 h with agitation. In panel A, PCR lanes are as follows, from left to right: M, 100-bp ladder DNA marker; L, *Listeria* primers targeting genes *actA* (395 bp) and *inlB* (146 bp); E, *E. coli* O157:H7 primers targeting genes stx_2 (584 bp), *eaeA* (482 bp), and stx_1 (348 bp); and S, *Salmonella* primers targeting genes *sefA* (310 bp) and *spv* (250 bp). An ICLFA also showed positive reactions with *Salmonella* serovar Enteritidis, *E. coli* O157:H7, and *L. monocytogenes* antigens for corresponding samples (see Fig. S1 in the supplemental material).

needs in the mandatory testing of food products for multiple pathogens prior to retail distribution. To aid in multipathogen detection, a suitable selective enrichment medium is necessary to improve detection utilizing methods such as multiplex PCR (5, 20, 42, 46), DNA array hybridization (15), array-based immunosorbent assays (14), and antibody-based array biosensor techniques (35, 50).

In this study, a selective enrichment broth, SEL, was developed and evaluated for its ability to enrich a test sample with multiple pathogens concurrently if or when the pathogens were present in the same sample. SEL was formulated by modifying the recipe for BLEB and contains four different antimicrobial agents, acriflavine, cycloheximide, fosfomycin, and nalidixic acid (Table 2), along with tryptic soy broth, yeast extract, sodium pyruvate, and sodium phosphate, which are proven to support the growth of healthy and injured food-borne pathogens (9, 11). Sodium pyruvate and sodium phosphates protect bacteria from pH changes and reactive oxygen atoms (32), and the selective antibiotics inhibit the growth of background resident microorganisms (31).

Overall, the individual growth patterns of the three target pathogens in SEL were satisfactory, and the performance of SEL as a selective enrichment broth was superior to those of mEC+n for *E. coli* and FB for *Listeria* and equivalent to that of RV broth for *Salmonella*. For *E. coli* O157:H7, SEL was able to support growth after inoculation with 10 and 1,000 CFU/ml, while mEC+n failed to support growth after inoculation with 10 CFU/ml. The inability of mEC+n to support growth at this inoculation level was in agreement with the findings in a previous report (25). The lack of growth may be due to the strain used or the incubation temperature or agitation conditions employed in this study. Moreover, bile salts and novobiocin present in mEC+n most likely exerted inhibitory effects culminating in reduced or no growth at lower cell numbers (28, 49). The inability of mEC+n to support growth at 10 CFU/ml or lower is unacceptable, since an infectious dose of *E. coli* O157:H7 is in the range of 10 to 100 CFU (25). The growth rate of *L. monocytogenes* in SEL was superior to that in FB (47). In addition, other bacteria, *Bacillus*, *Enterococcus*, and *Streptococcus* spp., which showed poor or no growth in SEL (Table 1) can grow in FB (12).

In a mixed-culture experiment, SEL allowed the concurrent growth of the three target pathogens and competition among the pathogens and their initial levels were determinants of their growth kinetics. When the pathogens were mixed in equal proportions, *E. coli* O157:H7 showed the shortest LPD and the highest maximum cell density, while *L. monocytogenes* showed the lowest maximum cell density and *Salmonella* growth was intermediate (Table 4). Lower cell numbers for *L. monocytogenes* were expected because this pathogen is a slow-growing and poor competitor (2). When the bacteria were mixed at various ratios, the growth pattern of each pathogen was proportional to the initial cell number. This detailed growth kinetics profile of each pathogen in a mixture in SEL would aid in the selection of a suitable method for the accurate detection of these three pathogens if present in the same sample.

The application of SEL as an enrichment medium for the detection of three target pathogens in inoculated meat samples by antibody-based lateral-flow immunoassays and nucleic acid-based PCR assays was investigated. As expected, individual-pathogen-specific ICLFA strips gave positive reactions when bacteria were grown in SEL, suggesting that all three pathogens can be detected using antibody-based methods. Moreover, the ICLFA reaction intensities in SEL were stronger than those in the respective individual selective enrichment broths (Fig. 3). This result suggests that SEL promoted increased expression of antibody-reactive antigens compared to the expression of these antigens in its counterparts. A selective- or nonselective-medium-mediated reduction in the expression of antigen or in an antigen-antibody reaction has been demonstrated previously for *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* (7, 21, 22, 24). Pathogen-specific multiplex PCR assays were also successful in detecting each pathogen from the mixture without producing any nonspecific amplification (Fig. 4 and 5). Furthermore, the growth of two nontarget bacteria, *Enterobacter aerogenes* and *Streptococcus mutans*, in SEL (Table 1) did not interfere with the PCR amplification of the specific target genes of the three pathogens (data not shown).

In the inoculated-meat experiments, both ICLFA and PCR assays were performed with SEL-enriched samples at various time intervals. In most cases, positive ICLFA reactions were obtained after 12 h of enrichment, with approximate cell populations of 6 to 8.2 log CFU/ml, while positive PCR results were obtained after 8 h of growth, with cell counts of 4.48 to 5.7

TABLE 6. Comparison of viable-cell counts for pathogens inoculated concurrently into turkey or salami and subjected to enrichment in UPB and SEL

Meat and organism	Mean $\log_{10}$ CFU/ml ± SD[a] at:									
	8 h		10 h		12 h		16 h		24 h	
	UPB	SEL	UPB	SEL	UPB	SEL	UPB	SEL	UPB	SEL
Turkey										
Salmonella serovar Enteritidis	5.52 ± 0.07 A	5.59 ± 0.32 A	6.98 ± 0.07 A	6.95 ± 0.08 A	7.24 ± 0.12 B	8.81 ± 0.02 A	7.50 ± 0.15 B	8.57 ± 0.06 A	8.05 ± 0.13 A	6.40 ± 0.17 B
E. coli O157:H7	6.56 ± 0.04 A	4.74 ± 0.23 B	8.48 ± 0.04 A	5.56 ± 0.07 B	8.20 ± 0.03 A	7.15 ± 0.09 B	7.76 ± 0.21 A	7.19 ± 0.06 B	6.06 ± 0.20 B	7.73 ± 0.05 A
L. monocytogenes	5.46 ± 0.03 A	5.08 ± 0.15 B	6.05 ± 0.08 A	6.21 ± 0.16 A	6.02 ± 0.06 B	7.30 ± 0.04 A	6.14 ± 0.17 B	7.24 ± 0.06 A	6.83 ± 0.13 B	7.47 ± 0.04 A
Salami										
Salmonella serovar Enteritidis	4.63 ± 0.06 B	4.84 ± 0.06 B	5.95 ± 0.07 A	5.70 ± 0.07 B	6.62 ± 0.15 B	7.04 ± 0.17 A	7.02 ± 0.06 B	7.56 ± 0.04 A	7.28 ± 0.07 B	8.47 ± 0.98 A
E. coli O157:H7	5.76 ± 0.15 A	5.75 ± 0.05 A	8.39 ± 0.07 A	7.09 ± 0.13 B	8.70 ± 0.02 A	8.70 ± 0.07 A	8.77 ± 0.01 B	9.20 ± 0.18 A	8.78 ± 0.16 A	8.90 ± 0.05 A
L. monocytogenes	4.48 ± 0.06 A	4.02 ± 0.21 B	5.49 ± 0.08 A	4.55 ± 0.12 B	5.65 ± 0.03 A	4.52 ± 0.07 B	6.10 ± 0.05 A	5.95 ± 0.10 A	6.67 ± 0.06 A	5.88 ± 0.03 B

[a] Counts in the same row labeled with the same letter (A or B) and corresponding to growth in UPB and SEL at a given time point are not significantly different at P of <0.05. Meat samples (25 g/225 ml of SEL) were inoculated with *Salmonella*, *E. coli*, and *Listeria* (ca. 3×10^2 CFU of each pathogen/ml) and assayed at 8, 10, 12, 16, and 24 h. Bacterial cell counts were determined by plating *Salmonella* cells onto XLD agar, *E. coli* cells onto CT-SMAC, and *Listeria* cells onto MOX.

log CFU/ml, irrespective of the type of meat sample (see Fig. S2 in the supplemental material), confirming that PCR is more sensitive than the ICLFA. (Note that 8 h is the earliest time point at which we tested.) In general, these limits of detection for ICLFA and PCR are in agreement with the data in previous reports (8, 19, 44).

The ability of selective enrichment broth to resuscitate temperature-, preservative-, salt-, and acid-stressed cells (1, 3, 33, 36) encountered during food processing, storage, or sanitization is critical for detection. It is also well known that injured cells can cause an improper estimation of the decimal reduction time (D value) and z value (temperature required to change the D value to transverse by 1 log) during thermal processing (41) and, most importantly, that the injured pathogens can revive and grow under favorable conditions (9). We have demonstrated that SEL allowed the recovery of acid (pH 5.5)- and temperature (4°C)-stressed cells and that, overall, the recovery rates were comparable to those in nonselective TSBYE broth (Table 5). However, the recovery rates for pH 4.5-stressed cells were variable; SEL successfully resuscitated *Listeria* cells but failed to resuscitate *E. coli* O157:H7 and *Salmonella* cells.

In this study, the performance of SEL was compared with that of UPB (2), a currently known universal enrichment broth for *Salmonella*, *Listeria*, *E. coli* O157:H7, and *Yersinia* spp. (43, 51, 54). Overall, SEL supported improved growth of all three target pathogens compared to that in UPB and the individual selective enrichment broths RV, mEC+n, and FB. In addition, SEL inhibited greater numbers of natural food-borne bacteria, including some nascent food isolates, than UPB (Table 1; see Table S2 in the supplemental material). Of the two ETEC strains tested, the O78:H11 strain did not grow in SEL, while the O25:K98:NM strain did. The lack of growth of O78:H11 was determined to be due to the presence of nalidixic acid and acriflavine (data not shown). Further research on the types and concentrations of antibiotics needed for all ETEC strains to grow in SEL is warranted.

When both SEL and UPB were used as enrichment broths with spiked meat samples, the overall bacterial cell counts in UPB were slightly better than those in SEL, but the differences were not statistically significant. Though PCR and ICLFA results for the two media were comparable, the PCR with UPB-enriched samples yielded slightly improved detection of *L. monocytogenes* at 8 h compared to that with SEL-enriched samples (see Fig. S2 in the supplemental material).

In summary, SEL, a selective enrichment broth, promoted the concurrent growth of three major food-borne pathogens, *S. enterica*, *E. coli* O157:H7, and *L. monocytogenes*, present at various cell numbers in cultures and meat samples. The performance of SEL was superior to that of UPB and the respective individual selective enrichment broths. Based on the data presented in this study, SEL can be considered a selective enrichment broth for the detection of three major food-borne pathogens by antibody- or nucleic acid-based assays. Currently, SEL is being evaluated for the detection of pathogens in naturally or artificially contaminated meat samples by biosensor-based assays, including light scattering and the use of fiber optic sensors.

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