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Differences in Survival among 13 *Listeria monocytogenes* Strains in a Dynamic Model of the Stomach and Small Intestine▽

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Thirteen *Listeria monocytogenes* strains were compared for the ability to survive in a dynamic gastrointestinal model. Strains displayed various degrees of susceptibility to gastric acidity; however, strain-to-strain variations became evident mainly after 90 min of exposure (pH 2.0). Cell levels transferred to the intestine depended on initial populations, while reductions during intestinal exposure were relatively small for all strains.

Strains of *Listeria monocytogenes* are diverse in serological and molecular features, while serotypes and genetic groups display great diversity in virulence and environmental distribution (14, 26). Variations in physiological responses of *L. monocytogenes* strains have also been reported (9, 10, 17) and may contribute to virulence heterogeneity, as resistance to stresses is critical for survival within the host (13).

The acidity of the stomach is considered a major defense barrier against food-borne infection (24). Subsequently, cells that survive gastric passage and reach the small intestine must withstand the presence of bile and high-osmolarity conditions (13). Various aspects of food-borne listeriosis have been examined using artificial gastrointestinal fluid broth systems (2, 11, 15, 22, 25); however, findings may not accurately reflect the specific stages of *L. monocytogenes* survival in the digestive tract, since those studies did not account for the changing conditions to which pathogens are subjected while in the digestive tract. In this respect, artificial gastrointestinal systems that closely simulate the dynamics of gastrointestinal transit may be valuable instruments for identification of factors affecting the gastrointestinal survival of *L. monocytogenes*, including strain-to-strain variations.

To our knowledge, the use of dynamic gastrointestinal systems in the study of food-borne pathogens is limited (3, 4, 16). In this study, we examined differences in gastrointestinal survival among 13 *L. monocytogenes* strains, representing different serotypes and three genotypic lineages (26), using a simulated model of the human stomach and small intestine.

Bacteria. To examine the potential contribution of the alternative sigma factor σ^B function in the gastrointestinal survival of *L. monocytogenes*, tested strains (Table 1) also included 10403S and its in-frame *sigB* deletion mutant strain, A1-254 (27).

Cultures of individual strains were prepared in 100 ml tryptic soy broth without dextrose (Difco, Becton Dickinson, Sparks, MD) supplemented with 0.6% yeast extract (Acumedia, Lansing, MI) (TSBYE-G), inoculated with active cultures of each strain (6.9 to 7.2 log CFU/ml), and incubated for 4 or 16 h (30°C) to investigate potential effects of the age of cells on the survival of each strain.

Simulated gastrointestinal fluids. An artificial saliva solution (6.2 g/liter NaCl, 2.2 g/liter KCl, 0.22 g/liter $CaCl_2$, and 1.2 g/liter $NaHCO_3$) (18, 19) was autoclaved and cooled to ambient temperature (25°C) before use. Gastric fluid (pH 2; 5N HCl) (20, 21) contained 0.4 g/liter glucose, 3.0 g/liter yeast extract, 1.0 g/liter Bacto peptone (Difco), 4.0 g/liter porcine mucin (Sigma-Aldrich, St. Louis, MO), 0.5 g/liter cysteine, 0.08 g/liter NaCl, 0.4 g/liter $NaHCO_3$, 0.04 g/liter K_2HPO_4, 0.04 g/liter KH_2PO_4, 0.008 g/liter $CaCl_2 \cdot 2H_2O$, 0.008 g/liter $MgSO_4 \cdot 7H_2O$, 1.0 g/liter xylan (Sigma-Aldrich), 3.0 g/liter soluble starch (Sigma-Aldrich), 2.0 g/liter pectin (Sigma-Aldrich), 1 ml/liter Tween 80, and 3 g/liter pepsin from porcine stomach mucosa (Sigma-Aldrich). Intestinal fluid (16) consisted of 0.1 g/liter porcine trypsin (type IX-S; Sigma-Aldrich) and 3.5 g/liter porcine pancreatin (Sigma-Aldrich), and the solution was filtered (0.45-μm-pore-diameter cellulose filter; Millipore Corp., Bedford, MA). Biliary secretions were simulated by preparing 2% or 4% porcine bile (Sigma-Aldrich) solution.

Dynamic gastrointestinal system. The simulated gastrointestinal tract (16) (Fig. 1) consisted of Erlenmeyer flasks (500 ml), representing the gastric (GC) and the intestinal (IC) compartments, kept in a water bath (shaking water bath model 50; Precision Scientific, Chicago, IL) at 37°C. Peristaltic pumps (variable-speed low-flow pump; Fisher Scientific) delivered gastric fluid into the GC (flow rate, 0.33 ml/min) and intestinal fluid (flow rate, 0.33 ml/min) and bile solutions (4% for the first 30 min and 2% for the remaining time; flow rate, 0.5 ml/min) into the IC. A multichannel peristaltic pump (205U; Watson-Marlow Limited, Cornwall, England) transferred the gastric contents into the IC (initiated 15 min after the beginning of the challenge) at a flow rate of 1.1 ml/min (7).

Gastrointestinal passage tolerance assay. Prior to each challenge, 10 ml of gastric fluid was added to the GC, whereas the IC contained 7 ml of 4% bile solution (19). Each 100-ml culture (4 or 16 h) was diluted (1:1 [vol/vol]) with artificial saliva. The pH of the GC was adjusted (with 5 N HCl) to 5.0, 4.0, 3.0, and 2.0 at 10, 28, 58, and 88 min, respectively, to reproduce in vivo gastric pH values corresponding to young adults after

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TABLE 1. *Listeria monocytogenes* strains used in this study

Strain	Serotype[a]	Lineage[a]	Origin (yr of isolation)	Reference
558	1/2	NK	Pork meat	
R2-500[b]	4b	1	Food, epidemic case, North Carolina (2000)	12
R2-501[b]	4b	1	Human, epidemic case, North Carolina (2000)	12
Scott A	4b	1	Human	
N1-225[b]	4b	1	Human, epidemic case, United States (1998–99)	12
N1-227[b]	4b	1	Food, epidemic case, United States (1998–99)	12
C1-056[b]	1/2a	2	Human, sporadic case	12
N3-031[b]	1/2a	2	Food (hot dog), sporadic case (1989)	12
J1-101[b]	1/2a	2	Human, sporadic case (1989)	12
10403S[c]	1/2a	2	NK	5
A1-254[c]	Δ*sigB* mutant of 10403S			27
J1-158[b]	4b	3	Goat	12
J1-168[b]	4a	3	Human, sporadic case	12

[a] NK, not known. Serotype and lineage designations were provided by the donor or reference (except for strains 558 and Scott A).
[b] Kindly provided by Martin Wiedmann (Department of Food Science, Cornell University, Ithaca NY).
[c] Kindly provided by Kathryn J. Boor (Department of Food Science, Cornell University, Ithaca NY).

ingestion of a standard meal (8); the gastric pH then remained constant until the end of the challenge (120 min). The intestinal pH was maintained at 6.5 ± 0.3 with 0.3 M $NaHCO_3$ (18, 19). The GC and IC pH conditions were monitored continuously (Ultra Basic; Denver Instrument, Arvada, CO). Secretion of gastrointestinal fluids continued for 120 min after the beginning of each challenge; however, the IC was maintained (statically) in the water bath for a final microbiological analysis at 240 min. *L. monocytogenes* populations were assessed before being mixed with saliva and at intervals during exposure to each compartment by diluting 1-ml samples with 0.1% buffered peptone water (9 ml; Difco) and plating, in duplicate, 0.1-ml portions onto tryptic soy agar (Difco) supplemented with 0.6% yeast extract (TSAYE) and Palcam agar (Difco) (30°C, 48 h).

Statistical analyses. The study was conducted three times. Cell counts were divided by dilution factors (16) to account for the continuous addition or removal of gastrointestinal fluids. Numbers obtained were converted into log CFU/ml and analyzed using the Glimmix procedure of SAS, version 9.2 (SAS Institute Inc., Cary, NC) (23). Independent variables included strain, time and age of the culture, and their interactions. Mean differences were separated at the significance level of 95%.

L. monocytogenes data (Palcam agar) were fitted to the model of Baranyi and Roberts (1), using DMFit software (Institute of Food Research, Norwich, United Kingdom), to determine shoulder durations (lags in death) and inactivation rates (IRs) of cultures. Inactivation kinetics were analyzed with the mixed procedure of SAS (23), with strain being the independent variable; additional analyses, in which serotype or lineage was the independent variable, were conducted to identify potential serotype/lineage-related effects on gastrointestinal survival.

Gastric survival. Overall, counts from Palcam agar (Fig. 2) and TSAYE (not shown) were similar (<0.3-log CFU/ml difference) throughout the challenge; thus, the reported results are for populations on Palcam agar. Although the reasons for a lack of detection of substantial sublethal injury are unclear, traits of individual strains and potential acid adaptation (due to

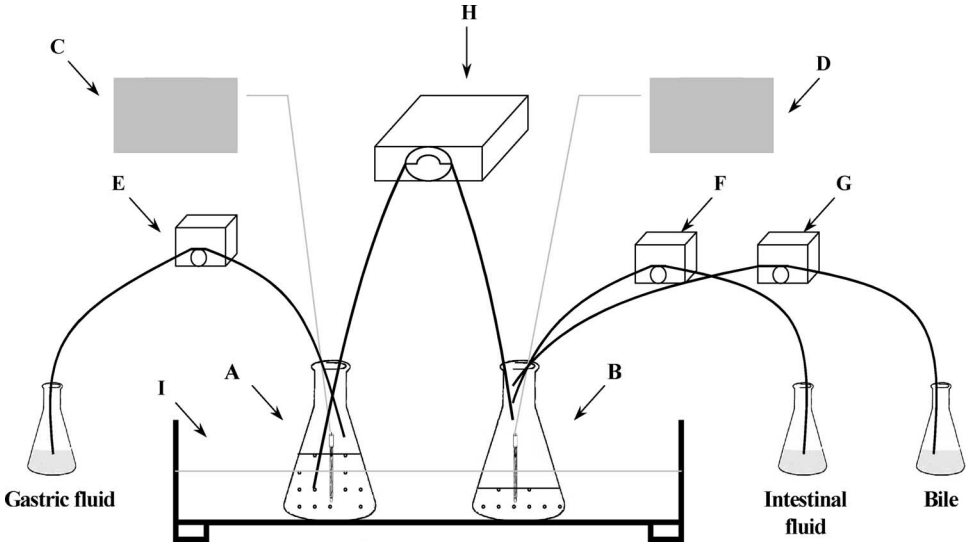

FIG. 1. Schematic diagram of the dynamic gastrointestinal model used in this study. (A, B) 500-ml Erlenmeyer flasks representing the GC and IC; (C, D) pH meters monitoring the pH in the GC and IC; (E, F, G) peristaltic pumps delivering gastric fluid (flow rate, 0.33 ml/min) in the GC and intestinal fluid (flow rate, 0.33 ml/min) and 2 or 4% bile (flow rate, 0.5 ml/min) in the IC; (H) peristaltic pump transferring the gastric contents (flow rate, 1.1 ml/min) in the IC; (I) shaking water bath stabilized at 37°C.

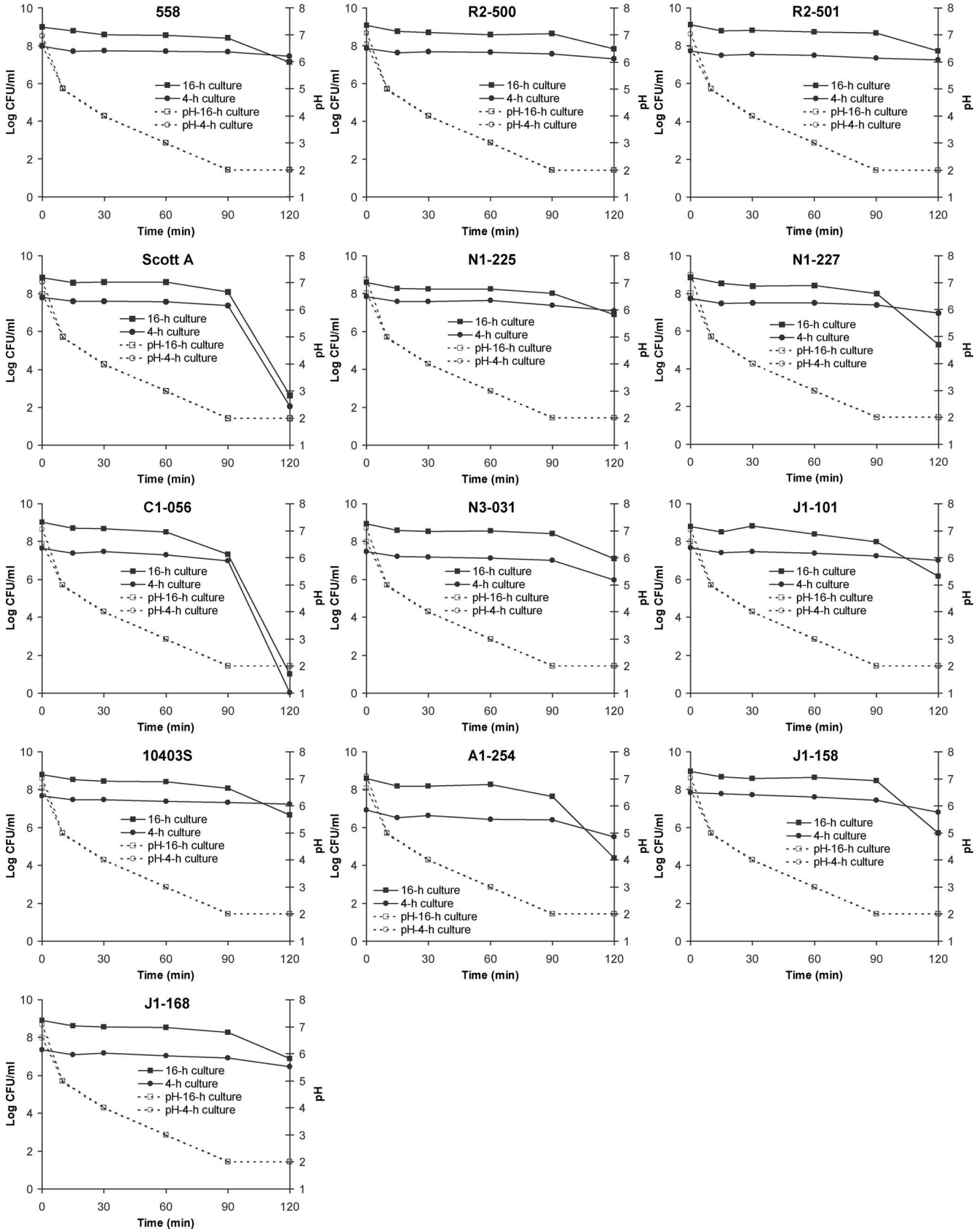

FIG. 2. Survival (log CFU/ml [Palcam agar counts]) of individual *L. monocytogenes* strains and pH values within the GC (37°C) during a simulated gastrointestinal challenge, conducted after inoculation into 100 ml of TSBYE-G and incubation for 4 or 16 h at 30°C. Experiments were conducted in triplicate, and the results are means.

TABLE 2. Mean (n = 3) shoulder durations and inactivation rates (± standard deviations) of individual *Listeria monocytogenes* strains in a simulated gastrointestinal system (GC at pH 2.0 within 88 min; IC at pH 6.5; 37°C) after inoculation (1 ml) into 100 ml of TSBYE-G and incubation for 4 h at 30°C[b]

Strain	GC		Maximum inactivation rate (log CFU/ml/min) in IC
	Shoulder duration (min)[a]	Maximum inactivation rate (log CFU/ml/min)	
558	0.00 ± 0.00^{A}	0.003 ± 0.001^{A}	0.008 ± 0.006^{A}
R2-500		0.003 ± 0.003^{A}	0.010 ± 0.009^{A}
R2-501	68.66 ± 42.85^{BC}	0.002 ± 0.003^{A}	0.004 ± 0.001^{A}
Scott A	77.48 ± 0.74^{B}	0.209 ± 0.019^{B}	0.006 ± 0.002^{A}
N1-225	40.77 ± 34.76^{C}	0.010 ± 0.007^{A}	0.009 ± 0.007^{A}
N1-227	71.49 ± 3.31^{B}	0.019 ± 0.006^{A}	0.011 ± 0.012^{A}
C1-056	76.88 ± 2.37^{B}	0.262 ± 0.022^{B}	0.004 ± 0.005^{A}
N3-031	29.64 ± 51.33^{D}	0.029 ± 0.047^{A}	0.009 ± 0.011^{A}
J1-101		0.005 ± 0.002^{A}	0.013 ± 0.011^{A}
10403S		0.002 ± 0.001^{A}	0.008 ± 0.007^{A}
A1-254	39.10 ± 32.04^{CD}	0.032 ± 0.018^{A}	0.016 ± 0.007^{A}
J1-158	51.94 ± 19.76^{BC}	0.042 ± 0.029^{A}	0.009 ± 0.003^{A}
J1-168	40.90 ± 31.77^{CD}	0.013 ± 0.009^{A}	0.006 ± 0.009^{A}

[a] Latency to death. For blank cells, no shoulder was observed (inactivation was immediate). No shoulder was observed for the IC.
[b] Different superior capital letters within a column show significantly different data ($P < 0.05$).

TABLE 3. Mean (n = 3) shoulder durations and inactivation rates (± standard deviations) of individual *Listeria monocytogenes* strains in a simulated gastrointestinal system (GC at pH 2.0 within 88 min; IC at pH 6.5; 37°C) after inoculation (1 ml) into 100 ml of TSBYE-G and incubation for 16 h at 30°C[b]

Strain	GC		IC	
	Shoulder duration (min)[a]	Maximum inactivation rate (log CFU/ml/min)	Shoulder duration (min)[a]	Maximum inactivation rate (log CFU/ml/min)
558	63.73 ± 16.28^{A}	0.043 ± 0.028^{AD}		0.002 ± 0.002^{AB}
R2-500		0.009 ± 0.001^{A}		0.000 ± 0.003^{Ac}
R2-501	71.17 ± 1.00^{A}	0.023 ± 0.001^{A}		0.003 ± 0.005^{AB}
Scott A	74.71 ± 1.59^{A}	0.197 ± 0.010^{B}		0.008 ± 0.005^{ABD}
N1-225	70.45 ± 3.30^{A}	0.039 ± 0.009^{AD}		0.003 ± 0.001^{AB}
N1-227	70.35 ± 2.94^{A}	0.092 ± 0.001^{BD}		0.004 ± 0.001^{AB}
C1-056	70.74 ± 4.99^{A}	0.221 ± 0.013^{C}		0.014 ± 0.010^{BC}
N3-031	86.48 ± 2.32^{A}	0.047 ± 0.007^{AD}	1.32 ± 2.29	0.016 ± 0.004^{BC}
J1-101	68.15 ± 1.55^{A}	0.062 ± 0.010^{AD}		0.018 ± 0.007^{CD}
10403S	67.35 ± 2.77^{A}	0.048 ± 0.003^{AD}		0.010 ± 0.005^{BC}
A1-254	83.68 ± 22.73^{A}	0.188 ± 0.113^{B}		0.021 ± 0.013^{C}
J1-158	76.66 ± 1.30^{A}	0.103 ± 0.019^{BD}		0.008 ± 0.006^{ABD}
J1-168	65.32 ± 7.12^{A}	0.049 ± 0.008^{AD}		0.009 ± 0.006^{BC}

[a] Latency to death. For blank cells, no shoulder was observed (inactivation was immediate).
[b] Different superior capital letters within a column show significantly different data ($P < 0.05$).
[c] The slope of the survival curve was zero, indicating that no inactivation occurred.

gradual gastric acidification) might have contributed to these findings.

With the exception of the Δ*sigB* mutant strain A1-254, all cultures had reached the exponential (7.4 to 8.0 log CFU/ml) and stationary (8.6 to 9.1 log CFU/ml) phases in TSBYE-G within 4 and 16 h, respectively. Strain A1-254 exhibited no apparent changes in its population during the 4-h incubation, suggesting that σ^B may affect the growth potential, even at 30°C.

Cell counts of all *L. monocytogenes* strains remained in the range of 6.4 to 7.6 (4-h cultures) and 7.3 to 8.7 (16-h cultures) log CFU/ml during the first 90 min of gastric exposure (Fig. 2). Major ($P < 0.05$) reductions in populations and strain differences were observed mainly at 120 min. The highest ($P < 0.05$) acid sensitivity was displayed by strain C1-056, while the second most acid-sensitive strain was Scott A. This was confirmed by IR data indicating that strain C1-056 displayed the highest IR, followed ($P \geq 0.05$) by Scott A (Tables 2 and 3). However, as suggested by their large shoulder durations (Tables 2 and 3), reductions in populations of these strains occurred mainly at later stages of exposure. The Δ*sigB* mutant, A1-254, declined faster ($P \geq 0.05$) than the wild-type strain, 10403S (Table 2). It should be noted, however, that under the examined conditions a valid comparison between the gastric resistances of 4-h cultures of A1-254 and 10403S may not be feasible, as the slow growth of the former strain prevented it from being in the same growth phase as the remaining strains. On the other hand, growth experiments (data not shown) suggested that both A1-254 and 10403S had reached stationary phase within 16 h of incubation, enabling a valid comparison between these strains. As a 16-h culture, strain A1-254 was more ($P < 0.05$) acid sensitive than 10403S and displayed an IR similar ($P \geq 0.05$) to that of Scott A (Table 3). Overall, IRs of 16-h cultures were not different ($P \geq 0.05$) between pairs (outbreak sets; strains belonging to each set were genetically close) of food and human isolates (Table 1) (12). Unlike findings of previous studies (6, 15), the IRs suggested that 4-h cultures were more resistant than 16-h cultures of the respective strains. A possible explanation for this discrepancy could be that the 4-h cultures were still in the early stages of the exponential phase and thus contained a substantial portion of the original stationary-phase cells of the inoculum used to initiate the culture, which could have exhibited more acid resistance (6).

Intestinal survival. Populations transferred to the IC within the first 30 min of gastric emptying were correlated with the initial level of each strain and ranged from 5.4 to 8.1 log CFU/ml and 7.0 to 9.1 log CFU/ml, for 4- and 16-h cultures, respectively (data not shown).

Reductions in populations during the intestinal challenge were not as drastic as those observed in the GC, as demonstrated by the intestinal IRs (Tables 2 and 3). Differences in intestinal IRs of strains A1-254 and 10403S (16-h cultures only) suggested that the contribution of σ^B to intestinal survival was small ($P \geq 0.05$) (Table 3). Combined isolates of serotype 1/2 and lineage 2 possessed significantly ($P < 0.05$) higher IRs than did combined serotype 4b and lineage 1 or 3 isolates, respectively. However, these serotype/lineage-related effects were present only in 16-h cultures. Moreover, differences among serotypes or lineages referred only to the combined observations, as the behaviors of individual strains within serotypes or lineages were not identical during intestinal exposure.

Overall, gradual acidification of the stomach contents together with gastric emptying resulted in cells being subjected to different levels of acidity. Thus, high cell numbers, even of acid-susceptible strains, survived gastric exposure while the pH was >3.0, reaching the intestine in a viable state. In their study, Dykes and Moorhead (9) reported increased acid resistance in all clinical *L. monocytogenes* strains, an observation that led them to remark on the importance of acid tolerance in the

infection process. In our study, clinical isolates C1-056 and Scott A were the most acid susceptible among the strains examined; however, the increased survival of these isolates during the initial stages of gastric exposure may help to explain their implication in human disease, particularly if high contamination levels were involved. The fact that all strains survived the gastrointestinal passage suggested that although acid resistance is a crucial element in terms of intrahost survival, assumptions regarding the gastrointestinal survival of the pathogen might be more accurate when other gastrointestinal tract-related aspects (e.g., gastric emptying) have also been considered.

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REFERENCES

1. **Baranyi, J., and T. A. Roberts.** 1994. A dynamic approach to predicting bacterial growth in food. Int. J. Food Microbiol. **23:**277–294.
2. **Begley, M., C. G. M. Gahan, and C. Hill.** 2002. Bile stress response in *Listeria monocytogenes* L028: adaptation, cross-protection, and identification of genetic loci involved in bile resistance. Appl. Environ. Microbiol. **68:**6005–6012.
3. **Bernbom, N., T. R. Licht, P. Saadbye, F. K. Vogensen, and B. Nørrung.** 2006. *Lactobacillus plantarum* inhibits growth of *Listeria monocytogenes* in an in vitro continuous flow gut model, but promotes invasion of *L. monocytogenes* in the gut of gnotobiotic rats. Int. J. Food Microbiol. **108:**10–14.
4. **Beumer, R. R., J. de Vries, and F. M. Rombouts.** 1992. *Campylobacter jejuni* non-culturable coccoid cells. Int. J. Food Microbiol. **15:**153–163.
5. **Bishop, D. K., and D. J. Hinrichs.** 1987. Adoptive transfer of immunity to *Listeria monocytogenes*: the influence of in vitro stimulation on lymphocyte subset requirements. J. Immunol. **139:**2005–2009.
6. **Davis, M. J., P. J. Coote, and C. P. O' Byrne.** 1996. Acid tolerance in *Listeria monocytogenes*: the adaptive acid tolerance response (ATR) and growth-phase-dependent acid resistance. Microbiology **142:**2975–2982.
7. **Doran, S., K. L. Jones, J. M. Andrews, and M. Horowitz.** 1998. Effects of meal volume and posture on gastric emptying of solids and appetite. Am. J. Physiol. **275:**1712–1718.
8. **Dressman, J. B., R. R. Berardi, L. C. Dermentzoglou, T. L. Russell, S. P. Schmaltz, J. L. Barnett, and K. M. Jarvenpaa.** 1990. Upper gastrointestinal (GI) pH in young, healthy men and women. Pharm. Res. **7:**756–761.
9. **Dykes, G. A., and S. M. Moorhead.** 2000. Survival of osmotic and acid stress by *Listeria monocytogenes* strains of clinical or meat origin. Int. J. Food Microbiol. **56:**161–166.
10. **Faleiro, M. L., P. W. Andrew, and D. Power.** 2003. Stress response of *Listeria monocytogenes* isolated from cheese and other foods. Int. J. Food Microbiol. **84:**207–216.
11. **Formato, G., I. Georgaras, I. M. Barmpalia, P. N. Skandamis, K. E. Belk, J. A. Scanga, P. A. Kendall, G. C. Smith, and J. N. Sofos.** 2007. Effect of acid adaptation on growth during storage at 10°C and resistance to simulated gastric fluid of *Listeria monocytogenes* inoculated onto bologna formulated with or without antimicrobials. J. Food Prot. **70:**65–69.
12. **Fugett, E., E. Fortes, C. Nnoka, and M. Wiedmann.** 2006. International Life Sciences Institute North America *Listeria monocytogenes* strain collection: development of standard *Listeria monocytogenes* strain sets for research and validation studies. J. Food Prot. **69:**2929–2938.
13. **Gahan, C. G. M., and C. Hill.** 2005. Gastrointestinal phase of *Listeria monocytogenes* infection. J. Appl. Microbiol. **98:**1345–1353.
14. **Kathariou, S.** 2002. *Listeria monocytogenes* virulence and pathogenicity, a food safety perspective. J. Food Prot. **65:**1811–1829.
15. **King, T., T. Ferenci, and E. A. Szabo.** 2003. The effect of growth atmosphere on the ability of *Listeria monocytogenes* to survive exposure to acid, proteolytic enzymes and bile salts. Int. J. Food Microbiol. **84:**133–143.
16. **Koo, J., D. L. Marshall, and A. DePaola.** 2001. Antacid increases survival of *Vibrio vulnificus* and *Vibrio vulnificus* phage in a gastrointestinal model. Appl. Environ. Microbiol. **67:**2895–2902.
17. **Lianou, A., J. D. Stopforth, Y. Yoon, M. Wiedmann, and J. N. Sofos.** 2006. Growth and stress resistance variation in culture broth among *Listeria monocytogenes* strains of various serotypes and origins. J. Food Prot. **69:**2640–2647.
18. **Marteau, P., M. Minekus, R. Havenaar, and J. H. J. Huis In't Veld.** 1997. Survival of lactic acid bacteria in a dynamic model of the stomach and small intestine: validation and the effects of bile. J. Dairy Sci. **80:**1031–1037.
19. **Minekus, M., P. Marteau, R. Havenaar, and J. H. J. Huis In't Veld.** 1995. A multicompartmental dynamic computer-controlled model simulating the stomach and the small intestine. Altern. Lab. Anim. **23:**254–258.
20. **Molly, K., M. V. Woestyne, I. De Smet, and W. Verstraete.** 1994. Validation of the simulator of the human intestinal microbial ecosystem (SHIME) reactor using microorganisms-associated activities. Microb. Ecol. Health Dis. **7:**191–200.
21. **Naim, F., S. Messier, L. Saucier, and G. Piette.** 2004. Postprocessing in vitro digestion challenge to evaluate survival of *Escherichia coli* O157:H7 in fermented dry sausages. Appl. Environ. Microbiol. **70:**6637–6642.
22. **Olier, M., S. Rousseaux, P. Piveteau, J.-P. Lemaitre, A. Rousset, and J. Guzzo.** 2004. Screening of glutamate decarboxylase activity and bile salt resistance of human asymptomatic carriage, clinical, food, and environmental isolates of *Listeria monocytogenes*. Int. J. Food Microbiol. **93:**87–99.
23. **SAS Institute Inc.** 2008. SAS/STAT 9.2 user's guide. http://support.sas.com/documentation/cdl/en/statug/59654/HTML/default/chap0_toc.htm.
24. **Smith, J. L.** 2003. The role of gastric acid in preventing foodborne disease and how bacteria overcome acid conditions. J. Food Prot. **66:**1296–1303.
25. **Stopforth, J. D., Y. Yoon, I. M. Barmpalia, J. Samelis, P. N. Skandamis, and J. N. Sofos.** 2005. Reduction of *Listeria monocytogenes* populations during exposure to a simulated gastric fluid following storage of inoculated frankfurters formulated and treated with preservatives. Int. J. Food Microbiol. **99:**309–319.
26. **Wiedmann, M., J. L. Bruce, C. Keating, A. E. Johnson, P. L. McDonough, and C. A. Batt.** 1997. Ribotypes and virulence gene polymorphisms suggest three distinct *Listeria monocytogenes* lineages with differences in pathogenic potential. Infect. Immun. **65:**2707–2716.
27. **Wiedmann, M., T. J. Arvik, R. J. Hurley, and K. J. Boor.** 1998. General stress transcription factor σ^B and its role in acid tolerance and virulence of *Listeria monocytogenes*. J. Bacteriol. **180:**3650–3656.