Journal of Food Protection, Vol. 77, No. 8, 2014, Pages 1298–1307
doi:10.4315/0362-028X.JFP-13-423

Low, Medium, and High Heat Tolerant Strains of *Listeria monocytogenes* and Increased Heat Stress Resistance after Exposure to Sublethal Heat†

QIAN SHEN,‡ PRIYANKA M. JANGAM,‡ KAMLESH A. SONI, RAMAKRISHNA NANNAPANENI,*
WES SCHILLING, AND JUAN L. SILVA

Department of Food Science, Nutrition and Health Promotion, P.O. Box 9805, Mississippi State University, Mississippi State, Mississippi 39762, USA

MS 13-423: Received 4 October 2013/Accepted 30 March 2014

ABSTRACT

A group of 37 strains representing all 13 serotypes of *Listeria monocytogenes* with an initial cell density of 10^7 CFU/ml were analyzed for their heat tolerance at 60°C for 10 min. These *L. monocytogenes* strains were categorized into three heat tolerance groups: low (<2 log CFU/ml survival), medium (2 to 4 log CFU/ml survival), and high (4 to 6 log CFU/ml survival). Serotype 1/2a strains had relatively low heat tolerance; seven of the eight tested strains were classified as low heat tolerant. Of the two serotype 1/2b strains tested, one was very heat sensitive (not detectable) and the other was very heat resistant (5.4 log CFU/ml survival). Among the 16 serotype 4b strains, survival ranged from not detectable to 4 log CFU/ml. When one *L. monocytogenes* strain from each heat tolerance group was subjected to sublethal heat stress at 48°C for 30 or 60 min, the survival of heat-stressed cells at 60°C for 10 min increased by 5 log CFU/ml ($D_{60°C}$-values nearly doubled) compared with the nonstressed control cells. Sublethal heat stress at 48°C for 60 or 90 min increased the lag phase of *L. monocytogenes* in tryptic soy broth supplemented with 0.6% yeast extract at room temperature by 3 to 5 h compared with nonstressed control cells. The heat stress adaptation in *L. monocytogenes* was reversed after 2 h at room temperature but was maintained for up to 24 h at 4°C. Our results indicate a high diversity in heat tolerance among strains of *L. monocytogenes,* and once acquired this heat stress adaptation persists after cooling, which should be taken into account while conducting risk analyses for this pathogen.

Listeria monocytogenes is a ubiquitous, gram-positive, and psychrotrophic foodborne pathogen. Infections have a high mortality of 20 to 30% *(32, 36)*. Thirteen serotypes of *L. monocytogenes* are known, of which 1/2a, 1/2b, and 4b are responsible for most listeriosis outbreaks in the United States. Strains of serotype 4b appear to be more virulent; epidemiological analysis has frequently associated these strains with listeriosis outbreak cases, whereas serotype 1/2a strains are typically confined to food processing environments and are not frequently isolated from clinical cases *(33, 44)*. *L. monocytogenes* has received extra attention because it can actively multiply under refrigeration conditions, unlike other foodborne pathogens such as *Salmonella* and *Escherichia coli* O157:H7 *(7, 17, 35)*. Therefore, *L. monocytogenes* is of utmost concern in ready-to-eat products because any postprocessing contamination at the processing plant will provide an opportunity for active growth under cold conditions, possibly reaching levels that can cause infection. Because of these risks, the United States has a zero tolerance policy for *L. monocytogenes* in ready-to-eat food products *(40)*.

Health risks associated with microbial stress adaptation is an important area of research with direct practical implications. Stress is defined as any physical, chemical, or biological condition that adversely affects bacterial growth and survival *(46)*. Commonly, sublethal exposure to any stress for 15 to 60 min is sufficient to induce cellular changes that enable foodborne pathogens to resist lethal inactivation treatments. For example, *L. monocytogenes* subjected to sublethal heating at 48°C for 60 min were more heat tolerant at 60°C than were cells that were not sublethally heated *(1)*. Cross-protection, where sublethal exposure to one stress confers resistance against other heterogeneous inactivation treatments, also has been found in *L. monocytogenes*. For instance, *L. monocytogenes* cells heat stressed at 45°C for 60 min had higher resistance to ethanol and osmotic stress conditions *(25)*. In another study, heat- or salt-stressed *L. monocytogenes* cells were not resistant to acid inactivation treatments *(20)*. This finding suggests that the stress tolerance mechanisms in *L. monocytogenes* are highly complex and depend on various stress parameters and conditions.

An understanding the heat stress response is critical because heating remains the most widely used measure for

* Author for correspondence. Tel: 662-325-7697; Fax: 662-325-8728; E-mail: nannapaneni@fsnhp.msstate.edu.
† Approved for publication as journal article J-12425 of the Mississippi Agricultural and Forestry Experiment Station, Mississippi State University.
‡ Both authors contributed equally.

protecting the food supply. The increase in thermal resistance after sublethal heating treatment was initially demonstrated in a broth model study and has been confirmed in food matrices *(1, 10, 31)*. An intriguing factor in the *L. monocytogenes* heat stress response is the genetic diversity associated with various strains of the different serotypes. Buncic et al. *(3)* found no inherent differences in heat tolerance at the phenotypic level between *L. monocytogenes* 1/2a and 4b isolates when cells were subjected to lethal inactivation at 60°C. In another study, high variability in heat tolerance was observed in 21 strains of *L. monocytogenes* of different serotypes, and lineage appeared to have some effect on the inherent heat tolerance of *L. monocytogenes (8)*. Lianou et al. *(22)* discovered that among 25 *L. monocytogenes* strains isolated from food or clinical outbreaks serotype 4b strains had the lowest thermal resistance, but this study did not include strains from all 13 known serotypes of *L. monocytogenes*. At a molecular level, the requirement for *sigB* to induce heat stress adaptation in *L. monocytogenes* can be strain or serotype dependent *(12, 28),* and the heat stress response in *L. monocytogenes* was not modulated based on the presence or absence of antibiotic resistance genes *(43)*.

Of practical relevance, in several scenarios heat stress adaptation may or may not be important. Initial acquired heat stress adaptation in broth can enhance the heat tolerance of *L. monocytogenes* during subsequent lethal heat treatments in food products. Carlier et al. *(6)* found that heat shocked (42°C) *L. monocytogenes* cells had higher survival rates than did nonheated control cells in ham at 60°C. Jorgensen et al. *(14)* found that *L. monocytogenes* survival in minced beef was higher at 60°C after cells were preheated at 46°C for 30 min. Farber and Brown *(10)* reported that preheating at 48°C for 60 min conferred increased heat tolerance to *L. monocytogenes* in meat products. Sublethal heat–induced adaptation to heat stress also may occur in food products subjected to slow and gradual heating. In large meat products such as intact ham, the rate of heat transfer is a crucial factor affecting the lethal heat inactivation of *L. monocytogenes (19, 39)*. Other thermal techniques such as microwaving and infrared heating in which heat transfer occurs mainly by conduction or convection can create areas with exposure to sublethal temperatures *(11, 21, 47)*. In contrast, Samelis et al. *(34)* reported that after heat treatment at 60°C for 30 s, *Listeria* in raw milk was reduced to a safe level. In this case, the inactivation effect could have been due to the rapid increase in temperature or low levels of *Listeria* contamination.

In the various reports of *L. monocytogenes* and heat stress, several aspects are not clear. None of the studies included evaluation of the heat tolerance of all 13 serotypes of *L. monocytogenes*. Studies of the effect of inherent variation in heat tolerance among *L. monocytogenes* strains on heat stress adaptation under a variety of sublethal heat stress conditions have not been attempted. An increase in the lag phase due to lethal heat shock (55 or 63°C) was reported by Vasseur et al. *(42)*. However, the effect of cell injury from sublethal heat treatment (48°C) as a measure of the increase in the lag phase of various strains with different levels of heat tolerance has not been examined. The stability (i.e., cellular imprint or memory) of heat stress adaptation is very important because heat-adapted cells may not immediately encounter homologous (i.e., heat) or heterogeneous (i.e., acid, antimicrobial, or high hydrostatic pressure) lethal inactivation treatments during food processing. The results of two previous studies suggested that heat stress adaptation may persist for 24 h in broth or meat products at 4°C *(10, 15)*. However, little information is available on whether the stability of heat stress adaptation in *L. monocytogenes* is strain dependent.

To improve current understanding of the heat stress adaptation of *L. monocytogenes*, we evaluated (i) the heat tolerance of 37 *L. monocytogenes* strains representing all known 13 serotypes of *L. monocytogenes*, (ii) the heat stress adaptation in representative *L. monocytogenes* strains with low, medium, and high heat tolerance after sublethal heat stress at 48°C, (iii) the growth rate of representative *L. monocytogenes* strains with low, medium, and high heat tolerance after sublethal heat stress at 48°C for different time periods, and (iv) the stability of heat stress adaptation in representative *L. monocytogenes* strains with low, medium, and high heat tolerance after sublethal heat stress at 48°C followed by cooling to 22 or 4°C before lethal heat stress at 60°C.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Thirty-seven *L. monocytogenes* isolates collected from different sources were used in this study. Details of strains, serotypes, and sources are given in Tables 1 through 3. Working stock cultures of these strains were maintained at 4°C in tryptic soy broth slants supplemented with 0.6% yeast extract (TSBYE; Sigma-Aldrich, St. Louis, MO). Ten milliliters of TSBYE was inoculated with one loopful of *L. monocytogenes* stock cultures (using sterile disposable loops) and incubated overnight in an incubator shaker (C24 classic series, New Brunswick Scientific, Edison, NJ) at 37°C to obtain stationary-phase cultures.

Heat tolerance of *L. monocytogenes* at 60°C. The *L. monocytogenes* strains were grown overnight at 37°C and thermally challenged at 60°C for 10 min. The optical density at 630 nm (OD_{630}) of the overnight culture for all the strains were 1.0 to 1.2, a level of approximately 9 log CFU/ml. These stationary-phase cultures were initially diluted to 10^8 log CFU/ml in TSBYE, and 100 μl of each culture was transferred to Eppendorf tubes on a digital thermal block (Accublock digital dry bath, Labnet International, Edison, NJ) containing 900 μl of TSBYE preheated to 60°C. The surviving cells were enumerated after 10 min at 60°C on tryptic soy agar (TSA) plates supplemented with 0.1% esculin and 0.05% ferric ammonium citrate (TSAEF). Compared with TSA or TSAYE, TSAEF provided better recovery of heat-stressed cells in our early stages of testing. Plates were incubated at 37°C for 36 to 48 h, and colonies were counted.

Effect of sublethal heating at 48°C on heat stress adaptation in *L. monocytogenes* strains. In this assay, cells of three representative *L. monocytogenes* strains, EGD (BUG 600; low heat tolerance), NRRL B-33157 (medium heat tolerance), and F4260 (high heat tolerance) were exposed to the sublethal temperature of 48°C for 0, 5, 15, 30, 60, and 90 min to evaluate

TABLE 1. L. monocytogenes *strains with low heat tolerance: survival of 0 to 2 log CFU/ml at 60°C for 10 min*

Strain	Serotype	Isolation	Source[a]	Mean ± SE survival (log CFU/ml)
BUG 600 (EGD)	1/2a	Human	Pasteur Institut, Paris	1.5 ± 0.2
BUG 1600 (EGDe)	1/2a	Derivative of BUG 600	M. Wiedmann, Cornell University	<1
ESL-A-254	1/2a	Derivative of 10403S	M. Wiedmann, Cornell University	<1
DP-L-1964	1/2a	Derivative of 10403S	M. Wiedmann, Cornell University	<1
10403S	1/2a	Human	M. Wiedmann, Cornell University	1.5 ± 0.2
NRRL B-33069	1/2a	Bovine milk	USDA ARS	1.8 ± 0.5
V7	1/2a	Raw milk	FDA	<1
NRRL B-33123	1/2b	Floor drain	USDA ARS	1.8 ± 0.5
V2	1/2c	Human cerebrospinal fluid	VICAM	<1
ATCC 19113	3a	Human, Denmark	ATCC	2.0 ± 0.11
ATCC 2540	3b	Human cerebrospinal fluid	ATCC	<1
SLCC 2479	3c	Unknown	ATCC	<1
ATCC 19114	4a	Bovine brain	ATCC	<1
NRRL B-33001	4b	Clinical strain	USDA ARS	<1
Scott A	4b	Human clinical strain	FDA	<1
F4393	4b	Cheese	CDC	<1
F5069	4b	Milk, cerebrospinal fluid	CDC	<1
ATCC 43257	4b	Mexican style cheese	ATCC	<1
F1057	4b	Milk	CDC	<1
F1109	4b	Milk	CDC	<1
F2385	4b	Epidemic strain, California	California, 1985	<1
NRRL 33083	4b	Outbreak strain	USDA ARS	<1
Murray B	4ab	Human	FDA	2.0 ± 0.3
ATCC 19116	4c	Chicken, England	ATCC	2.0 ± 0.6
ATCC 19118	4e	Chicken, England	ATCC	<1

[a] USDA ARS, U.S. Department of Agriculture, Agricultural Research Service; FDA, U.S. Food and Drug Administration; VICAM, Waters Corporation; ATCC, American Type Culture Collection; CDC, Centers for Disease Control and Prevention.

the effect of this sublethal treatment on the ability of the strains to withstand lethal heat treatment at 60°C. For the sublethal treatment, 1 ml of stationary-phase culture was added to 9 ml of preheated (48°C) TSBYE in 15-ml tubes. A reciprocal water bath shaker (model R76, New Brunswick Scientific) was used to heat the tubes, which were immersed upright in water to just below the lid. Additional precautions taken included adding inoculum directly into the preheated broth and using of serological pipettes to mix the samples so that cell inoculum did not adhere to the nonheated part of the inner tube wall and cap. After incubation at 48°C for 0 to 90 min, the tubes were vortexed for 30 s, and 100 μl of the heated sample was transferred to 900 μl of preheated TSBYE (60°C for 15 min) in Eppendorf tubes and incubated at 60°C in a digital heating block. Multiple Eppendorf tubes were prepared for each sample, and individual Eppendorf tubes were used for *L. monocytogenes* enumeration. For strain BUG 600, survival was determined every 5 min for 20 min. For strains NRRL B-33157 and F4260, the survival was determined every 10 min for 50 min. After heat treatments at 60°C, all samples were plated on TSAEF and incubated at 37°C for 24 to 36 h to obtain countable colonies.

Effect of sublethal heating at 48°C on the lag phase of *L. monocytogenes* strains. This experiment was conducted to determine the lag time of strains BUG 600, NRRL B-33157, and F4260 after exposure to 48°C. *L. monocytogenes* cells were exposed to sublethal treatment at 48°C for 0, 5, 15, 30, 60, and 90 min following the same protocol as for the previous experiment, and then 2 ml of each sample was distributed into duplicate 24-well

TABLE 2. L. monocytogenes *strains with medium heat tolerance: survival of 2 to 4 log CFU/ml at 60°C for 10 min*

Strain	Serotype	Isolation	Source[a]	Mean ± SE survival (log CFU/ml)
FSL-B2-002	1/2a	Derivative of 10403S	M. Wiedmann, Cornell University	2.3 ± 0.3
NRRL B-33015	4b	Monkey placenta	FDA	3.3 ± 0.3
NRRL B-33058	4b	Clinical strain	Halifax, Nova Scotia	2.3 ± 0.7
NRRL B-33094	4b	Big fruit bat	USDA ARS	4.0 ± 0.7
NRRL B-33109	4b	Cooler condensate	USDA ARS	3.7 ± 0.3
NRRL B-33155	4b	Sodium caseinate	USDA ARS	3.8 ± 0.5
NRRL B-33157	4b	Cheese plant	USDA ARS	2.6 ± 0.0
NRRL B-33389	4b	Human	USDA ARS	2.8 ± 0.1
ATCC 19117	4d	Sheep, USA	ATCC	3.4 ± 0.1

[a] FDA, U.S. Food and Drug Administration; USDA ARS, U.S. Department of Agriculture, Agricultural Research Service; ATCC, American Type Culture Collection.

TABLE 3. L. monocytogenes *strains with high heat tolerance: survival of 4 to 6 log CFU/ml at 60°C for 10 min*

Strain	Serotype	Isolation	Source[a]	Mean ± SE survival (log CFU/ml)
F4260	1/2b	Human cerebrospinal fluid and blood	CDC	5.4 ± 0.5
SLCC 2482	7	Human feces	ATCC	4.2 ± 0.1
46 NADC	Unknown	Chicken	A. Mendonca, Iowa State University	4.4 ± 0.2

[a] CDC, Centers for Disease Control and Prevention; ATCC, American Type Culture Collection.

microtiter plates. The plates were placed on a shaker at room temperature (22°C), and the OD_{630} was measured every hour for 12 h with a microtiter plate reader (EL_X 800_{NB} universal microplate reader, BioTek Instruments, Winooski, VT).

Effect of cooling step on stability of heat stress adaptation in *L. monocytogenes* strains. In previous experiments, the heat stress adaptation of *L. monocytogenes* was determined by exposure to sublethal heat at 48°C and then to lethal heat at 60°C. Here, we define the stability of heat stress adaptation as the ability of *L. monocytogenes* cells to maintain heat resistance up to a definite time after removal from the sublethal heat stress environment. *L. monocytogenes* cell suspensions (10 ml) were exposed to sublethal heat stress at 48°C for 60 min as described above and then held at room temperature for 0, 60, and 120 min. Subsamples (100 μl) were then mixed with 900 μl of TSBYE that had been heated at 60°C, and inactivation of BUG 600, NRRL B-33157, and F4260 was evaluated after 10, 15, and 20 min, respectively. For comparison, the non–heat-adapted samples from each strain without growth at room temperature were also subjected to direct challenge at 60°C. After the 60°C lethal exposure, surviving *L. monocytogenes* cells were enumerated on TSAEF.

The stability of heat stress adaptation in samples subsequently stored at 4°C was determined after 0, 2, 6, and 24 h of refrigeration of samples that had been first sublethally heat stressed at 48°C for 60 min or not sublethally heat stressed (control samples) and then exposed to 60°C. The BUG 600, NRRL B-33157, and F4260 strains were inactivated at 60°C after 10, 15, and 20 min, respectively. The protocol was similar to that described above for determining stability at room temperature, but the major difference was the duration of the incubation time (up to 2 h at room temperature versus 24 h at 4°C) and analysis of control samples at each time point during the 4°C incubation. Analysis of control samples simultaneously with heat stress–adapted cells at each time point was not possible for the room temperature experiment because of active growth of *L. monocytogenes* cells at room temperature.

Statistical analysis. Three replicates were conducted for all experiments in a completely randomized design structure. Data recorded were the mean ± the standard error of three individual trials. An analysis of variance with Tukey's honest significant difference test ($P < 0.05$) was performed to determine significant mean separation (SPSS version 12.0, SPSS, Chicago, IL).

RESULTS

Diversity of heat tolerance in *L. monocytogenes* strains at 60°C. Based on their survival at 60°C for 10 min, 37 *L. monocytogenes* strains representing all 13 serotypes were classified into three subgroups of low, medium, and high heat tolerance. Low heat tolerance was defined as survival of 0 to 2 log CFU/ml at 60°C for 10 min. Of the total 37 strains analyzed, 25 belonged to this subgroup (Table 1). Medium heat tolerance was defined as survival of 2 to 4 log CFU/ml at 60°C for 10 min; nine strains belonged to this subgroup (Table 2). High heat tolerance was defined as survival of 4 to 5 log CFU/ml at 60°C for 10 min; three strains belonged to this subgroup (Table 3).

Strains of *L. monocytogenes* representing serotype 1/2a had relatively low heat tolerance; seven of eight tested 1/2a strains were classified in the low heat tolerance group. Of these eight strains of serotype 1/2a, survival ranged from nondetectable to 2.3 log CFU/ml. Extensive variability was observed in heat tolerance at 60°C among serotype 1/2b and 4b strains. Of the two serotype 1/2b strains tested, one was nondetectable and the other survived at up to 5.4 log CFU/ml after the 60°C treatment. Among the 16 strains of serotype 4b, survival ranged from nondetectable to 4 log CFU/ml. For the other 10 serotypes, one serotype 7 strain had high heat tolerance and the rest were classified as having low heat tolerance. No specific serotype-associated heat tolerance was observed among the strains of those 10 serotypes.

Ability of *L. monocytogenes* to survive at 60°C is dependent on the exposure time to sublethal heat stress. Heat stress adaptation, defined as the increased resistance to lethal temperature after exposure to sublethal heat stress, was dependent on the duration of the exposure to the sublethal heat stress. Three strains of *L. monocytogenes*, BUG 600, NRRL B-33157, and F4260, were selected to represent low, medium, and high heat tolerance, respectively. Overall, *L. monocytogenes* stationary-phase cells that were preheated at 48°C for 5, 15, 30, 60, and 90 min were more heat tolerant than were the control cells, which were not preheated. The *D*-values at 60°C ($D_{60°C}$; defined as the time required to achieve 90% reduction in the *L. monocytogenes* population at 60°C) were calculated for survival curves from Figure 1 and presented in Table 4.

For strain BUG 600 (Fig. 1A), counts in control samples declined from an initial 7 log CFU/ml to nearly 1 log CFU/ml within 10 min of heating at 60°C, resulting in a $D_{60°C}$-value of 1.9 ± 0.2 min. BUG 600 cells that were heat stressed at 48°C for 5 min had approximately 3 log CFU/ml survival after 10 min of lethal (60°C) heat treatment, although the calculated $D_{60°C}$-value of 2.1 ± 0.0 min was not significantly different than that for the control ($P > 0.05$). Heat adaptation was more evident for cells treated at 48°C for 15, 30, 60, and 90 min, with survival of 4.5 to 5.5 log CFU/ml after 10 min at 60°C. The $D_{60°C}$-values calculated for cells that were preheated at 48°C for 15, 30, 60, and 90 min were 3.1 ± 0.2, 4.0 ± 0.5, 5.0 ± 0.5, and 2.9 ± 0.1 min, respectively. Preheating for either 15 or 90 min resulted $D_{60°C}$-values similar to those for the

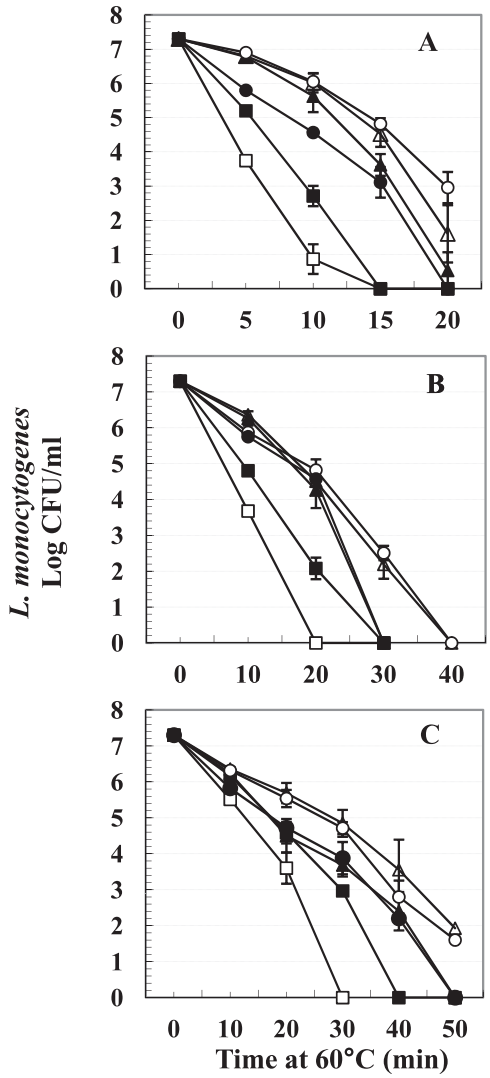

FIGURE 1. *Effect of sublethal heating at 48°C for 0 (□), 5 (■), 15 (▲), 30 (△), 60 (○), and 90 (●) min on survival at 60°C for three* L. monocytogenes *strains: (A) BUG 600 (serotype 1/2a), (B) NRRL B-33157 (serotype 4b), and (C) F4260 (serotype 1/2b).*

controls, which indicated that excessive preheating impaired the acquired heat stress adaptation. According to survival curves (Fig. 1) and $D_{60°C}$-values (Table 4), preexposure at 48°C for 60 min conferred the most protection from subsequent heat stress.

The NRRL B-33157 (medium heat tolerance) control (nontreated) survival was below the detection limit after 20 min of heating at 60°C (Fig. 1B), with a $D_{60°C}$-value of 2.8 ± 0.0 min. The cells that were preheated at 48°C for 5 min survived at about 2 log CFU/ml ($D_{60°C}$-value, 4.2 ± 0.0 min) after 20 min of heating at 60°C, suggesting mild heat adaptation. The heat adaptation for the cells that were preheated at 48°C for 15, 30, 60, and 90 min was much higher, with survival of 4.2 to 4.7 log CFU/ml after 20 min at 60°C. When the lethal exposure time at 60°C was increased to 30 min, the survival of cells preheated for 15 and 90 min at 48°C was nondetectable, whereas cells preheated for 30 and 60 min had survival of 2.5 log CFU/ml. Cells preheated at 48°C for 15 and 90 min had a similar heat tolerance, with $D_{60°C}$-values of 4.3 ± 0.3 and 5.0 ± 0.4 min, respectively. The maximum tolerance to 60°C was achieved when the samples were preheated at 48°C for 30 and 60 min, with $D_{60°C}$-values of 5.4 ± 0.1 and 5.7 ± 0.2 min, respectively.

The adaptation effect for high heat tolerant strain F4260 followed a pattern similar to that of the low and medium heat tolerant strains (Fig. 1C). The nonheated samples had a $D_{60°C}$-value of 4.3 ± 0.0 min, whereas the cells preheated at 48°C for 5, 15, 30, 60, and 90 min had $D_{60°C}$-values of 5.7 ± 0.5, 8.0 ± 0.7, 10.4 ± 0.5, 9.8 ± 0.2, and 8.1 ± 0.4 min, respectively. Control cells declined to nondetectable levels after 30 min, whereas samples preheated for 30 and 60 min survived even after 50 min of heating at 60°C.

To summarize the adaptation results, the effect of sublethal heating at 48°C on survival after lethal heating at 60°C was evident in all three strains representing different inherent thermal tolerance levels. For the three strains, sublethal heat stress at 48°C for 30 or 60 min induced the most effective heat stress adaptation. As the preheating time at 48°C was increased to 90 min, the heat stress adaptation effect diminished, and these cells were less tolerant to lethal heat stress.

Sublethal heating of *L. monocytogenes* cells at 48°C increased the growth lag phase. Exposure of *L. monocytogenes* to sublethal heating at 48°C interrupted the active growth cycle and resulted in an increase in the lag time. Based on the OD_{630}, BUG 600 control cells initiated growth after 1 h, and a growth plateau was reached within 10 h (Fig. 2A). BUG 600 cells that were heat stressed at 48°C for 5, 15, or 30 min also resumed growth after 1 h and reached a growth plateau within 10 h. In contrast, heat stress at 48°C

TABLE 4. D-*values of three* L. monocytogenes *strains with low (BUG 600), medium (NRRL B-33157), and high (F4260) heat tolerance*

48°C treatment time (min)	*D*-value (min) at 60°C[a]		
	BUG 600 (serotype 1/2a)	NRRL B-33157 (serotype 4b)	F4260 (serotype 1/2b)
0	1.9 ± 0.2 A	2.8 ± 0.0 A	4.3 ± 0.0 A
5	2.1 ± 0.0 A	4.2 ± 0.0 B	5.7 ± 0.5 AB
15	3.1 ± 0.2 AB	4.3 ± 0.3 B	8.0 ± 0.7 BC
30	4.0 ± 0.5 BC	5.4 ± 0.1 C	10.4 ± 0.5 C
60	5.0 ± 0.5 C	5.7 ± 0.2 C	9.8 ± 0.2 C
90	2.9 ± 0.1 AB	5.0 ± 0.4 BC	8.1 ± 0.4 BC

[a] Within each column, means followed by different letters are significantly different based on Tukey's analysis of variance ($P < 0.05$).

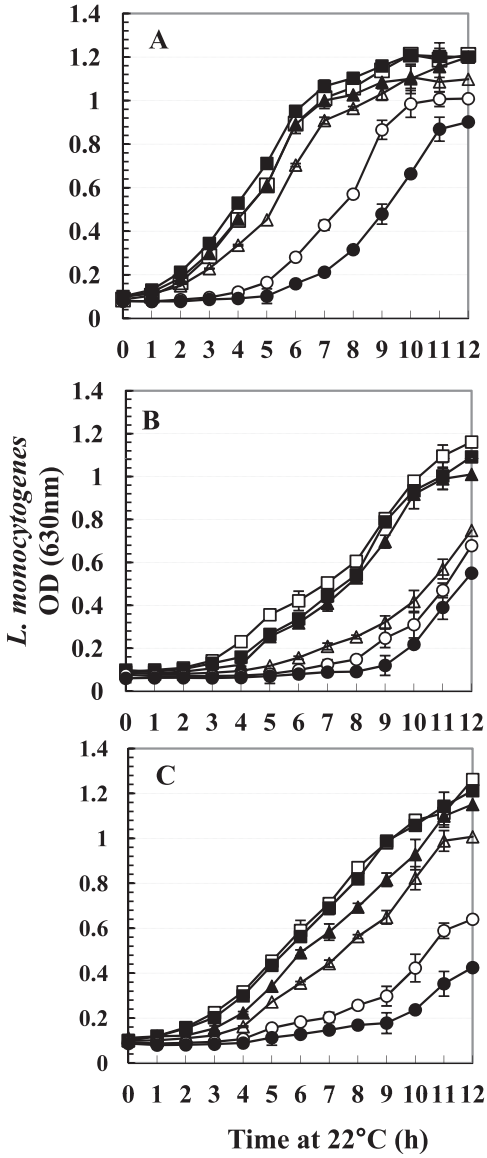

FIGURE 2. *Effect of sublethal heating at 48°C for 0 (□), 5 (■), 15 (▲), 30 (△), 60 (○), and 90 (●) min on lag phase at room temperature for three* L. monocytogenes *strains: (A) BUG 600 (serotype 1/2a), (B) NRRL B-33157 (serotype 4b), and (C) F4260 (serotype 1/2b).*

for 60 and 90 min extended the lag phase, so that cells did not initiate growth until 5 h.

Strain NRRL B-33157 has an inherent longer lag phase, and control cells did not initiate growth until 3 h (Fig. 2B). Heating at 48°C for 5 and 15 min led to a lag phase of 4 h, after which growth resumed. Cells heated at 48°C for 30, 60, and 90 min had a lag phase of 6 to 7 h, after which they grew slowly. NRRL B-33157 was the most slowly growing of the three representative strains, which was reflected in its inherent longer lag phase and subsequent lower growth rate during the lag phase.

The F4260 control cells and those stressed at 48°C for 5 min had similar behavior; growth resumed after 2 h. When the cells were stressed at 48°C for 15 and 30 min, the lag phase was extended to 3 h. Stress at 48°C for 60 and 90 min resulted in the longest lag phase of approximately 5 h (Fig. 2C).

Stability of heat stress adaptation after cooling step. Differences were noted in the retention of the heat stress adaptation response at room temperature (22°C) (Fig. 3) and 4°C (Fig. 4). At 22°C, a gradual decrease in the heat tolerance of *L. monocytogenes* cells was observed after removal of the sublethal heat stress. In contrast, heat stress–adapted cells when cooled to 4°C maintained heat stress resistance for up to 24 h.

The heat-adapted BUG 600 cells that were immediately inactivated at 60°C survived at approximately 6 log CFU/ml. With increased cooling time at room temperature up to 2 h before lethal heat inactivation, the survival of heat-adapted cells was reduced to 4 log CFU/ml (Fig. 3A). Heat stress adaptation in the BUG 600 cells was not completely lost after 2 h of cooling at room temperature; heat-adapted cells levels were about 2 log CFU/ml higher than those of nonadapted control cells. The heat stress adaptation of the medium heat tolerant strain NRRL B-33157 was relatively less stable at room temperature. Heat stress adapted cell survival was 5.3 log CFU/ml when there was no time lag between sublethal and lethal heating, but survival was reduced to 4.2 and 2.8 log CFU/ml after 1 and 2 h of cooling at room temperature, respectively (Fig. 3B). The decrease in the heat stress adaptation effect in high heat tolerant strain F4260 was similar to that of strain NRRL B-33157. At the end of 2 h of cooling at room temperature, the heat stress adaptation was completely lost, and survival was reduced from 5.2 to 2.8 log CFU/ml, similar to that of the nonadapted control cells (Fig. 3C).

During cooling at 4°C, the heat stress adaptation response was stable. For all three *L. monocytogenes* strains, no decline in heat tolerance for the heat stress adapted cells was observed up to 24 h during refrigeration storage. Recoveries of heat stress adapted cells were similar at 2, 6, and 24 h for all three strains. Differences between control and heat stress–adapted cells were approximately 2 log CFU/ml for the low heat tolerant BUG 600 strain (Fig. 4A), approximately 4 log CFU/ml for the medium tolerant NRRL B-33157 strain (Fig. 4B), and 1.0 to 1.5 log CFU/ml for the high heat tolerant F4260 strain at the 2-, 6-, and 24-h sampling times (Fig. 4C). For strains BUG 600 and F4260, the control cells without sublethal heat treatment had increased heat tolerance after cooling at 4°C. For example, the survival of BUG 600 control cells was 2 to 3 log CFU/ml higher after 2 and 6 h at 4°C than after of 0 h at 4°C (Fig. 4A).

DISCUSSION

In this study, we evaluated the heat tolerance of 37 *L. monocytogenes* strains representing all 13 known serotypes and identified a high diversity of heat tolerance in this pathogen within serotypes. Thirty-seven *L. monocytogenes* strains were categorized into three groups (low, medium, and high heat tolerance) based on their inherent heat tolerance at 60°C. Nearly 90% of the serotype 1/2a strains had relatively low heat tolerance (Table 1), whereas only 50% of the serotype 4b strains were in this subgroup. Serotype 4b contained both low and medium heat tolerant

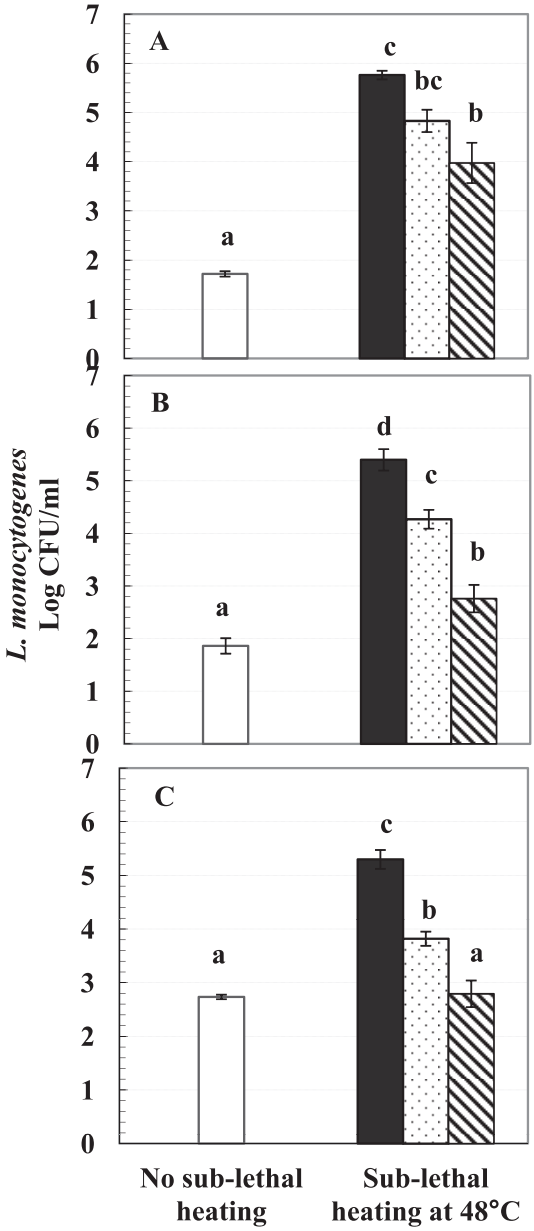

FIGURE 3. *Effect of sublethal heating at 48°C for 60 min followed by cooling at room temperature for 0 (■), 1 (⁘), and 2 (▨) h on survival at 60°C for three* L. monocytogenes *strains: (A) BUG 600 (serotype 1/2a), (B) NRRL B-33157 (serotype 4b), and (C) F4260 (serotype 1/2b). Means shown by bars with different letters are significantly different based on Tukey's analysis of variance (*$P < 0.05$*).*

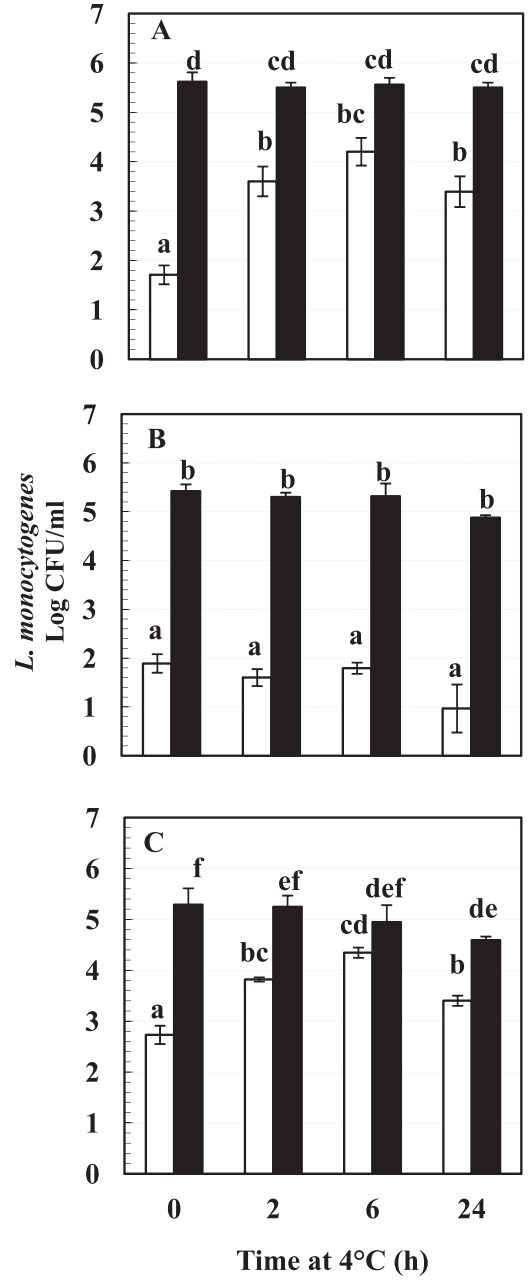

FIGURE 4. *Effect of sublethal heating at 48°C for 60 min followed by cooling at 4°C for 0, 2, 6, and 24 h on survival at 60°C for three* L. monocytogenes *strains: (A) BUG 600 (serotype 1/2a), (B) NRRL B-33157 (serotype 4b), and (C) F4260 (serotype 1/2b). Bars show values for samples that were not preheated (□) and samples that were preheated at 48°C (■). Means shown by bars with different letters are significantly different based on Tukey's analysis of variance (*$P < 0.05$*).*

strains, whereas serotype 1/2b strains had either low or high heat tolerance. In a previous study of 25 *L. monocytogenes* strains, serotype 4b strains had lower heat tolerance at 55°C than did strains of other serotypes *(22)*. In another study, no differences in inherent heat tolerance were found between serotype1/2a and 4b isolates, but serotype 4b isolates were more heat resistant than were serotype 1/2a isolates after cold storage at 4°C *(3)*. De Jesus and Whiting *(8)* and Sorqvist *(38)* also reported that no serotype-based heat tolerance pattern was observed among *L. monocytogenes* strains. Therefore, serotype may not be the sole factor that contributes to the differences in heat tolerance among *L. monocytogenes* strains. Other differences among protocols used in lethal heat challenge experiments (e.g., strain, growth medium, growth temperature, pH, and heating menstruum) could lead to the wide variation in heat tolerance found among *L. monocytogenes* strains *(9, 16)*.

In a majority of previous studies, heat stress adaptation was induced by exposing *L. monocytogenes* cells to sublethal temperatures for 1 to 2 h *(1, 4, 10, 37)*. However, this approach does not approximate actual food processing conditions, where during most heat treatments foods are

exposed to sublethal temperatures for less than 30 min. Therefore, any information on the sublethal heat exposure time that is most likely to induce heat stress adaptation would be valuable for the food processing industry. As little as 15 min was adequate to induce some heat stress adaptation in the *L. monocytogenes* strains in our study. This finding was consistent with the results obtained by Hassani et al. *(13)*. Molecular investigation revealed increased expression of class I and class III heat shock genes in *L. monocytogenes* cells exposed to 48°C for only 3 min *(41)*. In another study with *E. coli,* heat shock protein positive regulator σ^{32} was upregulated after 2 to 4 min of temperature increase from 30 to 42°C *(30)*. These results indicate that heat stress adaptation in *L. monocytogenes* may easily occur when a short-term sublethal heat stress is present during thermal processing. However, in the present study 30 to 60 min at 48°C was the condition that most enhanced heat tolerance in *L. monocytogenes* (Fig. 1). Similar patterns were observed for all strains regardless of their inherent low, medium, or high heat tolerance. Our finding differs slightly from that of Linton et al. *(24),* who found that 20 min of heating at 48°C was the optimal condition to induce heat stress adaptation in *L. monocytogenes*. This difference could be the result of strain variation or differences in the bacterial growth phase. In contrast, excessive (90 min) sublethal heat (48°C) exposure reduced heat stress adaptation in *L. monocytogenes*. In another study, excessively high heating temperatures or long heating times resulted in reduced heat stress adaptation in *L. monocytogenes (4)*. Such prolonged exposure to sublethal heat stress may provoke cell injury, which subsequently may negatively affect the heat stress adaptive mechanism. In contrast, Pagan et al. *(31)* reported that prolonged exposure (up to 9 h) to lower sublethal temperatures of 40 to 46°C did not impair heat stress adaptation of *L. monocytogenes*. However, 40 to 46°C may not be high enough to cause cell injury during sublethal heating.

Listeriosis outbreaks can occur when a small number of *L. monocytogenes* cells multiply to reach approximately 1,000 CFU in contaminated ready-to-eat food products. Hence, the ability of this pathogen to grow in ready-to-eat food products is closely associated with its infectious dose and virulence potential. Heat stress–induced cell injury impairs the ability of the cell to grow, which may increase the resuscitation time. Therefore, we investigated the lag phase of *L. monocytogenes* after different periods of sublethal heat exposure at 48°C. For all three representative strains, 60 or 90 min of sublethal heating at 48°C significantly delayed cell growth in TSBYE at room temperature. This finding suggests that 60 to 90 min of heating at 48°C may cause enough cell injury to prolong the lag phase of these strains. Cell injury triggered by heat treatment has been well documented in previous studies, and recovery of heat stressed *L. monocytogenes* cells has been enhanced by adding nutrients, reducing the agar concentrations, and using bilayer agar or cold preincubation *(18, 26, 38, 45)*. We found that 30 min at 48°C was sufficient to trigger heat stress adaptation in *L. monocytogenes* but had no adverse effect on the lag phase and thus did not impair the growth capability (Figs. 1 and 2). Therefore, potentially short periods (i.e., 30 min) of sublethal heating may not compromise *L. monocytogenes* cell integrity or growth potential and thus may pose a significant safety risk during food processing.

Heat stress adaptation in *L. monocytogenes* cells confers both greater resistance to lethal high temperatures and cross-resistance to commonly used antimicrobial agents or disinfectants *(23, 29)*. However, this enhanced resistance to lethal temperatures or against antimicrobial compounds was determined under conditions in which *L. monocytogenes* cells were exposed to sublethal heat and then immediately subjected to lethal thermal inactivation treatments. This situation may not be the typical risk scenario encountered during food processing. Hence, the stability of the acquired heat stress adaptation in *L. monocytogenes* must be determined before conducting a challenge with lethal inactivation steps, which is a critical point during food processing. In the stability assays, we observed that heat stress adaptation in *L. monocytogenes* was reversed within 2 h of cooling at room temperature (Fig. 3) but was highly stable for up to 24 h of cooling at 4°C (Fig. 4). The reversal of heat stress adaptation in *L. monocytogenes* could be attributed to a lower concentration of heat shock proteins after sublethal heat stress is removed. In *E. coli* O157:H7, the heat shock protein positive regulator σ^{32} was unstable in a steady state growth stage where sublethal heat stress was not present *(2)*. The high stability of *L. monocytogenes* heat stress adaptation after cooling at 4°C could be associated with the absence of active growth within 24 h at 4°C and thus stabilization of the activated heat shock proteins. This hypothesis must be tested by further transcriptional or proteomic analysis of heat-adapted *L. monocytogenes* cells held at 4°C.

In the *L. monocytogenes* BUG 600 and F4260 strains, control cells were more heat tolerant after exposure to 4°C (Fig. 4A and 4C). Other researchers reported that the expression of heat shock proteins (GroEL and DnaK) in *L. monocytogenes* was upregulated after exposure to 4°C for 11 days *(5)*. However, Miller et al. *(27)* found that cold shock at 0 to 15°C for 1 to 3 h decreased the heat resistance of *L. monocytogenes*. In another study, after cold storage at 4°C for 2 weeks 53% of 81 *L. monocytogenes* isolates did not have altered heat resistance, 41% had decreased heat resistance, and 6% had increased heat resistance *(3)*. Therefore, cold-induced heat resistance in *L. monocytogenes* is determined by multiple factors such as the cold storage time and temperature and the strain tested. Collectively, although storage at refrigeration temperatures usually delays the growth of *L. monocytogenes,* it may preserve an acquired heat stress adaptation. Under those circumstances, either a higher reheating temperature or longer reheating time may be needed to destroy heat stress adapted *L. monocytogenes* cells and mitigate any food safety risk after cold storage.

In conclusion, this study revealed extensive diversity in the heat tolerance response among strains of the 13 serotypes of *L. monocytogenes,* which were classified of low, medium, and high heat tolerant. After sublethal heat

stress adaptation, $D_{60°C}$-values of these three groups of heat tolerant strains increased by a maximum 2.5 times and the lag phase was prolonged by 3 to 7 h. This heat stress adaptation was reversed within 2 h at 22°C but was highly stable for up to 24 h at 4°C. Further studies are needed to determine the heat stress response of *L. monocytogenes* using a larger set of serotype 1/2a, 1/2b, and 4b strains under simulated thermal processing conditions or in real food substrates, which may have implications for the food safety risks associated with this pathogen.

ACKNOWLEDGMENT

This research was supported in part by Strategic Research Initiative and Food Safety Initiative awards to R. Nannapaneni from the Mississippi Agricultural and Forestry Experiment Station under project MIS-401160.

REFERENCES

1. Agoston, R., C. Mohacsi-Farkas, and S. D. Pillai. 2010. Exposure to sub-lethal temperatures induces enhanced heat resistance in *Listeria monocytogenes*. *Acta Aliment*. 39:327–336.
2. Arsene, F., T. Tomoyasu, and B. Bukau. 2000. The heat shock response of *Escherichia coli*. *Int. J. Food Microbiol.* 55:3–9.
3. Buncic, S., S. M. Avery, J. Rocourt, and M. Dimitrijevic. 2001. Can food-related environmental factors induce different behaviour in two key serovars, 4b and 1/2a, of *Listeria monocytogenes*? *Int. J. Food Microbiol.* 65:201–212.
4. Bunning, V. K., R. G. Crawford, J. T. Tierney, and J. T. Peeler. 1990. Thermotolerance of *Listeria monocytogenes* and *Salmonella typhimurium* after sublethal heat shock. *Appl. Environ. Microbiol.* 56:3216–3219.
5. Cacace, G., M. F. Mazzeo, A. Sorrentino, V. Spada, A. Malorni, and R. A. Siciliano. 2010. Proteomics for the elucidation of cold adaptation mechanisms in *Listeria monocytogenes*. *J. Proteomics* 73:2021–2030.
6. Carlier, V., J. C. Augustin, and J. Rozier. 1996. Heat resistance of *Listeria monocytogenes* (phagovar 2389/2425/3274/2671/47/108/340): *D*- and *z*-values in ham. *J. Food Prot.* 59:588–591.
7. Chavant, P., B. Martinie, T. Meylheuc, M.-N. Bellon-Fontaine, and M. Hebraud. 2002. *Listeria monocytogenes* LO28: surface physicochemical properties and ability to form biofilms at different temperatures and growth phases. *Appl. Environ. Microbiol.* 68:728–737.
8. De Jesus, A. J., and R. C. Whiting. 2003. Thermal inactivation, growth, and survival studies of *Listeria monocytogenes* strains belonging to three distinct genotypic lineages. *J. Food Prot.* 66:1611–1617.
9. Edelson-Mammel, S. G., R. C. Whiting, S. W. Joseph, and R. L. Buchanan. 2005. Effect of prior growth conditions on the thermal inactivation of 13 strains of *Listeria monocytogenes* in two heating menstrua. *J. Food Prot.* 68:168–172.
10. Farber, J. M., and B. E. Brown. 1990. Effect of prior heat shock on heat resistance of *Listeria monocytogenes* in meat. *Appl. Environ. Microbiol.* 56:1584–1587.
11. Fellows, P. J. 2000. Properties of foods and processing theory, p. 26–43. *In* P. J. Fellows (ed.), Food processing technology: principles and practice, vol. 1. CRC Press, Boca Raton, FL.
12. Ferreira, A., C. P. O'Byrne, and K. J. Boor. 2001. Role of sigma(B) in heat, ethanol, acid, and oxidative stress resistance and during carbon starvation in *Listeria monocytogenes*. *Appl. Environ. Microbiol.* 67:4454–4457.
13. Hassani, M., P. Manas, R. Pagan, and S. Condon. 2007. Effect of a previous heat shock on the thermal resistance of *Listeria monocytogenes* and *Pseudomonas aeruginosa* at different pHs. *Int. J. Food Microbiol.* 116:228–238.
14. Jorgensen, F., T. B. Hansen, and S. Knochel. 1999. Heat shock-induced thermotolerance in *Listeria monocytogenes* 13-249 is dependent on growth phase, pH and lactic acid. *Food Microbiol.* 16:185–194.
15. Jorgensen, F., B. Panaretou, P. J. Stephens, and S. Knochel. 1996. Effect of pre- and post-heat shock temperature on the persistence of thermotolerance and heat shock–induced proteins in *Listeria monocytogenes*. *J. Appl. Bacteriol.* 80:216–224.
16. Juneja, V. K., T. A. Foglia, and B. S. Marmer. 1998. Heat resistance and fatty acid composition of *Listeria monocytogenes*: effect of pH, acidulant, and growth temperature. *J. Food Prot.* 61:683–687.
17. Kallipolitis, B. H., and H. Ingmer. 2001. *Listeria monocytogenes* response regulators important for stress tolerance and pathogenesis. *FEMS Microbiol. Lett.* 204:111–115.
18. Kang, D.-H., and D. Y. Fung. 1999. Thin agar layer method for recovery of heat-injured *Listeria monocytogenes*. *J. Food Prot.* 62:1346–1349.
19. Kim, K., E. A. Murano, and D. G. Olson. 1994. Heating and storage conditions affect survival and recovery of *Listeria monocytogenes* in ground pork. *J. Food Sci.* 59:30–32.
20. Koutsoumanis, K. P., P. A. Kendall, and J. N. Sofos. 2003. Effect of food processing–related stresses on acid tolerance of *Listeria monocytogenes*. *Appl. Environ. Microbiol.* 69:7514–7516.
21. Krishnamurthy, K., H. K. Khurana, J. Soojin, J. Irudayaraj, and A. Demirci. 2008. Infrared heating in food processing: an overview. *Compr. Rev. Food Sci. F* 7:2–13.
22. 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.
23. Lin, C.-M., S. S. Moon, M. P. Doyle, and K. H. McWatters. 2002. Inactivation of *Escherichia coli* O157:H7, *Salmonella enterica* serotype Enteritidis, and *Listeria monocytogenes* on lettuce by hydrogen peroxide and lactic acid and by hydrogen peroxide with mild heat. *J. Food Prot.* 65:1215–1220.
24. Linton, R. H., J. R. Bishop, and M. D. Pierson. 1990. Increase in heat resistance of *Listeria monocytogenes* Scott A by sublethal heat shock. *J. Food Prot.* 53:924–927.
25. Lou, Y., and A. E. Yousef. 1997. Adaptation to sublethal environmental stresses protects *Listeria monocytogenes* against lethal preservation factors. *Appl. Environ. Microbiol.* 63:1252–1255.
26. Mackey, B. M., E. Boogard, C. M. Hayes, and J. Baranyi. 1994. Recovery of heat-injured *Listeria monocytogenes*. *Int. J. Food Microbiol.* 22:227–237.
27. Miller, A. J., D. O. Bayles, and B. S. Eblen. 2000. Cold shock induction of thermal sensitivity in *Listeria monocytogenes*. *Appl. Environ. Microbiol.* 66:4345–4350.
28. Moorhead, S. M., and G. A. Dykes. 2003. The role of the *sigB* gene in the general stress response of *Listeria monocytogenes* varies between a strain of serotype 1/2a and a strain of serotype 4c. *Curr. Microbiol.* 46:461–466.
29. Moorman, M. A., C. A. Thelemann, S. Zhou, J. J. Pestka, J. E. Linz, and E. T. Ryser. 2008. Altered hydrophobicity and membrane composition in stress-adapted *Listeria innocua*. *J. Food Prot.* 71:182–185.
30. Nagai, H., H. Yuzawa, and T. Yura. 1991. Interplay of two *cis*-acting mRNA regions in translational control of sigma 32 synthesis during the heat shock response of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 88:10515–10519.
31. Pagan, R., S. Condon, and F. J. Sala. 1997. Effects of several factors on the heat-shock–induced thermotolerance of *Listeria monocytogenes*. *Appl. Environ. Microbiol.* 63:3225–3232.
32. Ramaswamy, V., V. M. Cresence, J. S. Rejitha, M. U. Lekshmi, K. S. Dharsana, S. P. Prasad, and H. M. Vijila. 2007. *Listeria*—review of epidemiology and pathogenesis. *J. Microbiol. Immunol. Infect.* 40:4–13.
33. Saa, P., M. Cabo, and J. Rodriguez. 2009. Effects of mussel processing soils on the adherence of *Listeria monocytogenes* to polypropylene and stainless steel. *J. Food Prot.* 72:1885–1890.
34. Samelis, J., A. Lianou, A. Kakouri, C. Delbes, I. Rogelj, B. Bogovic-Matijasic, and M. C. Montel. 2009. Changes in the microbial composition of raw milk induced by thermization treatments applied

prior to traditional Greek hard cheese processing. *J. Food Prot.* 72: 783–790.
35. Sauders, B. D., J. Overdevest, E. Fortes, K. Windham, Y. Schukken, A. Lembo, and M. Wiedmann. 2012. Diversity of *Listeria* species in urban and natural environments. *Appl. Environ. Microbiol.* 78:4420–4433.
36. Siegman-Igra, Y., R. Levin, M. Weinberger, Y. Golan, D. Schwartz, Z. Samra, H. Konigsberger, A. Yinnon, G. Rahav, N. Keller, N. Bisharat, J. Karpuch, R. Finkelstein, M. Alkan, Z. Landau, J. Novikov, D. Hassin, C. Rudnicki, R. Kitzes, S. Ovadia, Z. Shimoni, R. Lang, and T. Shohat. 2002. *Listeria monocytogenes* infection in Israel and review of cases worldwide. *Emerg. Infect. Dis.* 8:305–310.
37. Skandamis, P. N., Y. Yoon, J. D. Stopforth, P. A. Kendall, and J. N. Sofos. 2008. Heat and acid tolerance of *Listeria monocytogenes* after exposure to single and multiple sublethal stresses. *Food Microbiol.* 25:294–303.
38. Sorqvist, S. 1994. Heat resistance of different serovars of *Listeria monocytogenes*. *J. Appl. Bacteriol.* 76:383–388.
39. Stephens, P., M. Cole, and M. Jones. 1994. Effect of heating rate on the thermal inactivation of *Listeria monocytogenes*. *J. Appl. Microbiol.* 77:702–708.
40. U.S. Food and Drug Administration. 2013. *Listeria monocytogenes* risk assessment. Available at: http://www.fda.gov/Food/FoodScienceResearch/RiskSafetyAssessment/ucm184052.htm. Accessed 29 December 2013.
41. van der Veen, S., T. Hain, J. A. Wouters, H. Hossain, W. M. de Vos, T. Abee, T. Chakraborty, and M. H. J. Wells-Bennik. 2007. The heat-shock response of *Listeria monocytogenes* comprises genes involved in heat shock, cell division, cell wall synthesis, and the SOS response. *Microbiology* 153:3593–3607.
42. Vasseur, C., L. Baverel, M. Hebraud, and J. Labadie. 1999. Effect of osmotic, alkaline, acid or thermal stresses on the growth and inhibition of *Listeria monocytogenes*. *J. Appl. Microbiol.* 86:469–476.
43. Walsh, D., J. J. Sheridan, G. Duffy, I. S. Blair, D. A. McDowell, and D. Harrington. 2001. Thermal resistance of wild-type and antibiotic-resistant *Listeria monocytogenes* in meat and potato substrates. *J. Appl. Microbiol.* 90:555–560.
44. Ward, T. J., T. Usgaard, and P. Evans. 2010. A targeted multilocus genotyping assay for lineage, serogroup, and epidemic clone typing of *Listeria monocytogenes*. *Appl. Environ. Microbiol.* 76:6680–6684.
45. Yan, Z., J. B. Gurtler, and J. L. Kornacki. 2006. A solid agar overlay method for recovery of heat-injured *Listeria monocytogenes*. *J. Food Prot.* 69:428–431.
46. Yousef, A. E., and P. D. Courtney. 2003. Basics of stress adaptation and implications in new-generation foods, p. 2–3. *In* A. E. Yousef and V. K. Juneja (ed.), Microbial stress adaptation and food safety, vol. 1. CRC Press, Boca Raton, FL.
47. Zhang, H., and A. K. Datta. 2001. Electromagnetics of microwave heating: magnitude and uniformity of energy absorption in an oven, p. 33–62. *In* A. K. Datta and R. C. Anantheswaran (ed.), Handbook of microwave technology for food application, vol. 1. CRC Press, Boca Raton, FL.