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Effect of Prior Heat Shock on Heat Resistance of *Listeria monocytogenes* in Meat

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The effect of prior heat shock on the thermal resistance of *Listeria monocytogenes* in meat was investigated. A sausage mix inoculated with approximately 10^7 *L. monocytogenes* per g was initially subjected to a heat shock temperature of 48°C before being heated at a final test temperature of 62 or 64°C. Although cells heat shocked at 48°C for 30 or 60 min did not show a significant increase in thermotolerance as compared with control cells (non-heat shocked), bacteria heat shocked for 120 min did, showing an average 2.4-fold increase in the $D_{64°C}$ value. Heat-shocked cells shifted to 4°C appeared to maintain their thermotolerance for at least 24 h after heat shock.

Microorganisms exposed to sublethal temperatures respond by synthesizing a set of proteins, the so-called heat shock proteins (1, 8). This heat shock response by the organism confers upon it, among other things, an increased resistance to a subsequent lethal heat treatment. This heat shock response appears to be universal and has been observed in bacteria such as *Escherichia coli* (13), *Bacillus* spp. (2, 17), *Legionella pneumophila* (6), and *Salmonella* spp. (9–11), as well as in eucaryotic cells such as yeasts (12), *Drosophila* (1), and mammalian tissue culture cells (1, 4, 8). Although heat shock proteins appear to be involved in protecting organisms from the toxic effects of heat and other stresses, their exact function is not fully understood (14, 18), and whether there is a direct cause-effect relationship between the synthesis of heat shock proteins and the induction of thermotolerance is not known (8, 14, 18, 19).

Regarding the applicability of the heat shock response in food microbiology, Mackey and Derrick (11) recently observed that mild heating (48°C, 30 min) of broth, liquid whole egg, or reconstituted dried milk contaminated with *Salmonella thompson* resulted in an induction of thermotolerance in these cells. In most instances, heat-shocked cells had to be heated about twice as long as control cells (non-heat shocked) to achieve the same degree of inactivation. These authors thought that the heat shock phenomenon could be important for survival of microorganisms in those foods which received only a minimal heat treatment or for bulk foods which were heated up slowly to a final internal temperature.

There have been only two previous reports on the heat shock response of *Listeria monocytogenes* and its effect on the heat resistance of the organism, and these were done with a broth culture and not a food menstruum (5; R. G. Crawford, J. T. Tierney, J. T. Peeler, and V. K. Bunning, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, Q112, p. 348). Thus, our study was conducted to determine whether heat shocking *L. monocytogenes* inoculated onto meat could induce thermotolerance in these cells.

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MATERIALS AND METHODS

Organisms. The 10 *L. monocytogenes* strains used in this study were isolated during routine analysis of meats in Health Protection Branch laboratories. Three serotype 4 and seven serotype 1 strains were used. Strains were identified as described previously (3).

Meat. A fermented sausage mix was obtained from a local supplier. It consisted of a pork and beef mixture (66% pork, 33% beef) with the addition of salt, white pepper, nitrite, dextrose, lactose, and corn syrup. It had been previously irradiated to give a total aerobic colony count of <100 CFU/g.

Inoculum. All 10 *L. monocytogenes* strains were grown up overnight at 30°C in 5 ml of tryptone soy broth (Oxoid Canada Ltd.) containing 0.6% yeast extract. The cells were spun down (2,000 × *g*, 30 min.) and suspended in 5 ml of 0.1% (wt/vol) peptone-water. A 1-ml portion from each of the 10 cultures was combined and used as inoculum for the meat.

Meat inoculation. Meat (500 to 1,000 g depending on the experiment) was inoculated with 5 to 10 ml of *L. monocytogenes* inoculum and then blended for approximately 5 min in an Oster blender (Oster Kitchen Center, Sunbeam Corp., Toronto, Ontario, Canada) to obtain a homogeneously inoculated meat mixture. The meat (20 g) was then packed into three-ply laminate flexible pouches (dimensions, 7.5 by 11.5 cm; Reynolds Metal Co., Montreal, Quebec) which were vacuum sealed (Swissvac; Knud Simonsen Industries Limited, Rexdale, Ontario) and left overnight at 4°C.

The following day the pouches were placed into wire racks, which were transferred into a Blue M constant-temperature bath (Blue M, Blue Island, Ill.). Water bath temperatures were monitored with a Kaye Digistrip 4c monitor/controller (Kaye Instruments, Bedford, Mass.) attached to copper-constantan thermocouples. The latter were calibrated with a platinum resistance temperature detector (model 373A RTD monitor; Kaye Instruments). In addition to pouches containing only meat, several bags also contained a thermocouple placed in the geometric center of the pouch for monitoring the temperature profile. Heat shock temperatures examined were 40, 44, 48, and 52°C, while the final process temperature used was either 62°C for 0, 2, 7.5, 15,

TABLE 1. Effect of length of heat shock on thermal resistance of *L. monocytogenes* in cured meat heated at 64°C

Expt	$D_{64°C}$ value (min) after given heat shock[a]			
	0 min (control)	30 min	60 min	120 min
1	3.40	3.85	4.24	11.52
2	3.82	4.73	5.63	6.35
3	2.66	3.93	4.28	6.20
Avg	3.3[b]	4.2[b,c]	4.7[b,c]	8.0[c]

[a] Cells were heat shocked at 48°C. All correlation values were in excess of 0.96.

[b,c] Values bearing a common superscript letter are not significantly different ($P > 0.05$). Data were analyzed by one-way analysis of variance (16).

25, and 35 min or 64°C for 0, 2, 4, 6, and 8 min, unless indicated otherwise.

Enumeration of *L. monocytogenes*. Pouches were opened, the contents were aseptically transferred to a stomacher bag containing 80 ml of tryptose phosphate broth (Difco Laboratories, Detroit, Mich.), and the bag was then stomached for 1 min. Suspensions (0.2 ml) were surface plated onto tryptose agar plates (Difco) which were incubated at 25 to 30°C for 7 days before counting. Typical *Listeria* colonies (bluish-gray, ground-glass appearance) were selected for confirmation by performing the hanging-drop motility test (22°C), the beta-hemolysis test on tryptic soy agar with 7% horse blood, and the catalase test.

***D*-value determinations.** *D* values were determined by plotting the $\log_{10}$ of the number of survivors against time at a specific temperature. The population found after the product samples had attained the required temperature were designated as the zero-time count. A best straight-line relation was developed by using regression analysis to derive a regression equation of the type $y = c + mx$. The slope of the best straight line is m and, when it is inverted and the sign is changed from $-$ to $+$, gives the *D* value in minutes for the specific temperature.

Statistical analysis. Results were evaluated with Student's two-tailed *t* test and one-way analysis of variance, using Tukey's HSD procedure (16). The level of significance was set at $P < 0.05$ for all comparisons.

RESULTS

Preliminary experiments, using heat shock temperatures of 40, 44, 48, and 52°C, demonstrated that 44 and 48°C were optimal for inducing the maximum heat tolerance of *L. monocytogenes*. In all subsequent experiments, 48°C was chosen as the heat shock temperature to allow comparison with previously published work on other bacteria (11). *D* values were not calculated for these preliminary experiments because of insufficient data points.

When meat was heat shocked at 48°C for 30 min before being heated at the final test temperatures, *D* values at 62 and 64°C averaged (triplicate experiments) 7.54 and 3.39 min, respectively, for control cells and 8.29 and 2.93 min, respectively, for heat-shocked cells (results not shown). When these data were analyzed by the *t* test (16), no significant differences (5% level) in *D* values were observed between heat-shocked and control cells. However, upon increasing the heat shock time from 30 to 120 min, a significant increase in the $D_{64°C}$ value was observed (Table 1).

In an additional series of experiments, after heat shocking *L. monocytogenes* at 48°C for 1 h, the inoculated meat was left for 24 h at 4°C before being tested at a final temperature of 64°C. Holding the meat at 4°C for this additional 24 h decreased the heat resistance of both the control (non-heat-shocked) and heat-shocked cells slightly (Table 2). However, the ratios of non-heat-shocked to heat-shocked cells recovered at each time point at 64°C remained approximately the same for both stored (24 h, 4°C) and unstored meat (Table 2). Thus, heat-shocked cells appeared to maintain their increased thermotolerance for at least 24 h (Table 2; Fig. 1).

TABLE 2. Effect of a 24-h interval between heat shock and final heating of cured meat at 64°C

Time (min)[a]	Heat shock[b]	$\log_{10}$ CFU of viable *Listeria* cells per g (non-heat-shocked/heat-shocked surviving cells, %)[c]	
		0 h	24 h after heat shock
0	+	6.1 (6.3)	5.7 (6.3)
	−	4.9	4.5
2	+	5.7 (4.0)	5.4 (2.5)
	−	4.3	3.8
4	+	5.5 (0.4)	4.8 (0.6)
	−	3.1	2.6
8	+	4.7 (0.2)	3.6 (0.5)
	−	1.9	1.3
Control (no 64°C heat treatment)	+	7.2	7.1

[a] Test temperature was 64°C, with time being recorded after an internal temperature of 64°C was reached.

[b] Indicates whether (+) or not (−) the cells were heat shocked at 48°C for 1 h.

[c] Initial *Listeria* count was 7.1 $\log_{10}$. Counts are the average of two experiments, each performed with duplicate samples.

DISCUSSION

Although the response of procaryotic and eucaryotic cells to heat shock has been studied extensively (1, 7, 8), there has been little work done on the impact or importance of the heat shock phenomenon in the area of food microbiology.

Mackey and Derrick (9–11) were among the first to investigate the survival of a potential foodborne pathogen (*S. typhimurium*) after a sublethal heat shock. They worked first with a model broth system (9) and later with food products such as liquid whole egg, reconstituted dried milk, and minced beef. In many instances, heat-shocked salmonellae had to be heated twice as long as non-heat-shocked cells to reach the same degree of inactivation (11). Knabel et al. (5) found an increased thermotolerance in *L. monocytogenes* cells which had been heat shocked in broth at 43°C for 5, 30, or 60 min as compared with non-heat-shocked cells. However, it could not be ascertained whether the differences were statistically significant. Interestingly, cells grown at 43°C demonstrated a greater heat resistance than those grown at 37°C and then heat shocked at 43°C (5).

In this study, we have shown that heat shocking *L. monocytogenes* present on meat can signficantly augment the heat resistance of these cells, providing the length of heat shock is between 1 and 2 h. In contrast, with a heat shock of 48°C for only 30 min, Mackey and Derrick (11) observed a greater than twofold increase in *D* values of *S. thompson*

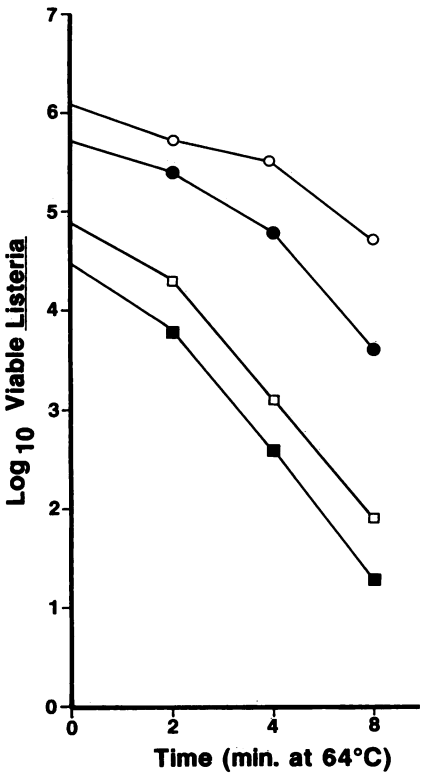

FIG. 1. Comparison of recovery of heat-shocked and non-heat-shocked cells on tryptose agar immediately after and 24 h after heat shocking at 48°C for 1 h. Symbols: ○, heat-shocked cells, 0 h/4°C; ●, heat-shocked cells, 24 h/4°C; □, non-heat-shocked cells, 0 h/4°C; ■, non-heat-shocked cells, 24 h/4°C.

heated in minced beef at either 54 or 60°C as compared with non-heat-shocked cells.

The optimum temperature for induction of thermotolerance in this study was between 44 and 48°C. Similarly, previous studies with mesophilic bacteria have demonstrated that a temperature range of 45 to 50°C was needed to give a maximum response (8). It is generally believed that organisms which can grow over a wide temperature range, such as *Listeria* spp., show a maximum response about 10 to 15°C above their optimum growth temperature (8). This also appears to be the case with *Listeria* spp. with a temperature optimum of 30 to 37°C (15).

The length of time that the elevated heat resistance in heat-shocked cells persists appears to be a function of many factors (8). Some of the variables known to influence the duration of the thermotolerance period include the temperature at which heat shocking is done, previous incubation temperature of the cell, and the metabolic state of the cell (8). Mackey and Derrick (9), working with *S. typhimurium* in tryptone soy broth, found that the augmented heat resistance of the heat-shocked cells (42, 45, or 48°C) remained at high levels for 10 h when the temperature was maintained and declined thereafter.

In our study, cells which had been heat shocked and then held at 4°C for 24 h appeared to lose some of their thermotolerance. For example, following a 24-h holding period, survival after exposure to 64°C for 0, 2, 4, and 8 min was 4.0, 2.2, 0.5, and 0.04%, respectively (Table 2). In comparison, survival of cells which were heat challenged immediately after heat shocking was 7.5, 3.1, 1.9, and 2.8%, respectively. Significantly, control (non-heat-shocked) cells also became more heat sensitive ($P < 0.05$) following a holding period at 4°C for 24 h (cf. Table 2, columns 3 and 4). This effect has also been observed with *L. monocytogenes* in raw milk (J. M. Farber, unpublished data). Thus, it appears that non-heat-shocked cells preconditioned at low storage temperatures become more sensitive to the lethal effects of heat. When one considers the latter effect, there is little or no loss of thermotolerance of the heat-shocked cells during the 24-h holding period. This conclusion is demonstrated by comparing the percent survival of non-heat-shocked and heat-shocked cells at each heating time (0, 2, 4, and 8 min; Table 2, columns 3 and 4).

The actual amount of time that cells were heat shocked appeared to influence the subsequent development of heat resistance in *L. monocytogenes*, with a 2-h heat shock period giving the greatest degree of protection to the cells. Heat shock times of >2 h were not examined. Our study shows a significant linear trend ($r = 0.81$), with thermotolerance increasing with increasing heat shock time. Knabel et al. (5) also found that increasing the length of heat shock increased the thermotolerance of *L. monocytogenes* cells, with 30- and 60-min heat shocks at 43°C giving more protection than a 5-min heat shock. The 30- and 60-min heat shocks appeared to recover equal numbers of cells. Heat shock times of >60 min were not examined (5).

It has been shown previously that a good correlation exists between the kinetics of heat shock protein synthesis and the development of thermotolerance in both eucaryotic and procaryotic cells (7, 8). Circumstantial evidence suggests that heat shock proteins are involved in the acquisition, maintenance, and decay of thermotolerance, but one cannot say with certainty that a direct causal relationship exists (7, 8, 13, 18). In addition to the increase in thermotolerance with increasing length of heat shock, it appeared that there developed a larger difference in recovery between heat-shocked and non-heat-shocked cells as the length of heating time at the standard challenge temperature (64°C) increased (Table 2). The reasons for this are unclear.

The significance of these results as far as the meat industry is concerned could be twofold. First, meats that are heated up slowly to a final internal temperature may contain microbial cells with an augmented heat resistance due to the heat shock response. It appears that the slower the temperature increase in the meat, the larger the increase in heat resistance (10). A recent example of a situation in which meats would be heated up slowly is in Sous-Vide-type products. Second, meats left on warming trays before being given a final reheating could possibly acquire an enhanced thermotolerant microbial population. Additional work is needed to assess the full impact of the heat shock response on the survival of various microorganisms in different foods. Further testing of the heat shock phenomenon and its relevance to dairy products is under way.

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