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Effects of Several Factors on the Heat-Shock-Induced Thermotolerance of *Listeria monocytogenes*

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The influence of the temperature at which *Listeria monocytogenes* had been grown (4 or 37°C) on the response to heat shocks of different durations at different temperatures was investigated. For cells grown at 4°C, the effect of storage, prior to and after heat shock, on the induced thermotolerance was also studied. Death kinetics of heat-shocked cells is also discussed. For *L. monocytogenes* grown at 37°C, the greatest response to heat shock was a fourfold increase in thermotolerance. For *L. monocytogenes* grown at 4°C, the greatest response to heat shock was a sevenfold increase in thermotolerance. The only survival curves of cells to have shoulders were those for cells that had been heat shocked. A 3% concentration of sodium chloride added to the recovery medium made these shoulders disappear and decreased decimal reduction times. The percentage of cells for which thermotolerance increased after a heat shock was smaller the milder the heat shock and the longer the prior storage.

Microbial thermotolerance varies very widely among different species and is influenced by a variety of factors (17, 44). Bacterial thermotolerance can also increase after exposure to different environmental stress conditions including heating at sublethal temperatures; viral infections; presence in the medium of chemical compounds such as ethanol, methylating agents, antibiotics (e.g., nalixidic acid), and amino acid restrictors (30); and acidic shock (10).

Any temperature above the optimum growth temperature is supposed to have some lethal effect. However, it has been shown that in most microbial species slow heating or heating for short periods of time at temperatures above the optimum temperature for growth induces higher thermotolerances (25–27). It is now believed that these temperatures trigger a physiological response (36) that leads to the synthesis of special proteins known as heat shock proteins (HSP) (21). Although HSP seem to play a role in the protection of microorganisms from the effects of heat and other stresses, the exact mechanism of action is not fully understood, and it is still not clear whether there is a direct cause-effect relationship between the synthesis of these proteins and the induction of a higher thermotolerance (21, 32, 36).

In food products requiring long heating lag phases, such as egg products, or those pasteurized at low temperatures for very long periods of time to retain flavor and texture, such as sous vide (processed refrigerated) foods (23), bacterial pathogens might respond to heat shock and increase thermotolerance. As a consequence, the risk of some of these microorganisms surviving heat treatment would increase (2, 23).

Most investigations of heat shock have been carried out with *Salmonella* spp. and *Escherichia coli*, but the importance of heat shock for public health could become even greater if the phenomenon occurred in other microorganisms like *Yersinia enterocolitica* and/or *Listeria monocytogenes*. As these species are psychrotrophic, any cell surviving heat treatment could grow even faster in foods during refrigerated storage than the saprophytic flora (16). *L. monocytogenes* has been found to contaminate different foods (7, 11) and has even been isolated from different pasteurized products (7, 11, 14, 15). Its thermotolerance is one of the highest among nonsporeformers (8, 14, 28). This quality and the ability to grow in a wide range of pHs and temperatures (even those of refrigeration [7, 11, 12]) make this microorganism one of the most dangerous for public health.

Despite the work carried out by many authors on the relationship between heat shock and the increase in thermotolerance and the production of HSP by *L. monocytogenes*, some aspects are still not clear. Whereas most authors have reported an increase in thermotolerance as a result of heat shock (9, 13, 18, 20, 22, 23, 34, 37, 43), Bunning et al. (2), after a detailed study on the effect of heat shocks of different durations at four different temperatures, were unable to detect any increase in thermotolerance and suggested that the effect reported by some authors (13) was most probably due to methodological problems.

The maximum thermotolerance that *L. monocytogenes* can attain as a response to heat shock is still unknown. It is also unknown whether the induced thermotolerance depends only on heat supplied by heat shock or on temperature or on both. The results of Linton et al. (23), from the most detailed study in the literature on the influence of temperature and duration of heat shock, showed that maximum thermotolerance was attained after a 20-min heating at 48°C. However, longer treatments and higher temperatures were not investigated.

There is also little knowledge about how the temperature at which cells were grown influences induced thermotolerance. There seems to be some disagreement between the only two reports in the literature on the effect of heat shock on *L. monocytogenes* grown at refrigeration temperatures. Jorgensen et al. (18) reported that, after the same heat shock, the thermotolerance of *L. monocytogenes* grown at 4°C was higher than that grown at 30°C. However, heat-shocked *L. monocytogenes* grown by Smith and Marmer (37) at 10°C did not even attain the thermotolerance of non-heat-shocked cells grown at 37°C. Results from Jorgensen et al. (18) are interesting as they sug-

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gest that the magnitude of the effect of heat shock treatments is highly dependent on the growth temperature of the cells.

In many investigations of the effect of heat shock on microorganisms, thermotolerance has been measured by the end point method. This method, unlike the survival curves method, does not provide information on thermal death kinetics that can indicate the mechanisms by which heat shock influences thermotolerance.

This investigation was carried out to determine the optimum combination of duration and temperature of heat shock at which *L. monocytogenes* grown at either 37 or 4°C attains maximum thermotolerance and also to collect data on the possible influence of heat shock in death kinetics.

MATERIALS AND METHODS

Bacterial culture and media. The strain of *L. monocytogenes* used was supplied by the Spanish Type Culture Collection (STCC 4031) and was maintained, during this investigation, on slants of tryptic soy agar with 0.6% yeast extract (TSAYE) (Biolife, Milan, Italy).

A broth subculture was prepared by inoculating, with one single colony, 5 ml of tryptic soy broth with 0.6% yeast extract (TSBYE) (Biolife) and incubating for 24 h at 37°C. Erlenmeyer flasks (250 ml) with 50 ml of sterile TSBYE were inoculated with this subculture to a final concentration of 10^6 cells/ml, and the cells were incubated at 37 or 4°C under agitation (130 revolutions/min) (Selecta; mod. Rotabit; Spain). Flasks were removed from incubation after 18 h (37°C incubation temperature) or 14 days (4°C incubation temperature). After this time the cultures had attained stationary growth phase and maximum thermotolerance. These fresh cultures were used in all experiments except where indicated. In these cases fresh cultures were stored for up to 14 days at 4°C without agitation. During this investigation no variation was observed in the thermotolerance of suspensions of non-heat-shocked cells during storage.

Heat shock treatments. Heat shock treatments were carried out in a test tube containing 5 ml of TSBYE preheated by immersion in a thermostated bath (mod. Digiterm; Selecta). Once the contents of this tube had stabilized at the selected heat shock temperature, the medium was inoculated with 0.1 ml of the cell suspension. After inoculation, one 0.2-ml aliquot was extracted at preset intervals and injected into a TR-SC thermoresistometer to measure thermotolerance. In experiments to determine the influence of storage at 4°C on the capacity of heat-shocked cells to maintain induced thermotolerance, two 0.2-ml aliquots, instead of one, were taken out during heat shock. In one of the aliquots the thermotolerance was measured immediately, and in the other it was measured after 24-h storage at 4°C.

Heat treatments. Heat treatments were carried out in the TR-SC thermoresistometer as previously described (6). Once the temperature of the heat treatment medium (350 ml of TSBYE) had attained stability (± 0.05°C), it was inoculated with 0.2 ml of the suspension. At preset intervals, one 0.1-ml sample for each heating time was directly collected into a tube of melted sterile TSAYE medium and immediately plated.

Incubation of heated samples and survival counting. Recovery of survivors of heat treatments was carried out by incubation at 37°C for 48 h on TSAYE medium. Previous experiments showed that longer incubation times did not influence the number of survivors. In experiments carried out to determine the capacity of heat damage repair, 3% sodium chloride (Panreac, Barcelona, Spain) was added to the recovery medium. After incubation, CFU were counted with an Image Analyzer Automatic Counter (Protos; Analytical Measuring Systems, Cambridge, United Kingdom) fitted with a 70-mm objective to facilitate the count on plates with a high density of CFU (5).

Thermotolerance parameters. Decimal reduction times (D_t values, minutes of heating at a given temperature [t] for the number of survivors to drop 1 log cycle) were calculated, as is usual, from the slope of the straight portion of survival curves obtained by plotting the log of the number of survivors versus their corresponding heating times. Time for the first log cycle reduction (TFLCR) was the time needed for the first 1 log reduction in the number of survivors. z values (increases in the temperature [°C] of treatment for the D_t values to decrease by 1 log cycle) were calculated from the decimal reduction time curve obtained by plotting log D_t versus the corresponding heating temperature.

Correlation coefficients ($r \geq 0.98$) and 95% confidence intervals were calculated with the appropriate statistical package (Statview 512; BrainPower Inc., Calabasas, Calif.). The statistical significance ($P \leq 0.05$) of differences between the D_t and z values was tested as described by Steel and Torrie (42).

RESULTS AND DISCUSSION

Effect of the intensity of heat shock on the thermotolerance of *L. monocytogenes* grown at 37°C. Figure 1 illustrates the influence of heat shock on *L. monocytogenes* thermotolerance at 65°C. This figure shows the relationship between log D_{65} and the duration of heat shock at 40, 43, and 46°C. As seen in the figure, regardless of the heat shock temperature, the thermotolerance increased with the increase in the duration of heat shock up to 120 min. Longer treatments (up to 540 min) did not increase thermotolerance. No data have been reported on the increase of *L. monocytogenes* thermotolerance after heat shocks of this duration. Most of the data in the literature were obtained with heat shocks not exceeding 30 to 60 min (2, 20, 23). Our results agree with those obtained by Mackey and Derrick (25) with *Salmonella typhimurium*. They also observed that thermotolerance increased with heat shocks of 120 to 180 min and that further increases of shock duration up to 600 min did not increase thermotolerance.

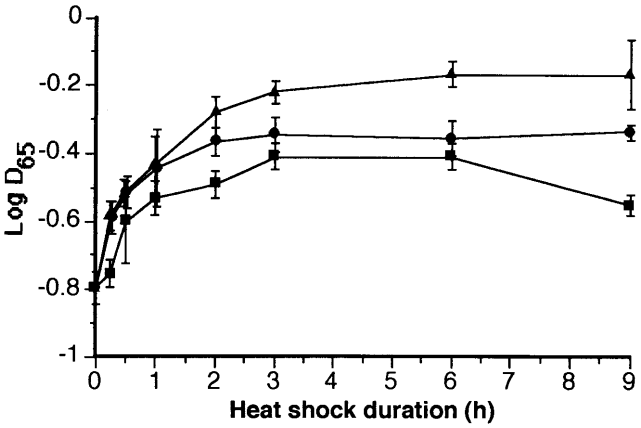

FIG. 1. Effects of temperature and duration of heat shock on thermotolerance at 65°C of *L. monocytogenes* grown at 37°C. The heat shock temperatures used were 40 (■), 43 (●), and 46°C (▲). Error bars indicate standard deviations.

According to our results the maximum thermotolerance induced by heat shock of *L. monocytogenes* cells depended on the temperature and the duration of the heat shock. However, it did not depend solely on the amount of heat supplied but also on the velocity of heat flow (temperature). If it depended only on the amount of heat supplied, the maximum thermotolerance attained after heat shocks at different temperatures would finally be the same, the only difference being in the duration of heat shock required.

It has been shown that *L. monocytogenes* can produce 12 to 14 different types of HSP (18, 41). If the increases in thermotolerance were due to HSP synthesized as a response to heat shock, as postulated by some authors (32, 46), the differences in thermotolerance could be due either to differences in the amount of HSP synthesized or to differences in the HSP synthesized at different temperatures.

The measurement of thermotolerance by the survival curves method provides detailed data on death kinetics that cannot be obtained by the end point method. Figure 2 shows survival curves at 65°C, after different heat shock treatments, of *L. monocytogenes* grown at 37°C. This figure illustrates the effect of the heat shock temperature on the profile of these curves. As seen in this figure, a higher heat shock temperature not only increased D_{65} values but also increased the duration of the shoulder. We also observed that the heat shock at 50°C inactivated approximately 94% of the cell population but that the remaining approximately 6% of cells were still capable of developing a higher thermotolerance (D_{65} of 0.36 min for heat-shocked cells versus D_{65} of 0.16 min for non-heat-shocked cells). This seemed to indicate that, during treatment, two phenomena were taking place simultaneously, one of inactiva-

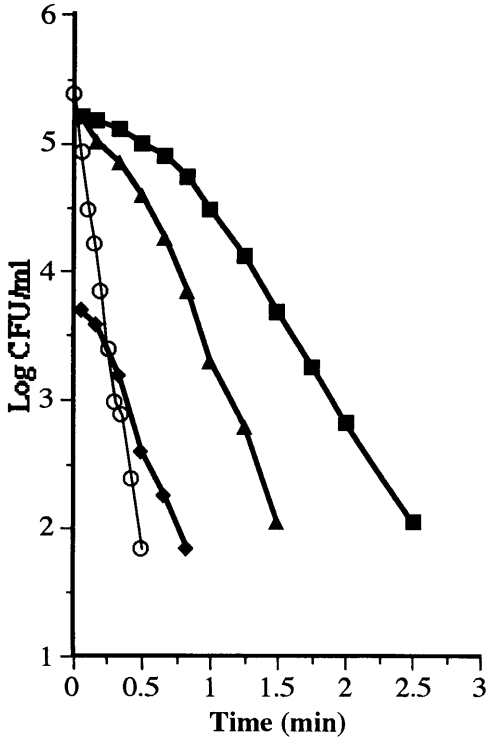

FIG. 2. Survival curves at 65°C, before (○) and after a 180-min heat shock at 40 (▲), 45 (■), or 50°C (◆), of *L. monocytogenes* grown at 37°C.

tion and the other of increase in thermotolerance. This increase in thermotolerance could perhaps explain the formation of tails in survival curves, as suggested by other authors (3, 29).

The relationship between heat shock temperature and thermotolerance at 65°C is shown in Fig. 3. As seen in this figure, thermotolerance increased with the heat shock temperature. However, conversely to that which occurred with the duration of heat shock (Fig. 1), after a maximum D_{65} was reached at 47.5°C (0.65 min) a further increase to 50°C caused a decrease in thermotolerance (D_{65} = 0.36 min). The heat shock treatment at 47.5°C for 180 min increased heat resistance over that of non-heat-shocked cells (D_{65} = 0.16 min) by approximately fourfold. This increase was bigger than those observed by other authors who reported increases in the range of zero- to three-

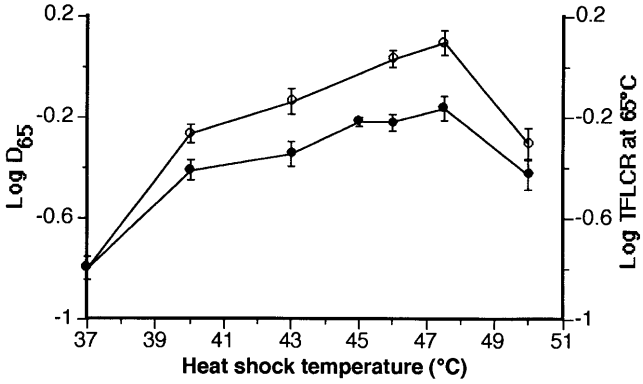

FIG. 3. Thermotolerance at 65°C after a 180-min heat shock at different temperatures of *L. monocytogenes* grown at 37°C. (●), log D_{65}; (○), log TFLCR. Error bars indicate standard deviations.

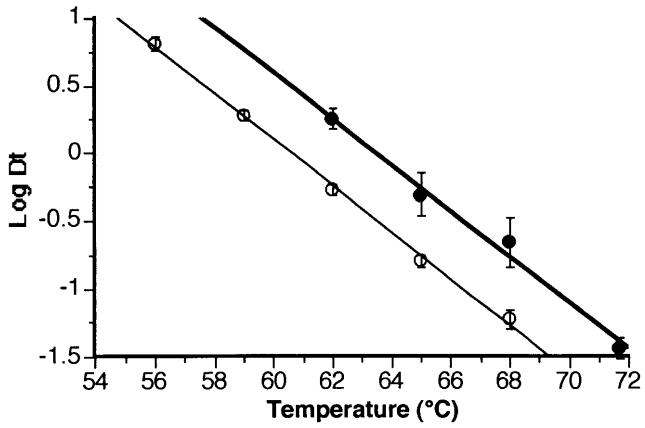

FIG. 4. Thermotolerance at different temperatures, before (○) and after (●) a 180-min heat shock at 42.5°C, of *L. monocytogenes* grown at 37°C. Error bars indicate standard deviations.

fold (13, 23, 34). This was probably because the heat shocks used by these authors were milder than those required for cells to develop maximum thermotolerance. Figure 3 also includes a plot in which thermotolerances were measured, not by the slope of the survival curve (D_t values), but by the TFLCR. The differences between the log D_{65} and the log TFLCR values corresponding to each heat shock temperature illustrates the duration of the shoulders of the curves. As observed, the duration of the shoulders increased with the increase in heat shock temperature.

The effect of a heat shock of 180 min at 42.5°C on the D_t values of *L. monocytogenes* at different treatment temperatures is illustrated in Fig. 4. This figure, which includes decimal reduction time curves of heat- and non-heat-shocked cells, shows that the increases in D_t values were the same over the range of temperatures tested. No statistically significant differences ($P \leq 0.05$) were found between the z values of the heat- and non-heat-shocked cells (z = 5.86). These results agree with the only data on *L. monocytogenes* (23) and *S. typhimurium* (25) reported in the literature.

Influence of growth temperature on the response of *L. monocytogenes* to heat shock. In Figure 5 the effect of heat shock at 42.5°C on the thermotolerance at 62°C of *L. monocytogenes* grown at either 4 or 37°C is shown. In Fig. 5A, thermotolerances are represented as log D_{62} values. In Fig. 5B, thermotolerances are represented as TFLCRs to show the effect of survival curve shoulders on the profile of this plot.

The significance of *L. monocytogenes* in relation to food safety is mainly due to its ability to grow in foods during refrigerated storage. The only data on the influence of the temperature at which *L. monocytogenes* was grown on its capacity to develop a higher thermotolerance are those from the work of Jorgensen et al. (18). According to these authors, after heat shock, cells grown at 4°C showed a thermotolerance that was higher than that of cells grown at 37°C. These results are surprising as different authors have demonstrated that in non-heat-shocked cells of different bacterial species, higher growth temperatures lead to higher thermotolerances (1, 31, 40, 45). As shown in Fig. 5A, we also observed that non-heat-shocked cells grown at 37°C (0 min of heat shock duration) were more thermotolerant than those grown at 4°C (D_{62} = 0.53 and 0.23, respectively). However, there was no detectable difference ($P \leq 0.05$) after a heat shock of 180 min at 42.5°C between the D_{62} values for cells grown at either 4 or 37°C. As also seen in

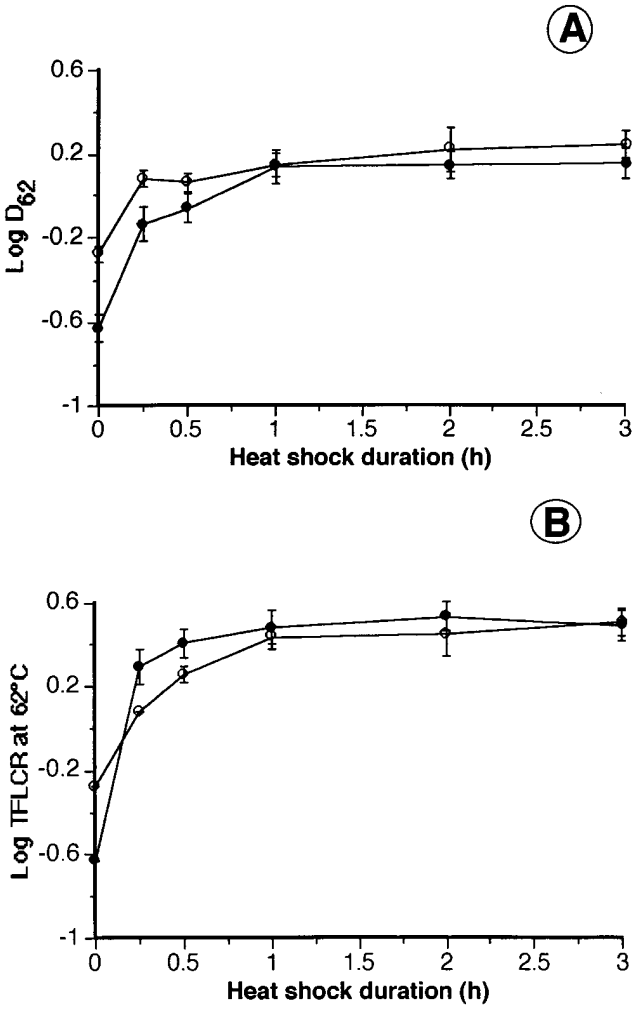

FIG. 5. Effects of heat shocks (42.5°C) of different durations on the thermotolerance at 62°C of *L. monocytogenes* grown at 4 (●) and 37°C (○). (A) Log D_{62}; (B) log TFLCR. Error bars indicate standard deviations.

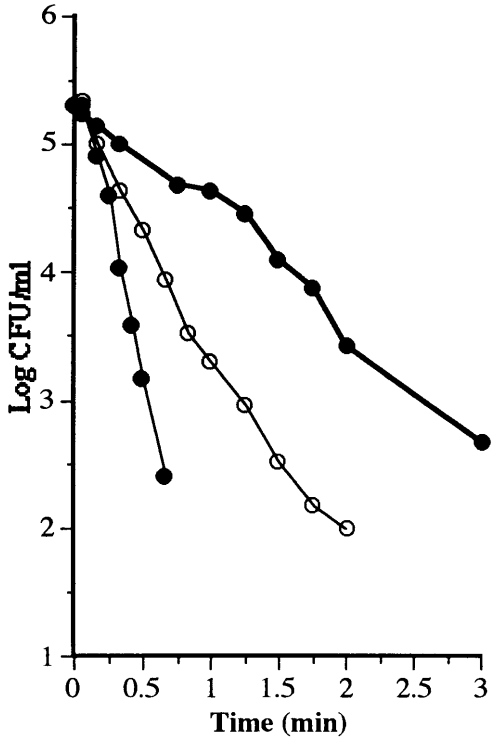

FIG. 6. Comparison of the thermotolerance, before (●) and after (●) a 180-min heat shock at 37°C, of *L. monocytogenes* grown at 4°C with that of non-heat-shocked cells grown at 37°C (○).

Fig. 5A, the increase in D_{65} values in response to heat shock was greater in cells grown at 4°C (sixfold increase) than in cells grown at 37°C (threefold increase). When thermotolerances were represented as TFLCRs (Fig. 5B), the thermotolerances after short-duration heat shocks (up to 60 min) of cells grown at 4°C were greater than those of cells grown at 37°C. This would explain why Jorgensen et al. (18) found that the thermotolerance after heat shock treatment of cells grown at 4°C was higher than that for cells grown at 37°C. The heat shock treatment given by these authors was rather short (30 min), and thermotolerances were measured not by D_t values (as in Fig. 5A) but by the end point method, which includes shoulders (as in Fig. 5B). The higher thermotolerance of non-heat-shocked cells grown at 37°C (Fig. 5) did not appear to be due to a possible synthesis of HSP during growth at 37°C because, as shown by Fig. 6, the thermotolerance of cells grown at 4°C (D_{62} = 0.23) and heat shocked for 180 min at 37°C (D_{62} = 1.1) was greater ($P \leq 0.05$) than that of cells grown at 37°C (D_{62} = 0.55). As no growth was observed during heat shock at 37°C of cells grown at 4°C, the differences between non-heat-shocked cells grown at 4 or 37°C could be due to other reasons such as differences in membrane composition, as suggested for other bacterial species by some authors (1, 19, 33).

In Fig. 7, the influence of 180-min heat shocks at different temperatures on the thermotolerance (D_{62}) of *L. monocytogenes* grown at 4°C has been plotted versus the corresponding heat shock temperatures. A plot has also been included representing thermotolerance as log TFLCR to illustrate the influence of survival curves' shoulders. As also occurred with cells grown at 37°C (Fig. 3), this figure shows that, within the range of temperatures studied, thermotolerance increased as heat shock temperature was increased, and that, after a maximum D_{62} was attained, a further increase in temperature made thermotolerance decrease. This figure also shows that the maximum thermotolerance was obtained at 45°C (an ap-

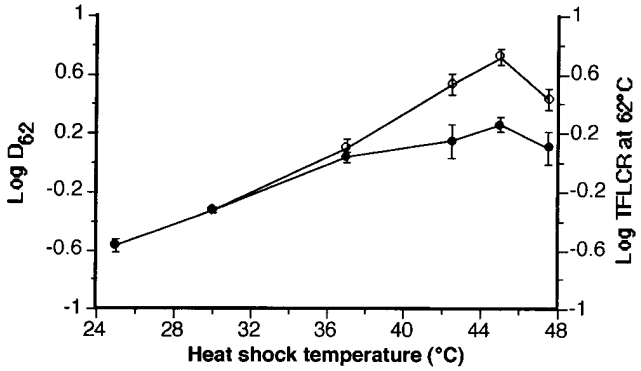

FIG. 7. Thermotolerance at 62°C, after a 180-min heat shock at different temperatures, of *L. monocytogenes* grown at 4°C. (●), log D_{62}; (○), log TFLCR. Error bars indicate standard deviations.

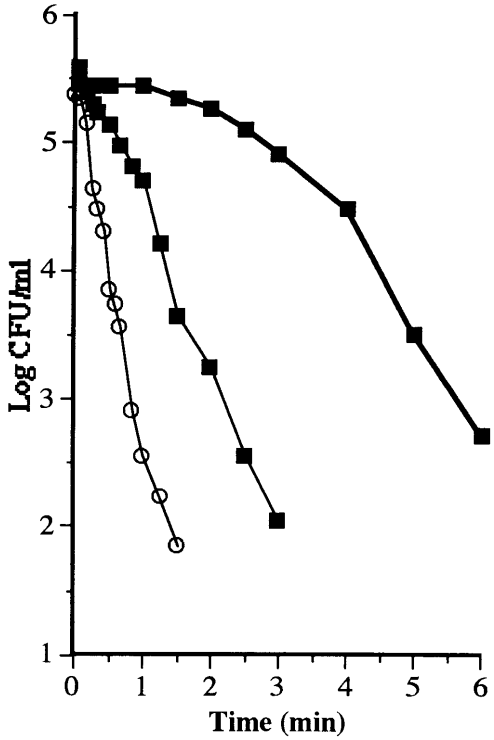

FIG. 8. Influence of storage at 4°C on the thermotolerance of *L. monocytogenes* grown at 4°C and heat shocked for 180 min at 42.5°C. (○), non-heat-shocked cells; (—■—), heat-shocked cells; (—■—), heat-shocked cells after 24 h of refrigerated storage.

proximately sevenfold increase). At 47.5°C, when heat shock started to be lethal, the thermotolerance decreased. There were differences between cells grown at 4°C (Fig. 7) or at 37°C (Fig. 3). Whereas the maximum thermotolerance of cells grown at 37°C was obtained after a heat shock at 47.5°C, that of cells grown at 4°C was obtained at 45°C. The optimum heat shock temperature for cells grown at 4°C was most probably due to their lower thermotolerance. For these cells a heat shock treatment at 47.5°C was already lethal. As previously discussed for those cells grown at 37°C, although shock temperatures higher than the optimum temperature for growth inactivated a proportion of cells (Fig. 2), the remaining cells exhibited an increase in thermotolerance. However, this increase was smaller than that induced by sublethal heat shock treatments. These results showed that the optimum heat shock depended on the temperature at which the cells were grown.

It was observed that cells grown at 4°C responded to heat shock temperatures as low as 30°C. This is in disagreement with the belief that only temperatures above the optimum growth temperature are capable of increasing thermotolerance (36).

Effect of storage on the capacity of *L. monocytogenes* to maintain the increase in heat-shock-induced thermotolerance. Data concerning the effect of storage of heat-shocked cells on the thermotolerance developed by heat shock do not make it possible to draw a clear conclusion. Whereas the thermotolerance developed by *S. typhimurium* after heat shock was lost during storage in just 1 h (24), *L. monocytogenes* still maintained, after a 24-h storage at 6°C, the thermotolerance developed after a 2-h heat shock at 48°C (9). According to Jorgensen et al. (18) the duration of the effect of heat shock on thermotolerance depends on storage temperature. Figure 8 shows our results concerning the effect of a 24-h storage at 4°C on the thermotolerance at 62°C, developed after a heat shock of 180 min at 42.5°C, of *L. monocytogenes* grown at 4°C. As shown in this figure by the slope of the straight portion of the survival curve, the heat shock increased thermotolerance but also induced the formation of a big shoulder that did not appear in the survival curve obtained with non-heat-shocked cells. The storage of heat-shocked samples for 24 h at 4°C before heat treatment made this shoulder disappear but did not influence the D_t value ($P \leq 0.05$). This suggests that, although the development of a higher D_t value and of a shoulder in the profile of survival curves are both induced by heat shock, they respond to different mechanisms. The role of HSP in bacterial heat damage repair seems to be well established (21, 23, 32, 37). Perhaps the shoulder and also the increase in D_t are due to the capacity of cells to repair heat damage through a mechanism involving some HSP, as discussed below.

Inactivation kinetics of heat-shocked *L. monocytogenes*. Heat inactivation kinetics are still not clearly understood, and although many deviations from the first-order reaction model have been reported (3, 29) and different mathematical models have been proposed (4, 35), the logarithmic order of death is still the model most widely used. The survival curves method of measuring thermotolerance has the advantage, over the end point method, of providing detailed information on phenomena occurring during heat inactivation that otherwise would go undetected.

Survival curves obtained during this investigation with non-heat-shocked cells grown at either 4 or 37°C followed the logarithmic order of death pattern, and the correlation coefficients of these lines were always higher than 0.99. However, the survival curves of heat-shocked cells always had shoulders, and the duration of these shoulders depended on different parameters. Shoulders of survival curves have been postulated to be due to spore activation (35), repair of heat injury (5), cell disaggregation (17), or even methodological problems (3). We carried out several experiments to collect information that could help to explain the appearance of shoulders and perhaps also the mechanisms of heat shock.

Influence of sodium chloride added to recovery medium on the profile of survival curves of heat- and non-heat-shocked *L. monocytogenes*. Sodium chloride has been reported to inhibit heat damage repair in different bacterial species (5, 22, 38–40). An experiment was carried out to determine the influence of sodium chloride on the heat damage repair capacity of heat-shocked cells of *L. monocytogenes*.

A heat treatment at 62°C was carried out with non-heat-shocked cells and with cells that had been heat shocked for 180 min at 42.5°C. After heat treatment, heat- and non-heat-shocked cells were incubated, for the recovery of survivors, in TSAYE medium and in TSAYE medium with 3% sodium chloride added to impair heat damage repair.

Results of this experiment are shown in Fig. 9. As seen in this figure, in heat-shocked cells, as well as in non-heat-shocked cells, some degree of heat damage repair occurred in TSAYE medium. This can be deduced by the difference, obtained for a given heating time, between the number of survivors after recovery in TSAYE medium and the number of survivors after recovery in TSAYE medium with 3% sodium chloride added. This difference in the number of survivors in each medium was much greater when cells had previously been heat shocked. The inhibitory effect of sodium chloride caused D_{65} to decrease from 0.23 to 0.1 min in non-heat-shocked cells and from 1.40 to 0.57 min in heat-shocked cells. However, what was most noticeable was the appearance of a prominent shoulder, after a short initial drop in the number of survivors, in the

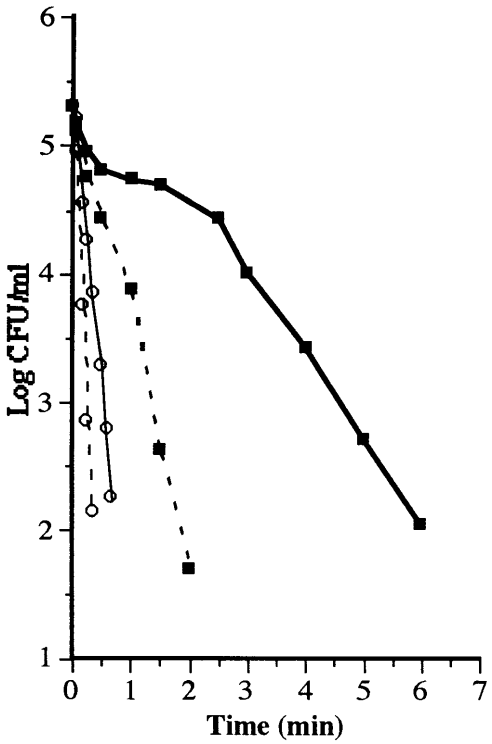

FIG. 9. Effects of sodium chloride on heat damage repair capacity after a heat treatment at 62°C of non-heat-shocked and heat-shocked *L. monocytogenes* grown at 4°C and stored at 4°C for 9 days. (○), non-heat-shocked cells; (■), heat-shocked cells (180 min at 42.5°C); (——), TSAYE recovery medium; (– – –), TSAYE with 3% sodium chloride added.

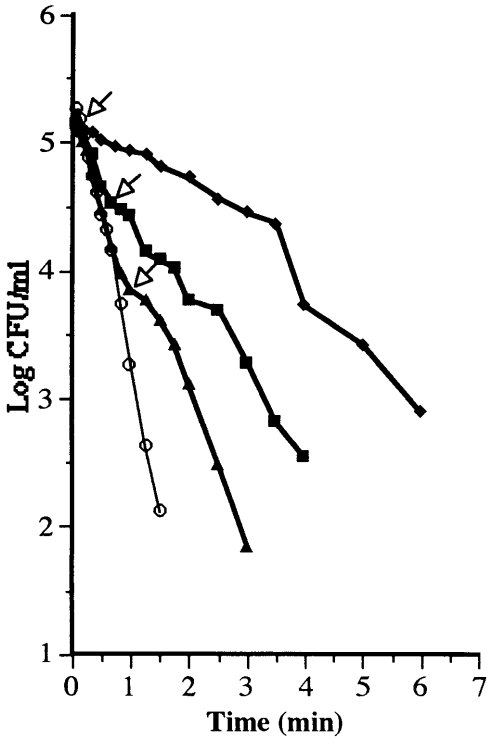

FIG. 10. Effects of the duration of heat shock at 42.5°C on the profile of survival curves at 62°C of *L. monocytogenes* grown at 4°C and stored at 4°C for 4 days. Non-heat-shocked cells (○) and cells heat shocked for 15 (▲), 30 (■), or 120 (◆) min are shown. Arrows indicate shoulders of survival curves.

survival curve of heat-shocked cells recovered in TSAYE medium. After this shoulder the survival curve followed the logarithmic order of death. The initial drop in the survival curve was also studied as described below. The shoulder did not appear when recovery was made in medium with 3% sodium chloride added. This was most probably due to the inhibiting effect of sodium chloride on heat damage repair mechanisms. These results would agree with the hypothesis that the shoulder is due to the repair capacity of HSP. During incubation for recovery of survivors, HSP would be capable of repairing small heat damages such as those inflicted during the heating period. But when these injuries were more severe these HSP would become incapable of repairing heat injury. Other HSP would be responsible for the increase in thermotolerance, as indicated by the increase in the slope (D_t value) of the straight portion of the survival curve compared with that of non-heat-shocked cells.

Influence of the duration of heat shock on the profile of survival curves of *L. monocytogenes* grown at 4°C and stored, prior to heat shock, at 4°C for 4 days. There is no data available to explain the occurrence of the initial straight portion, before the shoulder, in survival curves of heat-shocked cells. This was observed in those cells grown at 4°C which had been stored at this temperature (Fig. 9). This drop was possibly due to the proportion of cells unable to respond to heat shock and to synthesize HSP.

Experiments were performed to determine whether the duration of heat shock could be responsible for a higher proportion of cells responding. The results of these experiments are shown in Fig. 10. As seen in this figure, the shoulder of the survival curve of cells that had been heat shocked at 42.5°C for 120 min started from the very beginning of heat treatment. In contrast, the shoulder of survival curves of cells given milder heat shock treatments appeared after an initial straight portion that followed the slope of the survival curve of non-heat-shocked cells. This straight portion was shorter the longer the heat shock treatment. The survival curves had a straight final portion, after the shoulder. D_t of this final portion was always greater than that of non-heat-shocked cells. The straight portion before the shoulder could be due to the proportion of cells unable to respond to heat shock, which would be smaller the longer the duration of heat shock. If this were so, a 15-min heat shock at 42.5°C would only make 5% of the population respond, whereas a 30-min heat shock would make 18% of cells respond and a 120-min heat shock would make approximately 90% of the population respond. This would explain why the first portion of survival curves of heat-shocked cells matched the survival curve of non-heat-shocked cells and why the duration of the first straight portion decreased as the duration of heat shock was increased.

Influence of the duration of a previous storage at 4°C on the response of *L. monocytogenes* to heat shock. As the failure of cells to respond to heat shock was only observed in cells stored at 4°C (Fig. 9 and 10), several experiments were carried out to determine the possible influence of the duration of storage on the capacity of cells to respond to heat shock. The responses of fresh cell suspensions to heat shocks at 42.5°C of different durations (15, 30, 60, and 120 min) were measured as were the responses after 7 and 14 days of storage at 4°C.

Figure 11 shows survival curves corresponding to 15- and 120-min heat shocks. As seen in this figure, the proportion of cells responding to heat shock not only depended on the duration of the heat shock but also on the duration of the pre-

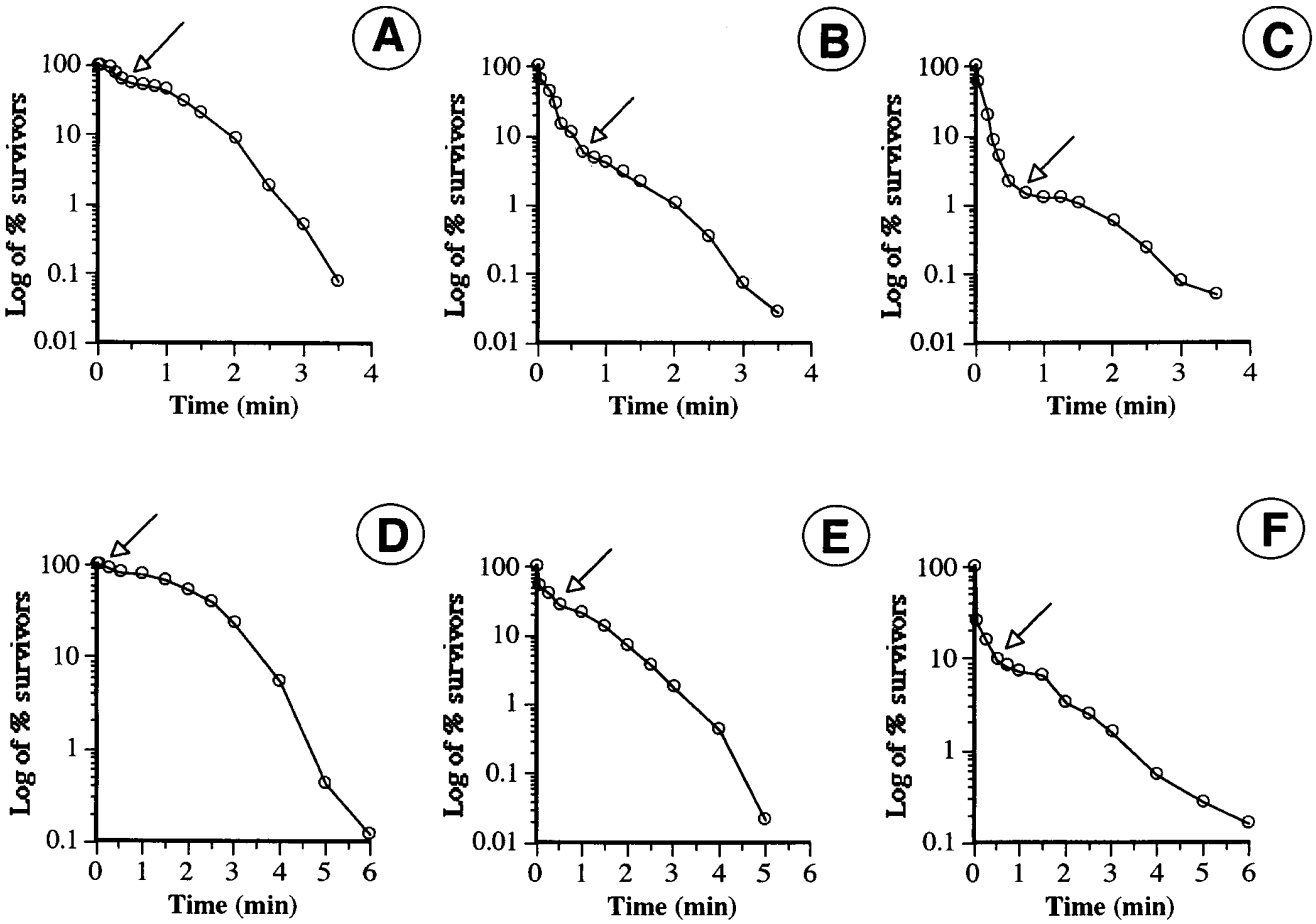

FIG. 11. Effects of storage duration at 4°C and duration of heat shock at 42.5°C on the profile of survival curves at 62°C of *L. monocytogenes* grown for 14 days at 4°C under agitation. The duration of storage was 0 (A and D), 7 (B and E), or 14 days (C and F). The duration of heat shock was 15 (A, B, and C) or 120 (D, E, and F) min at 42.5°C. Arrows indicate shoulders of survival curves.

vious storage. The longer the duration of heat shock and the shorter the duration of storage, the greater the proportion of cells responding to heat shock. Although the duration of the heat shock determined the proportion of cells responding, the influence of storage was also important. The proportion of cells able to respond decreased almost exponentially with time. After a 14-day storage only approximately 10% of cells given the longest heat shock (120 min) responded, and only 1.5% of those given the mildest heat shock (15 min) did so.

Our results have shown that the percentage of cells of *L. monocytogenes* responding to heat shock not only depended on the duration of heat shock but also on the previous storage of cells. The optimum heat shock temperature depended on the temperature at which cells were grown. The thermotolerance attained depended not only on the amount of heat but also on the temperature at which heat was supplied. Only the survival curves of heat-shocked cells had shoulders. Shoulders did not appear when recovery of survivors was carried out in medium with 3% sodium chloride added.

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