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**ABSTRACT**

The heat resistance of *Listeria monocytogenes* (1992 French outbreak strain) in ham and the effect of sublethal heat shock were studied. Experiments were carried out on bacterial cultures in three different physiological states: cultures at the end of the log phase, cultures heat shocked at 42°C for 1 h, and subcultures of cells resistant to prolonged heating. For these three cultures, we obtained D-values at 55 and 60°C of 17.8 and 1.82 min, 19.2 and 3.48 min, and 13.4 and 0.97 min, respectively. The corresponding z-values were 5.05°C, 6.74°C and 4.38°C, respectively. As reported in a previous study in artificial medium, an increased thermal tolerance could be induced by a sublethal heat shock. In this study, however, the increased thermal tolerance did not appear to be transmittable to subcultures.

The logarithmic form of the survivor curves obtained in this study was likely to be related to the use of a selective enumeration medium (Palcam) which was unfavorable to the recovery of the most heat-injured organisms. Consequently, the D-values obtained should be used with caution, since certain bacteria could under favorable storage conditions recover from damage sustained during heat treatment and regain their ability to multiply.

Key words: *L. monocytogenes*, heat resistance, ham

*Listeria monocytogenes* has been known to be a food-borne pathogen for the last 15 years. The organism has been implicated as the causative agent in several recent outbreaks of food-borne listeriosis worldwide, in particular in France in 1992. This outbreak of 279 cases was caused by a serovar 4b (phagovar 2389/2425/3274/2671/47/108/340) and implicated contaminated pork tongue in jelly as the major vehicle of the outbreak (6).

*L. monocytogenes* is an unusually thermotolerant gram-positive, non-spore-forming bacterium. Consequently, the 1992 French outbreak led to a reexamination of the effectiveness of heat treatments of processed foods. A study of the heat resistance of that particular strain of *L. monocytogenes* was needed.

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**MATERIALS AND METHODS**

**Bacterial strains and culture**

The strain of *Listeria monocytogenes* 4b phagovar 2389/2425/3274/2671/47/108/340 was supplied by Dr. J. Rocourt (Institut Pasteur, Paris, France) and maintained at 4°C on plate count agar (Difco Laboratories, Detroit, MI). Cultures were grown in tryptone soya (Oxoid, Unipath, Ltd., Basingstoke, Hampshire, England) plus 0.6% yeast extract (TSYE) (AES, Combourg, France) broth at 30°C for 24 h.

**Physiological states**

Experiments to characterize the acquired thermal tolerance of the cells in response to sublethal heat shock were carried out on...
bacterial cultures in the following three physiological states: (i) normal state: cultures grown in TSYE broth at 30°C for 24 h; (ii) heat shocked state: normal cultures heat shocked at 42°C for 1 h (1); (iii) resistant state: subcultures of cells from a normal culture which survived a heat treatment of 60°C for 20 min, subsequently grown in TSYE broth at 30°C for 24 h. The concentration of the cultures was approximately 10⁹ cells per ml.

Preparation of the samples

Raw ground brined ham was inoculated with 10 ml of vortexed culture per 100 g of meat and blended with a Stomacher (model 400; Seward Medical UAC, London, England). Portions (4.5 g) of the inoculated ground meat were placed in sterile glass 10 by 100 mm thermal death time (TDT) tubes (Seval, Paris, France).

The composition of the mixture of raw ground brined ham plus 10% (vol/wt) TSYE broth was as follows: water, 77.41%; lipids, 1.59%; proteins, 18.24%; total phosphorus in P₂O₅, 4.23 g/kg; collagen, 0.58%; total soluble carbohydrates, 0.31%; sodium nitrate, 0; potassium nitrate, 120 mg/kg; and sodium chloride 1.36%. Water content of the fat-free product was 78.66%, the average pH 5.5, and a₄ 0.98.

Heating and cooling the samples

For each study, the TDT tubes were sealed and first completely immersed in a Polystat constant-temperature water bath (Bioblock, Illkirch, France) for different lengths of time at 55 and 60°C. They were then immersed in cold water (12°C). Surviving bacteria from 2 tubes were enumerated at each time; two trials were conducted at each temperature.

The equations which describe the rise and fall of the temperature in ham were determined using a temperature recorder probe (Microlide S.A., St Léonard de Noblat, France) inserted into a TDT tube. The following equations were obtained:

\[ T = (T_s - T_h) \cdot e^{-kt} + T_h \text{ (heating);} \]
\[ T = (T_s - T_c) \cdot e^{-kt} + T_c \text{ (cooling, where zero time is taken to be the beginning of the cooling phase);} \]

where \( T \) is the temperature of the medium (°C); \( T_i \), initial temperature of the medium (°C); \( T_h \), heating temperature (°C); \( T_n \), maximum temperature attained by the medium (°C); \( t \), time (min); and \( k \) is 1.50 (min⁻¹).

Enumeration of surviving bacteria

After cooling, the contents of the TDT tubes were diluted 10-fold with buffered peptone diluent (AES) and blended with a Stomacher. The blends were serially diluted as required in Bacto-Tryptone (Difco) plus 0.85% sodium chloride (Prolabo; Paris, France). The appropriate dilutions were then plated on Palcam agar (Oxoid) either with a model DS spiral plater (Interscience, St Nom la Bretèche, France) or by surface plating 0.2 ml. Plates were incubated at 37°C for 48 to 72 h before enumeration. The log of noninjured survivors per gram was then plotted as a function of the duration of heating.

Identification of survivors

A morphological and biochemical confirmation of survivors of the prolonged heat treatments was carried out using the CAMP reaction (Christie, Atkins, and Munch-Pedersen) and carbohydrate (rhamnose and xylose) fermentation tests, followed by serotyping into serogroups 2 or 4, using sera prepared in our laboratory. In addition, the ham used in these studies was systematically screened to ensure that concentrations of Listeria spp. in the samples were lower than 25 cells/g before inoculation.

Modeling the survivor curves

Two models of the thermal inactivation kinetics were tested, based on the following hypotheses: that the bacterial population decreases logarithmically, and that the population decline is a sigmoidal function with a shoulder and a tail. These models are described below as follows:

(i) The linear model. This model is described by the following equation:

\[ \log(CFU) = \frac{t}{D} + \log(N_0), \]

where \( CFU \) is the number of survivors; \( D \), the decimal reduction time (min); \( N_0 \), the initial concentration of the population; and \( t \) is the heating time (min).

(ii) The "logit" model. This model assumes a logistic decay for the log of population as a function of the log of time:

\[ \% \log(CFU) = \frac{1}{1 + e^{(x-m)/s}}, \]

where \( m \) and \( s \) are the regression parameters of the model for heat resistance distribution within the population of L. monocytogenes, and \( x = \log(t) \), where \( t \) is the heating time.

Determining actual heating durations from the applied heating durations

The duration of the applied heat treatment was corrected for the lengths of the rising and falling phases of the temperature of the sample. The corrected duration was calculated to be the length of heat treatment at the given temperature which would have the same effect on the organisms as the actual heat treatment applied, assuming instantaneous heating and cooling of the sample.

In reference to the \( F \) concept, the following expression for the corrected duration of the heat treatment \( t_c \), was obtained:

\[ t_c = \int_{t_{45}}^{t_45} 10^{(T-T_0)/10} \, dt + \int_0^{t_{45}} 10^{(T-T_0)/10} \, dt', \]

where \( t \) is the time of the applied heat treatment; \( T \), temperature of the sample during heating at time \( t \); \( T_h \), heating temperature; \( T' \), temperature of the sample during cooling; \( t_{45} \), time needed for the sample to reach a temperature of 45°C during heating (heat injury begins at temperatures above 45°C (20)); \( t'_{45} \), time needed for the sample to cool to 45°C; and the z-value was taken to be 7°C, which is a typical value for L. monocytogenes (11).

Statistical analysis and comparison of the models

The parameters of the different models were determined by regression analysis using Statistical Analysis Systems (SAS Institute Inc., Cary, NC).
RESULTS

The fit of the different models to the general model (means of the experimental values obtained) was estimated by comparing the residual sum of squares (RSS). Combining the results of all of the studies, we obtained RSS values of 36.1 for the linear model, 62.5 for the "logit" model (and 10.3 for the general model). The model which best describes the thermal inactivation of \textit{L. monocytogenes} in this study is therefore the linear model (Figures 1 and 2).

Decimal reduction times at 55 and 60°C for each culture could be calculated (Table 1). For the untreated culture, average D-values were 17.8 and 1.82 min at 55 and 60°C, respectively. For the heat-shocked culture, average D-values were 19.2 and 3.48 min; for the resistant culture, they were 13.4 and 0.97 min. Corresponding z-values were 5.05°C for the untreated culture, 6.74°C for the heat shocked culture, and 4.38°C for the resistant culture. There is no significant \((P > 0.05)\) difference between the D-values of the three cultures at 55°C. On the other hand, at 60°C, the D-value of the heat-shocked culture is significantly \((P < 0.05)\) higher than the D-value of the untreated culture, which is significantly higher than the D-value of the resistant one.

DISCUSSION

The first-order inactivation kinetics obtained in this study were consistent with a homogeneous distribution of heat resistance within the bacterial population and cell death due to the inactivation of a single site per bacterium. This is in contrast with a previous study in TSYE broth, in which the authors found evidence for a log-normal distribution of heat resistance within this strain. This discrepancy could be due to the inactivation of a single site per bacterium. This is consistent with previously published results (4.6 to 7.4°C).

An increased thermal tolerance is induced by a sublethal heat shock, as is evidenced by higher decimal reduction times. However, the decimal reduction times of the resistant and untreated cultures were comparable in this study.

![Figure 1](http://meridian.allenpress.com/doi/pdf/10.4315/0362-028X-59.6.588)

**FIGURE 1.** Survivor curves of \textit{L. monocytogenes} at 55°C in ham.

![Figure 2](http://meridian.allenpress.com/doi/pdf/10.4315/0362-028X-59.6.588)

**FIGURE 2.** Survivor curves of \textit{L. monocytogenes} at 60°C in ham.

curves in TSYE broth can be explained if the bacteria subject to a prolonged heating had a better regenerative ability on a nonselective medium than on a selective medium. These bacteria would be unable to repair the heat damages sustained when placed in selective media containing inhibitors. In this case the thermal inactivation kinetics would be linear. In support of this hypothesis, we have also observed an increase in the number of colonies in those samples that were incubated for 1 day longer than usual, even for the samples subject to the most severe heat treatment. The log of survivors obtained from cultures on Palcam agar was 10% higher after an incubation of 72 h than it was after an incubation of 48 h. This was observed for survivor populations between 100 and 1,000 CFU/g. It seems then that the populations of \textit{L. monocytogenes} studied here have a heterogeneous distribution of heat injury, but this heterogeneity only becomes apparent when certain recovery methods are used.

D-values obtained for the three states are in the range of the values previously reported for studies in meat (0.75 to 28 min at 60°C), and do not indicate that this strain has a particularly high heat resistance. Similarly, z-values are consistent with previously published results (4.6 to 7.4°C).

An increased thermal tolerance is induced by a sublethal heat shock, as is evidenced by higher decimal reduction times. However, the decimal reduction times of the resistant and untreated cultures were comparable in this study.

![Table 1](http://meridian.allenpress.com/doi/pdf/10.4315/0362-028X-59.6.588)

**TABLE 1.** D and z-values of \textit{L. monocytogenes} in ham at 55 and 60°C

<table>
<thead>
<tr>
<th>Culture</th>
<th>55°C</th>
<th>60°C</th>
<th>z-values (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>17.8 ± 6.01(^a)</td>
<td>1.82 ± 0.06</td>
<td>5.05</td>
</tr>
<tr>
<td>Heat shocked</td>
<td>19.2 ± 3.90</td>
<td>3.48 ± 0.64</td>
<td>6.74</td>
</tr>
<tr>
<td>Resistant</td>
<td>13.4 ± 5.06</td>
<td>0.97 ± 0.45</td>
<td>4.38</td>
</tr>
</tbody>
</table>

\(^a\) Average of 2 replicates ± standard deviation.
HEAT RESISTANCE OF LISTERIA MONOCYTOGENES IN HAM

contrary to the observations reported in a previous study in TSYE broth.

ACKNOWLEDGMENTS

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REFERENCES