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# Thermal Resistance of *Listeria monocytogenes* in Milk

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**ABSTRACT**

The thermal resistance of *Listeria monocytogenes* associated with a milkborne outbreak of listeriosis was determined in buffer and whole milk. Thermal resistance was stable over a 2-year period and could not be altered by selecting heat-stressed survivors. The rate of inactivation was linear and did not differ significantly between pH 5.5 and 9.0. When portions of whole milk containing $1 \times 10^5$ cells of *L. monocytogenes*/ml were heated at seven temperatures from 52.2 to 74.4°C, the D-values ranged from 1683.7 to 0.7 s, respectively. The $z_D$-value was 6.3°C. The D-value at 71.7°C was 0.9 s. *L. monocytogenes* would not survive the pasteurization process.

Between June 30 and August 30, 1983, 49 patients in Massachusetts developed listeriosis. Seven cases occurred in infants and 42 occurred in immunosuppressesd adults; 14 patients (29%) died. The majority of *Listeria monocytogenes* patient isolates tested were serotype 4b. Epidemiological studies (*7*) revealed that the illness was strongly associated with drinking a specific brand of pasteurized whole or 2% milk. The implicated milk came from farms where listeriosis in dairy cows was detected. Inspection of the dairy plant where the milk was processed revealed no evidence of faulty pastuerization. These findings raised the question of whether *L. monocytogenes* in contaminated cows' milk had not been destroyed by pasteurization.

Data on the thermal inactivation parameters of *L. monocytogenes* are limited, although unusual heat resistance of the organism in meat (*9*) and in whole milk (*2,5,11*) had been reported. In recent studies, *Listeria* suspended in skim milk at initial levels of approximately $1 \times 10^5$/ml has been shown to survive heating provided during the manufacture of nonfat dry milk (*6*) and cottage cheese (*13*).

The present study was undertaken to provide information on the thermal inactivation of *L. monocytogenes* in whole milk; such information would be useful in evaluating the potential of this organism to survive the pasteurization process.

## MATERIALS AND METHODS

### *Cultures and culture conditions*

Four *L. monocytogenes* cultures associated with the outbreak were obtained from D. W. Fleming, Centers for Disease Control, Atlanta, GA. Two patient isolates, Scott A from blood culture and Murray B from cerebrospinal fluid, were serotyped as 4b. Two strains were recovered after the outbreak from raw milk collected in bulk tanks from farms supplying the subject dairy. Strain V7 was serotype 1a, whereas strain V37 was type 4b.

Stock cultures were grown in Trypticase soy-0.6% yeast extract (TSYE) broth (BBL Microbiology Systems, Cockeysville, MD) at 37°C for 24 h and maintained at 4°C with monthly transfers. A test culture was incubated in TSYE broth at 37°C for 24 h in three serial transfers. The cell density of the final culture was adjusted at 625 nm to an absorbance of 0.20, yielding approx $10^9$ CFU/ml.

### *Media*

Heating media included, in addition to sterile and raw whole milk, 0.1 *M* Tris-Maleic acid buffer, pH 3.5 to 9.5 (at increments of 0.5 pH unit). Sterile milk was a commercial product (Dairymen, Inc., UHT Division, Savannah, GA). Raw milk was taken from individual cows' milks, which was prescreened to obtain milk free of antibiotics and had a standard plate count of less than 1000 CFU/ml.

### *Thermal resistance studies*

To determine thermal resistance, an adjusted culture was serially diluted in phosphate-buffered water (*1*) and inoculated into heating media to yield approx $1 \times 10^5$ *L. monocytogenes* cells/ml. Portions of 1.5 ml were dispensed into 13 × 100-mm borosilicate glass tubes, which were then sealed and heated in a water bath at various temperatures between 52.2 and 71.7°C according to methods described previously (*3*). Triplicate tubes were used at each time interval. Tests at each temperature were repeated at least twice. Inoculated raw milk was also heated to temperatures of 71.7 and 74.4°C in a slug flow heat exchanger according to the procedure of Stroup et al. (*14*).

### *Microbiological procedures*

Total plate counts were determined in duplicate for heat exchanger samples and for pooled contents of tubes at each heating interval on TSYE agar after 48 h of incubation at 37°C.

*Listeria* counts from heated raw milk samples were obtained by biochemically verifying ten randomly selected colonies per plate. Four principal biochemical tests performed on cultures grown on appropriate media for 24 h at 37°C were used to distinguish *Listeria* colonies from indigenous milk flora. Motility was observed as "tumbling" motion in wet mounts from TSYE broth and "umbrella" growth around SIM (BBL) agar stabs. Growth on 5% horse blood agar plates was used to demonstrate the presence of hemolysin as well as for negative oxidase and positive catalase tests.

*Statistical methods*

The rates of thermal inactivation were determined and a linear regression of $\log_{10}$ counts/ml vs. heating times was computed (*10*) for *L. monocytogenes* tested at each temperature. The least-square estimate of slope was calculated and is the rate of thermal inactivation at constant temperature. An estimate of D-value was obtained by taking the absolute value of the inverse of the slope. A linear regression was computed from $\log_{10}$ D-value vs. temperature, and the $z_D$ value was computed as the absolute value of the inverse of the slope. The estimated D-values were corrected for heating and cooling (*4,14*).

## RESULTS AND DISCUSSION

In a preliminary investigation, the thermal resistance of the four *L. monocytogenes* outbreak strains in sterile milk was compared in duplicate heat trials at 66.1, 68.9 and 71.7°C. Strain Scott A proved to be somewhat more resistant than the other three strains and was selected for all subsequent heat studies.

The rate of thermal destruction of strain Scott A heated in buffer at 68.9°C was linear and did not differ significantly (at the $\alpha = 0.05$ level) between pH 5.5 and 9.0. Thermal resistance declined above and below this pH range. Figure 1 shows the limiting inactivation rates within the pH range of greatest heat resistance.

The thermal resistance characteristics of *L. monocytogenes* strain Scott A determined by heating bacteria suspended in raw whole milk at temperatures between 52.2 and 74.4°C are shown as D-values in Table 1. The $z_D$-value is 6.3°C. The D-values obtained in milk were plotted against the various heating temperatures, as shown in Fig. 2.

The thermal resistance of the Scott A strain was a stable characteristic, remaining unchanged during the nearly 2-year duration of this and subsequent heat studies. Attempts to select for greater heat resistance by testing populations grown from heat-trial survivors picked near the terminal interval were unsuccessful.

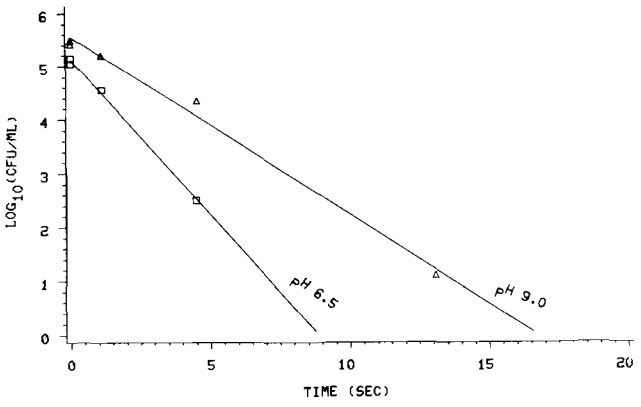


Figure 1. *Rate of thermal inactivation of* L. monocytogenes *strain Scott A at 68.9°C in buffer at selected pH values.*

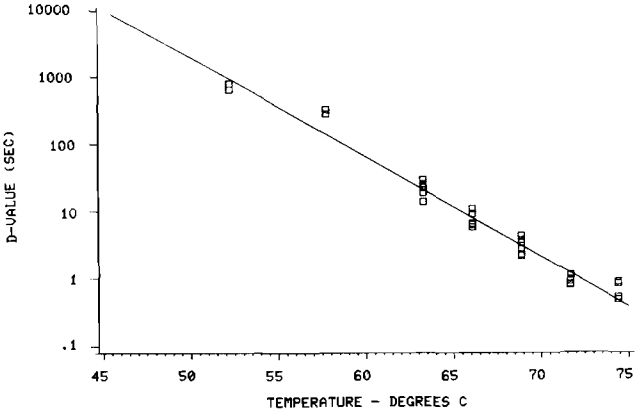


Figure 2. *Thermal inactivation curve for* L. monocytogenes *strain Scott A in raw milk.*

The heat resistance of strain Scott A in raw milk was only slightly less than that of *Salmonella senftenberg* 775W at 71.7°C in sterile whole milk. The D-value for Scott A at 71.7°C was 0.9 s, whereas 775W had a D-value of 1.2 s (*12*). However, Scott A was more heat resistant at 68.3°C (4.0 s from Fig. 2) than six other *Salmonella* strains reported by Read et al. (*12*).

TABLE 1. *D-value estimates for* L. monocytogenes *strain Scott A in raw milk*[a].

| Temperature (°C) | D-value (s) | Range (s) | Coefficient of variation (%) |
|---|---|---|---|
| 52.2 | 1683.7 (2)[b] | 1612.9 - 1754.4 | 5.9 |
| 57.8 | 289.6 (2) | 269.5 - 309.6 | 9.8 |
| 63.3 | 19.9 (6) | 13.4 - 28.4 | 30.3 |
| 66.1 | 7.3 (6) | 6.2 - 10.1 | 24.5 |
| 68.9 | 3.0 (6) | 2.1 - 4.2 | 27.6 |
| 71.7 | 0.9 (5) | 0.8 - 1.1 | 14.2 |
| 74.4 | 0.7 (4) | 0.5 - 0.9 | 30.7 |

[a]$Z_D$ = 6.3°C.
[b]Number of determinations.

The results of these studies support the conclusion that the current pasteurization process guidelines of the Food and Drug Administration (*8*) are adequate to destroy *L. monocytogenes* in whole milk. Our data show that 15 $\log_{10}$ of *L. monocytogenes* suspended in contaminated raw milk would be inactivated at 71.7°C (161°F) for 15 s. They further demonstrate that heat resistance of *L. monocytogenes* is a stable characteristic. We found no evidence that the heat treatment provided by the pasteurization process would select for a highly heat-resistant mutant.

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