

A Research Note
**Growth Temperature and Action of Lysozyme on
*Listeria monocytogenes***

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ABSTRACT

Listeria monocytogenes Scott A grown at 37°C were 1.8–2.5 fold more resistant to lytic action of lysozyme than cells grown at 19, 12, or 5°C. Results suggest that lysozyme may be an effective preservative for controlling *L. monocytogenes* in refrigerated foods.

INTRODUCTION

LYSOZYME is an antibacterial enzyme widely distributed in nature. The enzyme lyses certain gram-positive bacteria by hydrolyzing the beta-1, 4-glucosidic linkages between N-acetylmuramic acid and N-acetylglucosamine of the peptidoglycan in the cell wall (Imoto et al., 1972). An important foodborne pathogen, *Listeria monocytogenes*, was shown susceptible to lysozyme (Hughey and Johnson, 1987) and addition of lysozyme to certain food products led to inhibition of growth or to destruction of *L. monocytogenes* present in those foods (Hughey et al., 1989).

The effect of growth temperature on thermoresistance in *L. monocytogenes* has been studied (Smith et al., 1991). The results indicated that low growth temperatures ($\leq 28^\circ\text{C}$) resulted in cells with increased susceptibility to destruction by heat. Using *L. monocytogenes* Scott A as a representative strain of the species, the objective of our study was to determine if the growth temperature of an organism affected resistance to the lytic effect of lysozyme.

METHODS & MATERIALS

Microorganism and growth conditions

Listeria monocytogenes Scott A was maintained in brain heart infusion broth (BHI; Difco) stored at 5°C and transferred monthly. A seed culture was prepared by inoculating 50 mL of BHI in 250 mL flasks with *L. monocytogenes* and incubating on a rotary shaker (150 rpm) at 28°C for 18–20 hr. The 28°C grown cells were used a source of inoculum for experimental flasks (125 mL) consisting of 25 mL BHI supplemented to contain 0.5% (w/v) glucose. Inoculated flasks were incubated shaken (150 rpm) at 5 to 42°C. Early stationary phase cells ($1\text{--}2.5 \times 10^9$ CFU/mL) from each incubation temperature were harvested by centrifugation, washed once with distilled water, and resuspended in distilled water.

Lysozyme studies

Lysis of *L. monocytogenes* was studied using polystyrene 1 cm square cuvettes (4.5 mL) containing 1.5 mL tris(hydroxymethyl)aminomethane (Sigma), 0.2M, pH 8.8; 0.9 mL distilled water; and 0.5 mL washed cells (previously diluted to give zero time absorbance about 1.2 when added to 2.9 mL volume in the cuvette). Lysozyme dissolved in distilled water (0.1 mL containing 50 μg Sigma egg white lysozyme) was then added to the cuvettes. Each cuvette was capped with parafilm, mixed by inversion, and absorbance immediately determined using a Beckman DU-6 UV-Visible Spectrophotometer at 650 nm. Cuvettes were incubated at 42°C and absorbance determined every 10 min for 60 min. Time required for a decrease of

0.5 absorbance units was determined by plotting the best-fit regression curve for each experimental determination using a curve fitting program (Egraph; Great Basin Associates, Morrison, CO).

Statistical analysis of data

Data were analyzed by one-way analysis of variance using the Ecstatic (Someware in Vermont, Montpelier, VT) and Number Crunching Statistical System (J.L. Hintze, Kaysville, UT) software programs.

RESULTS & DISCUSSION

LYSOZYME exhibited anti-listerial activity with a definite increase in susceptibility of *L. monocytogenes* to lysozyme as growth temperature decreased (Table 1). About 33 min was required for reduction in absorbance of 0.5 units with cells grown at 37°C whereas those grown at 19 or 12°C were lysed in about 18 min. *L. monocytogenes* grown at 5°C showed reduction in absorbance of 0.5 units in about 13 min when lysozyme was present (Table 1). Results in Table 1 were similar to data on the effect of low growth temperature ($\leq 28^\circ\text{C}$) on death of *L. monocytogenes* by 1 hr at 52°C on which lethality was low for cells grown at 37°C (Smith et al., 1991). The increase in death for *L. monocytogenes* Scott A grown at 5 to 28°C was $10^3\text{--}10^4$ times higher than for cells grown at 37°C.

The theoretical explanation for increased heat sensitivity of bacteria grown at low temperature is that it is due to corresponding increase in the level of unsaturated fatty acids in the cytoplasmic membrane. The resultant increased fluidity of the membrane lipids is a major cause of decreased thermotolerance (Beuchat and Worthington, 1976; Yatvin, 1977). *L. monocytogenes* grown at low temperatures showed an increase in level of unsaturated fatty acids (Tadayon and Carroll, 1971). Thus, an increased concentration of unsaturated fatty acids may explain the increase in heat-induced death in *L. monocytogenes* grown at low temperatures. However, lysozyme action is on the bacterial cell wall (Imoto et al., 1972). Low temperature-induced unsaturated fatty acid synthesis in the cytoplasmic membrane is not a likely explanation for increased susceptibility of low temperature grown cells to the lytic action of lysozyme. Thus, at present there is no obvious answer as to why low temperature grown *L. monocytogenes* are more easily lysed by lysozyme

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Table 1—Effect of growth temperature on lysis of *Listeria monocytogenes* Scott A by lysozyme

| Growth temperature °C | n ^a | min ^b | Standard deviation of means |
|--------------------------|----------------|-------------------|--------------------------------|
| 42° | 22 | 30.6 ⁴ | 2.9 |
| 37° | 20 | 33.1 ⁵ | 3.6 |
| 28° | 27 | 27.8 ³ | 3.4 |
| 19° | 25 | 18.8 ² | 2.8 |
| 12° | 26 | 18.3 ² | 2.8 |
| 5° | 24 | 13.3 ¹ | 1.5 |

^a n = replicates

^b Values represent means of min to give decrease in absorbance of 0.5; means followed by different superscript are significantly different at $p \leq 0.05$ (Fisher's LSD Comparison).

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In a recent review, Proctor and Cunningham (1988) discussed use of lysozyme as a food preservative and Hughey et al. (1989) demonstrated that lysozyme killed or prevented growth of *L. monocytogenes* Scott A in foods. Our results suggested that food manufacturers must consider the temperature history of a food if they plan to use lysozyme to control *L. monocytogenes*. Use of lysozyme would be more effective for control of *L. monocytogenes* if it were added to food systems after attainment of refrigerated conditions.

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