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Thermal Inactivation of *Yersinia enterocolitica* in Milk

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Three strains of *Yersinia enterocolitica* isolated from milk had *D* values at 62.8°C from 0.24 to 0.96 min and *z* values of 5.11 to 5.78°C. Since the pasteurization processes for dairy products recommended by the Food and Drug Administration are adequate to destroy large concentrations of these organisms, *Y. enterocolitica* in pasteurized milk probably results from substandard processing or recontamination after pasteurization.

The frequent association of *Yersinia enterocolitica* with raw milk (6, 11) and the ability of this organism to grow in milk at refrigeration temperatures (3, 13) have been well documented. This organism has infrequently been isolated from pasteurized milk products (2, 7, 10) and from cooked meats (14). At least four investigations of the thermal resistance of *Y. enterocolitica* have been reported (3–6), and the results of Hughes (6) indicated that a few of the Australian milk isolates could survive pasteurization. No one has made a broad survey or complete characterization of the heat resistance of *Y. enterocolitica* strains isolated from milk. This work was an attempt to do both.

We have in our *Y. enterocolitica* culture collection Canadian milk isolates (11) obtained from D. A. Schiemann, Montana State University, Bozeman, and the more heat-resistant isolates (6) of D. Hughes, University of Sydney, Sydney, New South Wales, Australia. Additionally, we obtained from D. L. Zink, University of Arizona, Tucson, *Y. enterocolitica* strains isolated during the investigation of the 1976 Oneida County, New York, epidemic, including the strain isolated from chocolate milk (2).

A total of 30 Canadian strains, 6 Australian strains, and 12 American strains were screened for heat resistance at 62.8°C. From these 48 strains, 2 Australian strains (Aus 3 and Aus 31) and 1 Canadian strain (C 1017) were chosen for further characterization because of their unusual heat resistance. All three strains were reported to be sucrose and lactose positive and rhamnose negative. Strain C 1017 was reported by Schiemann to be nontypable, whereas Hughes reported that the Aus 3 and Aus 31 strains are serotypes 5b and 13/15, respectively (personal communication).

All cultures tested for heat resistance were grown for 48 h at 25°C in sterile whole milk to produce a concentration of 1×10^9 to 2×10^9 colony-forming units per ml. A 1-ml portion of test culture was added to 99 ml of sterile whole milk, and 1.5 ml of the mixture was heat sealed in a series of Pyrex tubes (13 by 100 mm). The sealed tubes were submerged in a water bath and heated at three of four temperatures (51.7, 57.2, 62.8, or 68.3°C) long enough for total inactivation of the bacteria. At 30-s intervals beginning at $t = 0$, duplicate tubes were removed from the water bath, cooled in an ice bath, and after appropriate dilution in phosphate-buffered dilution water, plated on Trypticase (BBL Microbiology Systems) soy agar with 0.6% yeast extract. The plates were counted after 7 days of incubation at 25°C.

The observed heating times were corrected for heating and cooling lethality by the method described by Anellis et al., (1). The estimates of thermal resistance were obtained by fitting the linear regressions (8) of the $\log_{10}$ number of survivors at each time interval. *D* values are the absolute value of the inverse slope of the regression line. These *D* values, in minutes, were used to fit plots of $\log_{10} D$ value versus temperature. The absolute value of the inverse slope of the line of $\log_{10} D$ value versus temperature is the *z* value (12).

The thermal resistances of the three *Y. enterocolitica* strains chosen for characterization were estimated (8, 12) and are given in Table 1. The correlation coefficients for *D*-value estimates ranged from −0.97 to −0.99. The replicate estimates of *D* values had observed percent coefficients of variation from 0 to 20.

Schmidt (12) has noted that $z = 10 \pm 2$°F (5.56 ± 1.1°C) is typical for vegetative cells in the temperature range of 130 to 160°F (54.4 to 71.1°C). Estimates of *z* values for strains Aus 3, Aus 31, and C 1017 were 5.78, 5.22, and 5.11°C, respectively. A plot of these curves is given in Fig. 1. Although the slopes of the three lines do not differ significantly ($\alpha = 0.05$), the *D* values of strain C 1017 are about twice those of the other two strains. The heat resistance of C 1017 is only

TABLE 1. Thermal resistance results for three *Y. enterocolitica* strains

Strain	Temp (°C)	*D* value (min)	% Replicate coefficient of variation	*z* value (°C)	Correlation coefficient (*r*)
Aus 3	51.7	23.6 23.4	0.6	5.78	−0.975
	57.2	6.5 5.8	8.0		
	62.8	0.31 0.26	12.4		
Aus 31	51.7	25.6 29.9	11.0	5.22	−0.988
	57.2	4.6 4.6	0.0		
	62.8	0.24 0.18	20.2		
C 1017	57.2	14.7 12.1	13.7	5.11	−0.999
	62.8	0.96 0.87	7.0		
	68.3	0.09 0.09	0.0		

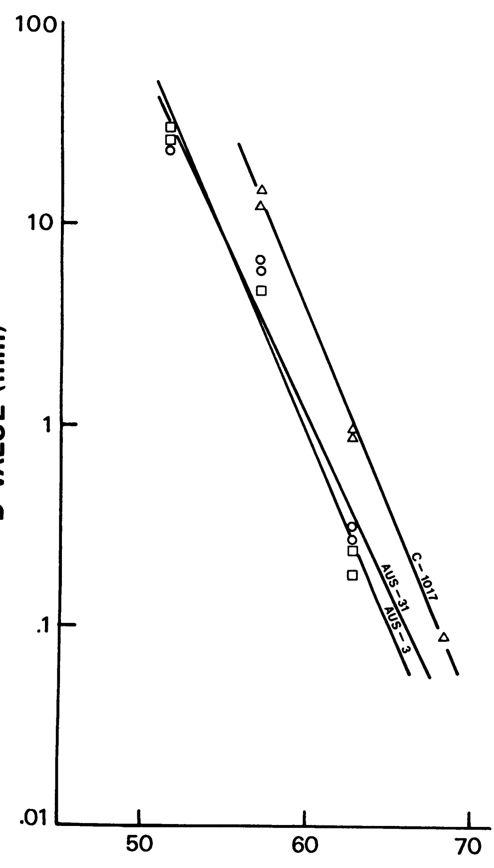

FIG. 1. Thermal resistance of three strains of *Y. enterocolitica*. Symbols: ○, Aus 3; □, Aus 31; △, C 1017.

slightly less than that of *Salmonella senftenberg* 775W at 68.3°C in sterile whole milk. The *D* value for C 1017 at 68.3°C is 0.09 min, whereas 775W has a *D* value of 0.17 min (9). All three strains studied were more heat resistant at 68.3°C than the six *Salmonella* strains reported in the study by Read et al. (9).

This does not mean that there is a danger of *Y. enterocolitica* strains surviving pasteurization, however, since the recommended U.S. Public Health Service pasteurization process exceeds the 10-*D* process recommended for destruction of *S. senftenberg* 775W (9). Investigations of the isolation of *Y. enterocolitica* from properly pasteurized milk should be directed toward post-heat contamination.

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