

Stability of the virulence plasmid in *Yersinia enterocolitica* at elevated temperatures

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This report presents information about the stability of the Yersinia enterocolitica virulence plasmid at elevated temperatures. Though considerable variation existed in the heat resistance of five strains (representing four serotypes), exposure of the different plasmid-bearing virulent strains to temperatures of 45, 50 and 55°C did not cause the loss of the virulence plasmid from the surviving cells. Based on the crystal violet (CV) binding test, these surviving cells were still virulent.

Human pathogenic strains of *Yersinia enterocolitica* harbor a 40-48 megadalton plasmid which is directly involved in the virulence of this organism (Heesemann et al. 1984, Portnoy and Martinez 1985, Cornelis et al. 1987). Several temperature dependent phenotypic properties associated with the virulence plasmid have been described (Zink et al. 1982, Portnoy and Martinez 1985, Cornelis et al. 1987). Loss of this plasmid results in loss of virulence and the concomitant disappearance of the associated phenotypic characteristics. Such loss of this plasmid is facilitated by culturing at 37°C (Zink et al. 1982, Portnoy and Martinez 1985, Cornelis et al. 1987), which indicates the possible instability of plasmid in *Y. enterocolitica* at higher temperatures. Although some literature is available on the effect of elevated temperature on this organism (Hanna et al. 1977, Restaino et al. 1980), no information is known about the heat-stability of its resident plasmid. The

present study was initiated to evaluate the stability of the virulence plasmid of *Y. enterocolitica* to heating by using the CV-binding technique (Bhaduri et al. 1987).

Five different plasmid-bearing virulent strains GER (Serotype 0:3), EWMS (Serotype 0:3), PT18-1 (Serotype 0:5, 0:27), 0:TAC (Serotype 0:TAC-OMA) and WA (Serotype 0:8) representing four serotypes of *Y. enterocolitica* were used to study time-temperature effects on the stability of the virulence plasmid. Storage of cultures, preparation of inocula, and incubation conditions have been described previously (Bhaduri et al. 1987). These plasmid-bearing strains were grown in brain heart infusion broth (BHI; Difco Laboratories, Detroit, MI) for 18 h at 25°C with shaking. The cells were diluted to a concentration of 10^3 cells ml⁻¹ (determined by A₆₀₀) in BHI broth and individually heated at 45, 50 and 55°C for 0-60 min. At intervals, aliquots were removed and 36 µl of samples were surface plated onto BHI agar (Difco Laboratories, Detroit, MI) with a spiral plater (Spiral

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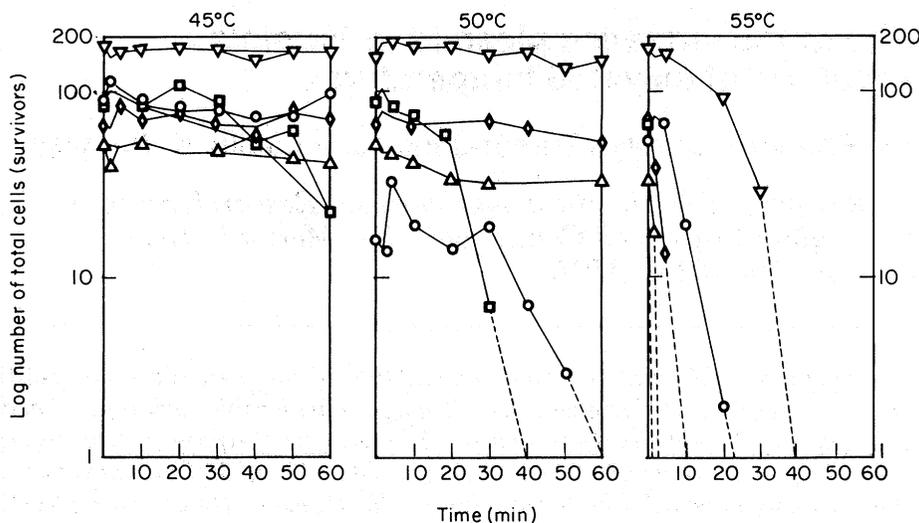


Fig. 1. Effect of heat on recovery of virulent strains of *Yersinia enterocolitica*. All colonies detected were P⁺ based on CV binding assays. Dotted lines indicate that no viable cells were detected at the indicated heating times. GER (○—○); EWMS (△—△); P18-1 (□—□); WA (▽—▽); AND 0:TAC (◇—◇).

System Model B, Cincinnati, OH). The plates were incubated at 37°C for 30 h. Total viable cells (survivors) were counted and plasmid-bearing clones were enumerated by CV-binding (Bhaduri et al. 1987).

The data in Fig. 1 show the effect of temperature on the recovery of the virulent strains of *Y. enterocolitica* at 45, 50 and 55°C, respectively. At 45°C, the number of viable cells remained unchanged with increasing time of exposure to heat except for strain PT18-1 in which the number of surviving cells was reduced after 30 min of heat exposure. All surviving cells retained the resident plasmid. At 50°C (Fig. 1), strains GER and PT18-1 were somewhat more heat sensitive with a reduction in their respective viable counts after 10 min. Strains GER and PT18-1 did not survive after 30, and 50 min of heating, respectively. In contrast, the same heat treatment caused very little reduction in the cell count of the strains EWMS, 0:TAC and WA. The surviving cells in both heat sensitive strains and heat resistant

strains retained the resident plasmid. Exposure to elevated temperature at 55°C showed that a considerable variation existed in the heat resistance of all five strains. Heating for 2–30 min at 55°C caused great reductions in number in all of the strains tested. There were no surviving cells with any of the strains tested after maximum exposure of heating for 30 min at 55°C. The surviving cells from each strain again retained the resident plasmid.

These results indicate that when plasmid-bearing virulent cells of *Y. enterocolitica* are exposed to heating at temperatures 45, 50 and 55°C, respectively, the surviving cells retained their resident plasmid and presumably would be virulent. The data suggest that few, if any, survivors can be expected to be found in foods heated and/or kept at >55°C. However, of greater significance is the observation that those surviving cells of *Y. enterocolitica* have retained the virulence plasmid and are potentially capable of causing food poisoning under the appropriate conditions.

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