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Inactivation of *Vibrio parahaemolyticus* in Distilled Water

J. S. LEE

Department of Food Science and Technology, Oregon State University, Corvallis, Oregon 97331

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Vibrio parahaemolyticus cells are readily inactivated in distilled water. The time of exposure required to inactivate 90% of the cells was between 0.9 and 4.4 min.

Vibrio parahaemolyticus requires NaCl for growth and for viability (3, 5). The osmotic fragility of this organism has been the basis of the recommendation by the public health authority of Japan that seafoods and utensils should be thoroughly washed with tap water (5).

There is a discrepancy in the literature as to the sensitivity of *V. parahaemolyticus* to distilled water. Yanagizawa (5) showed that an exposure of less than 10 min resulted in 10^5-fold reduction of viable cells. Hidaka and Kakimoto (1), on the other hand, reported that the inactivation process could be reversed if the cells were returned to a salt solution within 10 min.

This investigation was undertaken to determine the kinetics of *V. parahaemolyticus* inactivation in hypotonic solutions.

Three strains of *V. parahaemolyticus* were employed. Strain SAK-4 was received from R. Sakazaki, Japan; strain T-3765-1 was from H. Zen-Yoji, Japan; and strain ATCC 17749 was received from J. Liston, University of Washington, Seattle.

Cultures were grown on Brain Heart Infusion agar (Difco; BHI) supplemented with 2% NaCl, and the *p*H was adjusted to 7.8. A loopful of cells grown on BHI at 37 C for 24 hr was suspended in sterile glass-distilled water, and 1 ml of this suspension was withdrawn and resuspended in 99 ml of sterile 3% NaCl solution in 1-min intervals for 30 min. Dilutions were made in 3% NaCl, and 0.1 ml each of appropriate dilutions was spread-plated on BHI, in triplicate, and incubated at 37 C for 24 hr. Cells suspended in 3% NaCl and having an optical density at 400 nm adjusted to a corresponding distilled water suspension served as the control.

Table 1 compares the time required to inactivate 90% of the *V. parahaemolyticus* strains in distilled water. Ninety per cent inactivation (D_{10}) was observed within 4 min for all three strains.

The effect of cultural age on the sensitivity to distilled water was tested with 4-, 24-, and

TABLE 1. *Time of exposure to distilled water required to inactivate 90% of Vibrio parahaemolyticus*

V. parahaemolyticus strains	D_{10} (min)[a]
SAK-4	2.7 ± 0.3
T-3765-1	3.2 ± 0.3
ATCC 17749	3.5 ± 0.2

[a] Average of three separate experiments.

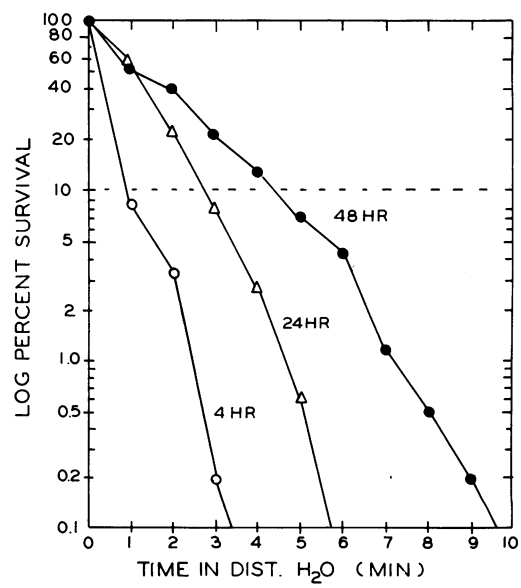

FIG. 1. *Effect of culture age on the viability of Vibrio parahaemolyticus SAK-4 in distilled water.*

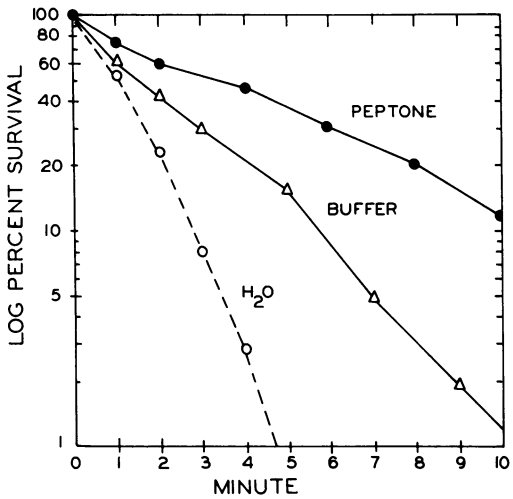

FIG. 2. *Effect of diluents on viability of Vibrio parahaemolyticus SAK-4.*

48-hr-old cells, which corresponded to log, stationary, and negative log phases of growth. Typical inactivation curves for strain SAK-4 are illustrated in Fig. 1. The other two strains showed essentially the same inactivation pattern. Cultural age beyond 48 hr did not result in further increase in resistance. The 96-hr cultures tested were, in fact, slightly more sensitive than 48-hr cells.

The osmotic fragility of *V. parahaemolyticus* was further observed in diluents commonly used for microbiological examinations of foods (Fig. 2). Although the loss of viability was not as rapid as in distilled water, 90% of the *V. parahaemolyticus* cells were inactivated within 11 min in 0.2% peptone (2) and within 7 min in Butterfield's phosphate buffer (4).

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