

Notes

Interacting Effects of pH, Temperature, and Salt Concentration on Growth and Survival of *Vibrio parahaemolyticus*

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Thermal resistance and minimal pH and temperature conditions for growth of *Vibrio parahaemolyticus* in artificial media containing 3 and 7% sodium chloride were studied. Growth was observed at pH 4.8 and at 5 C.

Vibrio parahaemolyticus was demonstrated to grow at 22 and 42 C, at pH 5 to 11, and at NaCl concentrations of 1 to 7% (4). Sodium chloride was demonstrated to have a protective effect on the viability of *V. parahaemolyticus* in Trypticase-soy-broth (TSB; BBL) held at 48 C for 40 min or at -18 C for up to 30 days, and fish homogenate was protective when compared to TSB (1). The organism was shown to be very sensitive to pH values below 6.0, but not to pH values ranging from 6 to 10 in shrimp homogenates (5). Some survivors were noted after heating of homogenates at 60 or 80 C for 15 min. Temmyo (3) reported that *V. parahaemolyticus* was killed after heating at 55 C for 10 min or at 60 C for 5 min in peptone-water.

The present study was designed to establish minimal initial pH values at which six strains of *V. parahaemolyticus* would grow in an artificial, liquid medium. Six strains of *V. parahaemolyticus* (107914, 8700 O4:K11, 284-72, O, T-3765-1 O3:K7, and 4750 O2:K3) were activated by transfer to either TSB containing 3% NaCl or TSB containing 7% NaCl and incubated on a rotary shaker at 37 C. The broth containing either 3 or 7% NaCl was adjusted to pH values ranging from 4.7 to 8.0 in 0.1-unit increments by adding HCl or NaOH and dispensed in 10-ml samples in screw-cap tubes. Changes in pH values were measured after sterilization. Ten tubes of each pH and NaCl combination were tempered at 2, 5, 9, 13, 21, and 30 ± 0.2 C in walk-in incubators. A standardized loopful of an 18-h culture grown in TSB with 3% NaCl was

transferred to tubes containing tempered TSB with 3% NaCl at various pH values; similarly, *V. parahaemolyticus* was cultured in TSB with 7% NaCl before its growth was tested in TSB with 7% NaCl at various pH values. Tubes were visually examined for up to 6 days of incubation, and results were recorded as extensive, moderate, or negative. The viable number of organisms contained in the loopful of inoculum was determined to be 10⁷, thus resulting in an initial population of 10⁸ in the 10 ml of test medium. This level of organisms produced no visual change in turbidity and was judged to have had no increase in cell numbers after 6 days of incubation. A population of approximately 10⁷ to 5 × 10⁷ was recorded as moderate growth, and populations exceeding 5 × 10⁷ were considered extensive. These populations had been determined previously by plating on Trypticase-soy-agar (TSA).

Thermal resistance of *V. parahaemolyticus* was studied. A 0.3-ml portion of a 24-h culture was dispensed into tubes containing 19.7 ml of TSB with 3% NaCl which had been tempered at 53 ± 0.2 C (128 F) in a water bath. Heating menstua had been adjusted to pH values ranging from 5.0 to 8.0 in 0.5-unit increments. Samples were withdrawn, and dilutions were made immediately by using a 3% NaCl in water diluent before plating in TSA with 3% NaCl tempered at 45 C. Recovery was at 37 C, and counts were made after 24 h of incubation. Two separate experiments were performed in duplicate.

TABLE 1. Growth of *Vibrio parahaemolyticus* in Trypticase-soy-broth

Strain	Temp. (C)	Minimum pH for growth	
		3% NaCl	7% NaCl
107914	5	(7.3) ^a	(7.6)
	9	7.6 ^b (7.2)	7.8 (7.1)
	13	5.4 (5.2)	6.1 (6.0)
	21	5.2 (4.9)	5.4 (5.3)
	30	5.2 (4.8)	5.3 (5.2)
8700	5	(7.7)	(7.6)
	9	7.8 (7.5)	7.7 (7.3)
	13	5.6 (5.2)	6.1 (6.0)
	21	5.1 (4.9)	5.7 (5.6)
	30	5.2 (4.8)	5.4 (5.3)
284-72	5	(7.8)	(7.7)
	9	7.7 (7.6)	7.8 (7.5)
	13	5.7 (5.6)	6.1 (5.7)
	21	5.3 (5.1)	5.6 (5.5)
	30	5.2 (5.0)	5.4 (5.3)
O	5	(7.3)	(7.3)
	9	7.6 (7.1)	7.7 (7.3)
	13	5.6 (5.2)	6.0 (5.5)
	21	5.4 (5.0)	5.4 (5.1)
	30	5.1 (5.0)	5.3 (5.0)
T-3765-1	5	(7.4)	(7.5)
	9	7.6 (7.1)	7.6 (7.2)
	13	5.8 (5.2)	5.9 (5.6)
	21	5.4 (5.2)	5.3 (5.2)
	30	5.1 (4.9)	5.3 (5.0)
4750	5	(7.7)	(7.5)
	9	7.8 (7.7)	7.8 (7.3)
	13	5.8 (5.6)	5.9 (5.7)
	21	5.5 (5.4)	5.5 (5.4)
	30	5.2 (5.1)	5.2 (5.0)

^a Including moderate growth.

^b Extensive growth.

Table 1 shows the minimum pH values at which *V. parahaemolyticus* was observed to grow. Values are listed regardless of the number of tubes out of 10 which showed increased population. Extensive growth at 5 C was not observed at the pH values examined; no growth was observed at 2 C. Growth at 5 and 9 C was only at an alkaline pH. There was a tendency for growth at lower pH's as the incubation temperature was increased, regardless of NaCl concentration. With the exception of strain 4750, *V. parahaemolyticus* was demonstrated to grow at lower pH values in the TSB with 3% NaCl than in TSB with 7% NaCl. Strains 107914 and 8700 both grew in TSB with 3% NaCl at pH 4.8 when incubation was at 30 C, the lowest pH tolerance observed in the study.

Figure 1 indicates the decimal reduction times for *V. parahaemolyticus*. The six strains were least sensitive to heat at pH 7.0 and most

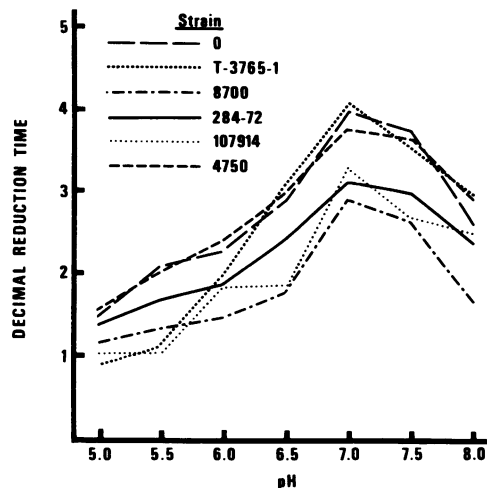


FIG. 1. Decimal reduction times at 53 ± 0.2 C for *Vibrio parahaemolyticus* heated in TSB with 3% NaCl and adjusted to pH 5.0 to 8.0.

sensitive at pH 5.0. Differences in lethal effects of heat on the six strains do occur, however, at the same pH. Strain T-3765-1 was the least resistant to heat at pH 5.0, but was most resistant at pH 7.0. Experimental design may lack the sophistication required to permit emphases on actual numbers; however, *V. parahaemolyticus* was extremely sensitive to heat treatment at 53 C in a TSB with 3% NaCl medium adjusted to pH 5.0 to 8.0. The organism was least sensitive at pH 7.0.

Data presented here were derived by using liquid media, and some caution must be taken if comparisons are to be made with similar pH-temperature-salt conditions in food products or other natural substrates. Strain O, for example, was reported to survive in shrimp homogenate after heating at 60 or 80 C for 15 min (5). An initial decrease in viable population of strain O in whole shrimp, followed by an increase between 4 and 8 days at 7 C, was noted. Kaneko and Colwell (2) reported that 10 C was the minimum temperature for growth for *V. parahaemolyticus* in a natural environment. Results reported from the conditions observed in this study therefore do not necessarily reflect minimum tolerances to adverse pH and temperature in all systems. However, data may be useful when determining the causative organism in foodborne outbreaks where *V. parahaemolyticus* is suspect.

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