Survival of Vibrio parahaemolyticus in Cooked Seafood at Refrigeration Temperatures

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The growth and survival of two strains of Vibrio parahaemolyticus isolated during food-borne gastroenteritis outbreaks in Japan and surface inoculated on cooked shrimp, shrimp with sauce, or cooked crab were tested at various refrigeration temperatures during a 48-h holding period. On cooked shrimp and crab, the vibrios grew well at 18.3 C, but their numbers declined gradually at 10 C and below. At 12.8 C, vibrios remained static for the most part. Thus, it appeared that 12.8 C was the borderline temperature for growth of the organism on cooked seafood. When cocktail sauce was added to surface-inoculated shrimp at a ratio of 2:1, the vibrio die-off rate was accelerated. In the shrimp and sauce few cells remained after 48 h, but in the sauce alone die-off was complete at 6 h.

The facultative halophile, Vibrio parahaemolyticus, is recognized as a public health hazard in seafoods of United States and foreign origin (13). Recent outbreaks of food poisoning in the United States were associated with the consumption of crab or shrimp contaminated with this organism (4–7). Foods contaminated with V. parahaemolyticus through inadequate preparation or handling may be held under conditions that favor the growth of bacteria and increase the risk to the consumer. Generation times as low as 12 min were observed when these organisms were inoculated into sea fish (11). Minimal temperatures reported for V. parahaemolyticus multiplication were 5 C (3) and 8 C (1) in artificial media, and 10 C in oyster homogenate (15) and the marine environment (10). Under favorable growth temperatures, extensive multiplication may occur that probably increases the risk of food-borne illness.

Restaurants and cafeterias commonly prepare seafood salads in advance of serving time and store them in the refrigerator until they are displayed on the serving line. Temperatures as high as 12.8 C in refrigerated showcases (2), 10 C in domestic refrigerators (19), and 15.6 C in coolers used in the blue crab industry (12) have been recorded. Such deviations from optimum refrigerator temperatures might well permit multiplication of contaminating V. parahaemolyticus.

A few studies (8, 9, 15, 18) of the survival of V. parahaemolyticus inoculated into the tissues or into homogenates of shrimp and oysters have been conducted. But such studies have limited applicability to the hazards of storing poorly refrigerated, contaminated seafood. This study was therefore undertaken to determine the effects of storage temperatures representative of both good and poor refrigeration on the growth and survival of V. parahaemolyticus on the surface of cooked seafoods such as shrimp and crab.

MATERIALS AND METHODS

Cultural methods. The two cultures of V. parahaemolyticus used in this study were Yanagisawa, O4: K9 (obtained from H. Zen-Yoji, Tokyo Metropolitan Research Laboratory of Public Health, Tokyo) and 9382, O4: K11 (obtained from Y. Miyamoto, Kanagawa Prefectural Public Health Laboratory, Yokohama). Both cultures were isolated during food-borne gastroenteritis outbreaks in Japan. The media and culture conditions used for maintenance of stock cultures as well as for verification of strains have been previously described (16). Both strains exhibited properties that have been well established for identifying pathogenic V. parahaemolyticus (14). Kanagawa hemolysis was determined on Wagatsuma agar (Eiken) that contained 10% washed human red cells. Serotyping was accomplished by slide agglutination with 8 monovalent O, 8 polyvalent K, and 52 monovalent K antisera (Toshiba Kazaku Co., Ltd., Tokyo). Inocula for a given experiment were prepared from strains grown on Trypticase soy agar (BBL) containing 3% NaCl at 35 C for 18 to 22 h. Cells were washed twice and resuspended in buffered physiological saline to a known optical density.

Preparation of seafood. Frozen whole jumbo shrimp in shells and frozen pasteurized Alaskan king crab meat were used in this study. Samples were cooked at 100 C for 6 min; the cooking water was then
Twenty-five grams of poured off, and cold, sterile distilled water was added. Shells were aseptically removed from the shrimp. Twenty-five grams of either crab or shrimp was added to sterile, 3-oz (88.69-ml) plastic bottles. Samples were equilibrated at 35 ± 1 °C and surface inoculated with 1 ml of cell suspension adjusted to provide initial concentrations of approximately 10^4 organisms/g. Bottles were sealed in plastic bags and immersed in water baths set at the desired test temperatures. Duplicate plates were prepared from appropriate dilutions of duplicate samples in modified Twedt medium (17) at 0, 6, 12, 24, 36, and 48 h. Plates were incubated at 35°C for 42 to 44 h. V. parahaemolyticus were never detected in uninoculated control samples plated at 0 and 48 h. In certain assays, 50 g of a commercial sauce (Seafood Cocktail Sauce, Crosse & Blackwell Co., White Plains, N.Y.) was added to 25 g of inoculated shrimp to approximate a commercial seafood cocktail that is produced and then frozen for retail sale. In other assays, samples of sauce were inoculated and tested for survival.

Verification. Five colonies from each of four countable plates at each incubation temperature in every experiment were picked to test in Trypticase soy-broth for verification of V. parahaemolyticus. The biochemical tests performed on each included growth in 0, 1, 8, and 10% NaCl; production of acetoin, indole, and H2S; and fermentation of glucose and sucrose. Serotyping was performed by slide agglutination.

RESULTS

The growth and survival of two strains of V. parahaemolyticus that were surface inoculated on whole shrimp held at six refrigeration temperatures are shown in Table 1. The vibrios grew well at 18.3 °C, but their numbers declined from 0.5 to 1 log at 10 °C and below during the 48-h holding period. At 12.8 °C, strain 9382 declined 1 log, and strain Yanagisawa remained relatively static. Table 2 illustrates the effect of added cocktail sauce on the growth and survival.

| Strain   | Temp (°C) | Expt | Counts |
|----------|-----------|------|--------|
|          |           |      | 0     | 3     | 6     | 12    | 24    | 36    | 48    |
| Yanagisawa | 1.6       | 1    | 7.2   | 7.9   | 6.5   | 6.0   | 7.1   |       |       |
|          |           | 2    | 13.8  | 12.4  | 10.9  | 9.0   | 8.7   |       |       |
|          |           | 3    | 9.4   | 8.2   | 6.7   | 6.1   | 3.8   |       |       |
|          |           | 4    | 5.5   |       | 3.5   | 4.4   | 2.7   | 2.1   | 1.8   |
|          | 4.4       | 1    | 8.0   | 6.3   | 5.6   | 6.6   |       |       |       |
|          |           | 2    | 12.8  | 12.7  | 11.9  | 8.5   |       |       |       |
|          |           | 3    | 8.2   | 8.3   | 6.8   | 4.0   |       |       |       |
|          | 7.2       | 1    | 6.1   | 7.3   | 6.9   | 5.7   |       |       |       |
|          |           | 2    | 13.3  | 10.6  | 11.0  | 6.7   |       |       |       |
|          |           | 3    | 8.0   | 6.8   | 6.1   | 3.0   |       |       |       |
|          |           | 4    | 3.6   | 3.3   | 2.9   |      | 1.9   | 1.3   |       |
|          | 10.0      | 1    | 5.9   | 6.5   | 7.4   | 5.6   |       |       |       |
|          |           | 2    | 12.1  | 11.5  | 9.5   | 6.4   |       |       |       |
|          |           | 3    | 9.4   | 7.0   | 6.4   | 2.8   |       |       |       |
|          | 12.8      | 1    | 6.3   | 6.4   | 7.2   | 15.6  |       |       |       |
|          |           | 2    | 13.0  | 10.9  | 7.5   | 4.1   |       |       |       |
|          |           | 3    | 7.5   | 5.8   | 6.3   | 2.6   |       |       |       |
|          |           | 4    | 4.3   | 3.1   | 3.1   |      | 3.3   | 49.6  |       |
|          | 18.3      | 4    | 5.3   | 930.9 | 9,100.0 | 890,000.0 | 770,000.0 |       |       |
| 9382     | 1.6       | 5    | 11.2  | 10.6  | 8.1   | 6.1   | 5.8   |       |       |
|          |           | 6    | 10.7  | 8.8   | 9.9   | 6.6   | 5.6   | 1.2   |       |
|          |           | 7    | 10.8  | 8.5   | 7.8   | 7.4   | 2.1   | 2.1   |       |
|          | 4.4       | 5    | 9.7   | 6.6   | 6.8   | 5.1   |       |       |       |
|          |           | 6    | 9.0   | 8.6   | 5.6   | 3.4   | 1.5   |       |       |
|          | 7.2       | 5    | 9.3   | 6.6   | 6.1   | 3.9   |       |       |       |
|          |           | 6    | 11.4  | 7.7   | 7.3   | 2.0   | 1.0   |       |       |
|          |           | 7    | 9.8   | 6.3   | 6.7   | 2.2   | 0.8   |       |       |
|          | 10.0      | 5    | 7.9   | 5.6   | 6.6   | 1.1   |       |       |       |
|          |           | 6    | 9.6   | 7.3   | 4.7   | 2.7   | 1.2   |       |       |
|          | 12.8      | 5    | 7.9   | 5.4   | 2.5   | 0.7   |       |       |       |
|          |           | 6    | 10.4  | 4.8   | 1.0   | 1.6   | 1.3   |       |       |
|          |           | 7    | 7.3   | 3.7   | 0.8   | 2.4   | 0.3   |       |       |
|          | 18.3      | 7    | 18.5  | 22.4  | 6,700.0 | 2,280.0 | 2,880.0 |       |       |

* Number of hours held.

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of vibrios on shrimp. In most cases counts fell more than 1 log in 24 h at all temperatures. Few organisms remained at 48 h. The rate of decline seemed less at 18.3°C than at the other temperatures. Indeed, multiplication occurred after 36 h at this temperature in one experiment (strain 9382). In sauce alone, few viable cells were present at 6 h, and die-off was complete at 24 h at all temperatures. The growth and survival of *V. parahaemolyticus* on cooked crabmeat were similar to those observed on shrimp (Table 3). The organisms grew well at 18.3°C, remained

### Table 2. Counts of *V. parahaemolyticus* per gram of whole cooked shrimp in commercial sauce incubated at various temperatures over a 48-h period

| Strain      | Temp (C) | Expt | Counts       |
|-------------|----------|------|--------------|
|             |          | 0*   | 6  | 12  | 24  | 36  | 48  |
| Yanagisawa  | 1.6      | 1    | 3,700 | 460 | 300 | 250 | 60  | 10  |
|             |          | 2    | 9,900 | 270 | 580 | 200 | 15  | 20  |
|             |          | 3    | 8,400 | 200 | 520 | 20  | 8   | —   |
|             | 7.2      | 1    | 1,000 | 500 | 190 | 15  | 13  | —   |
|             |          | 2    | 570  | 190 | 70  | 20  | 35  | —   |
|             |          | 3    | 630  | 400 | 60  | 4   | —   | —   |
|             | 12.8     | 1    | 2,100 | 360 | 770 | 230 | 25  | —   |
|             |          | 2    | 450  | 500 | 190 | 40  | —   | —   |
|             |          | 3    | 170  | 90  | 80  | 40  | —   | —   |
|             | 18.3     | 1    | 850  | 600 | 2800 | 2200 | 300 | —   |
|             |          | 2    | 1,600 | 370 | 8100 | 100  | 6   | —   |
|             |          | 3    | 330  | 260 | 140 | 22  | —   | —   |
| 9382        | 1.6      | 1    | 12,300 | 1,280 | 540 | 110 | 50  | —   |
|             |          | 2    | 4,600 | 190 | 100 | 20  | —   | —   |
|             |          | 3    | 6,900 | 120 | 30  | 25  | 10  | —   |
|             | 7.2      | 1    | 110  | 610 | 63  | 60  | 30  | —   |
|             | 2        | 230  | 250  | 10  | —   | —   | —   | —   |
|             | 3        | 350  | 60   | 10  | 10  | —   | —   | —   |
|             | 12.8     | 1    | 240  | 420 | 75  | 56  | 40  | —   |
|             | 2        | 130  | 210  | 50  | 130 | 10  | —   | —   |
|             | 3        | 120  | 190  | 10  | 10  | 10  | —   | —   |
|             | 18.3     | 1    | 1,260 | 2,030 | 60  | 11,700 | 570,000 | —   |
|             |          | 2    | 1,600 | 370 | 8100 | 100  | 6   | —   |
|             |          | 3    | 330  | 260 | 140 | 22  | —   | —   |

* Number of hours held.  
*—, None recovered.

### Table 3. Counts of *V. parahaemolyticus* Yanagisawa strain (in thousands) per gram of cooked crab meat incubated at various temperatures over a 48-h period

| Temp (C) | Expt | Counts       |
|----------|------|--------------|
| 1.6      | 1    | 12.0 | 9.0 | 7.3 | 6.2 | 10.1 | 2.2 |
|          | 2    | 16.4 | 7.9 | 15.4 | 5.3 | 1.4 | 0.2 |
|          | 3    | 18.9 | 14.5 | 12.0 | 6.6 | 8.3 | 0.3 |
| 7.2      | 1    | 9.4 | 11.5 | 8.2 | 11.2 | 5.7 |
|          | 2    | 13.4 | 10.5 | 16.2 | 1.3 | 3.8 |
|          | 3    | 15.4 | 12.8 | 11.4 | 9.0 | 6.9 |
| 12.8     | 1    | 11.1 | 9.0 | 7.4 | 22.8 | 86.0 |
|          | 2    | 17.1 | 9.8 | 10.2 | 1.1 | 4.4 |
|          | 3    | 19.6 | 13.3 | 4.0 | 15.3 | 17.6 |
| 18.3     | 1    | 14.5 | 133.0 | 1,330.0 | 300,000.0 | 500,000.0 |
|          | 2    | 16.0 | >30.0 | 49,000.0 | 410,000.0 | 140,000.0 |
|          | 3    | 15.1 | 166.0 | 42,700.0 | 510,000.0 | 198,000.0 |

* Number of hours held.
static (except for the small increase in experiment 1 at 48 h) at 12.8 C, and declined approximately 0.5 log at 7.2 C and 1 to 2 logs at 1.6 C during the 48-h holding period.

**DISCUSSION**

Our results indicate that *V. parahaemolyticus* inoculated onto the surface of cooked shrimp or crab gradually decline in numbers at incubation temperatures of 10 C and below and multiply if held at 18.3 C. Apparently, 12.8 C is the borderline temperature for growth of this organism on cooked seafood. The Yanagisawa strain remained nearly static, and the 9382 strain declined 1 log during the 48-h storage period.

By utilizing different experimental menstrua and methods, other investigators have obtained analogous results. Vanderzant and Nickelson (18) observed a decrease of 2 logs during the first 2 days of storage at 3, 7, and 10 C of approximately $10^8$ *V. parahaemolyticus* cells injected into whole uncooked shrimp. In shrimp homogenates inoculated with approximately $5 \times 10^4$ cells/ml, results were quite different. An initial slight increase in numbers over the first 12 h at all three temperatures was followed by a gradual decrease. Johnson and Liston (8) reported a slow decline in numbers of a *V. parahaemolyticus* strain after 2.5 days of storage at 11 C and below in depurated oysters naturally contaminated with $5.8 \times 10^4$ cells/g. Thomson and Thacker (15) inoculated *V. parahaemolyticus* strains into oyster homogenates at an initial level of approximately $5 \times 10^3$ cells/ml. They observed multiplication at 10 C and above and no change at 8 C, with a gradual decline of 1 to 2 logs at 4 C and below during 1 week of storage. Johnson et al. (9) reported little or no apparent decrease of *V. parahaemolyticus* in naturally contaminated oyster shellstock stored for 3 weeks at 4 C.

Reports in the literature demonstrate that domestic and commercial refrigerators exhibiting fluctuating temperatures capable of supporting *Vibrio* survival or multiplication on cooked seafood are not uncommon. Bauman (2) reported refrigerator showcases to cycle between 4.4 and 12.8 C during a 12-h period. When van Walbeek et al. (19) tested domestic refrigerators in the early morning hours to avoid fluctuation caused by frequent opening, they recorded temperatures as high as 10 C. Commercial coolers exhibited temperatures up to 15.6 C during a survey of the blue crab industry by Phillips and Peeler (12).

When commercial sauce was added to surface-inoculated shrimp, *Vibrio* decline was accelerated. In most cases, the die-off rate was greater than 1 log in 24 h. Only minimal numbers were present at 48 h. The explanation for this rapid decline may lie with the acidity of the sauce (pH 3.3 to 3.4). In sauce alone, *Vibrio* die-off was virtually complete in only 6 h. Since the shrimp was surface inoculated before admixing with sauce, it is very likely that the bacteriocidal effects of the acid pH were modified by buffering that would result from the association of vibrios with shrimp tissue.

The gradual decline of *V. parahaemolyticus* on the surface of cooked seafood at refrigerator storage temperatures of 10 C and below can hardly be considered to eliminate the public health hazard inherent in a contaminated product. The danger of gastroenteritis is still present for the consumer, either from massive numbers of *V. parahaemolyticus* or from modest numbers of a highly infectious strain.

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