WHAT SEAFOOD PROCESSORS SHOULD KNOW ABOUT *VIBRIO PARAHAEMOLYTICUS*[1]

J. S. Lee
Department of Food Science and Technology
Oregon State University, Corvallis, Oregon 97331

(Received for publication March 16, 1973)

Abstract

Information on *Vibrio parahaemolyticus* that is pertinent for its control in food processing operations is compiled and discussed in this paper. The growth potential of this organism and requirement for NaCl are discussed in some detail. Effects of temperature, pH, and antimicrobial agents are also presented.

Repeated outbreaks of gastroenteritis, caused by *Vibrio parahaemolyticus* in the United States *(24, 25, 26)* point to the need for control measures. Despite the numerous publications on *V. parahaemolyticus* scattered throughout the literature, information pertinent to the control of this organism is scarce. The intent of this publication is to bring together information that will help guide the seafood processors in meeting this new challenge.

Readers interested in additional information are referred to two recent reviews by Fishbein and Olson *(9)*, and Nickerson and Vanderzant *(27)*. Selected publications on the distribution and incidence in seafoods *(3, 4, 8, 14, 17, 31, 33, 35)* and the isolation and identification methods *(2, 23, 29, 30, 32, 33)* are listed in the references.

Growth

The amazingly rapid growth rate of *V. parahaemolyticus* is perhaps one of the most important characteristics to be considered. Aiso *(1)* grew *V. parahaemolyticus* strain No. 7 in brain-heart infusion broth (pH 7.9), plus 1.5% NaCl, on a shaker at 37 C. Growth was measured spectrophotometrically at 470 nm and by the plate count. The generation time thus obtained at the logarithmic growth phase was 7.6 min. This was claimed to be the shortest ever recorded for any bacteria. He also noted that growth of *V. parahaemolyticus* was equally rapid in seafoods, and the generation time of this organism in inoculated squid was 13 min.

Although we could not duplicate the 7.6-min generation time under identical conditions with 4 different strains of *V. parahaemolyticus*, the average generation time obtained was still a remarkably short 13.6 min (*unpublished data*).

The rapid growth of this organism might also have been responsible for the incriminated seafoods' often fresh and unspoiled appearances *(1)*. Aiso *(1)* showed that at 37 C, *V. parahaemolyticus* strain No. 7 increased in number from 10^2 to 10^4 within 1.5 hr in the inoculated squid; and it increased to 10^7 after 4.5 hr. In the same time period, the count of indigenous microorganisms barely increased from 10^2 to 10^3; and the total volatile bases, or the chemical indices of spoilage, had hardly increased.

After the Louisiana outbreak of *V. parahaemolyticus* gastroenteritis *(25)*, no mention of the incriminated shrimp having an off odor or flavor was made by those interviewed. A number of them, however, noted that the shrimp they ate appeared undercooked (Center for Disease Control, Atlanta, *personal communication*). Our unpublished data on volatile degradation products of *V. parahaemolyticus* in sterile fish showed that the compounds identified were similar to those reported for naturally spoiling fish *(22)*.

The lack of putrefactive or spoiled appearance of incriminated seafoods, therefore, appears to be due to quantitative rather than qualitative difference, i.e., the *V. parahaemolyticus* population could have reached dangerous proportions before signs of their presence might be detected.

Sodium Chloride Requirement

V. parahaemolyticus strains require NaCl for growth and maintenance of viability *(10, 18)*. The halophilism of this organism was one of the early characteristics recognized by the Japanese investigators, and it helped to establish this organism as a new agent of food poisoning *(10)*.

The requirement for NaCl perhaps reflects the marine origin of this organism. As with the other marine bacteria, *V. parahaemolyticus* requires NaCl partially for osmotic balance, and lysis in hypotonic solutions *(5, 13)*. Hidaka and Kakimoto *(12)* compared the osmotic fragilities of a non-marine bacterium, *P. fluorescens*, a marine bacterium, *Pseudomonas* 1055-1, and *V. parahaemolyticus*. *V. parahaemolyticus* lysed in NaCl or KCl concentration of 100 mM or less but required 1 mM or less of divalent cations, $MgCl_2$ or $CaCl_2$, before lysis could be induced.. *P.*

[1]Technical Paper No. 3549, Oregon Agricultural Experiment Station.

fluorescens was not sensitive to hypotonic conditions. The marine *Pseudomonas* 1055-1, however, was more sensitive than *V. parahaemolyticus;* and lysis could be induced by 600 mM or less of NaCl or KCl and 10 mM or less of $MgCl_2$ or $CaCl_2$.

The concentration of NaCl tolerated by suspected isolates serves as an important differential criterion *(2, 30)*. The maximum NaCl concentration tolerated by *V. parahaemolyticus* is 8%, while the closely related organism, *V. alginolyticus* can grow in 10% NaCl. The optimum concentration of NaCl for *V. parahaemolyticus,* however, is between 2 and 4%. In fact, *V. parahaemolyticus* was reported to grow poorly in foods containing 5% or more of NaCl *(16)*.

Reaction of the Substrate

V. parahaemolyticus prefers an alkaline pH. The recommended pH for the culture media is 7.4 to 8.6 *(2)*.

Kodama *(16)* claimed a correlation between the ability of a food to support growth of *V. parahaemolyticus* and its pH. Among 26 seafoods; 27 vegetables and pickles; and 9 meat, poultry, and dairy products common to the Japanese diet, he demonstrated that no food with a pH below 5.8 supported growth of *V. parahaemolyticus.* Maximum growth was obtained in uncooked octopus and marinated egg, and their respective pH values were 7.7 and 8.5.

The pH, however, may be one of many factors that influence *V. parahaemolyticus.* Some foods that did not support maximum growth of *V. parahaemolyticus* still had pH values in the range of 6.0 to 7.9. Cooked beans, despite their pH of 7.9, failed to support growth of *V. parahaemolyticus.*

Susceptible foods according to Kodama, are "proteinatious," with NaCl contents of 1 to 3%, and pH above 5.8. A food with a pH value below 5.0 and NaCl content above 5% is not likely to support growth of *V. parahaemolyticus.* He noted, however, that some foods might not attain the inhibitory concentrations of salt and pH, shown by the finished product, during processing. Therefore, one must also take the history of each food into account.

The effect of pH on *V. parahaemolyticus* strain O in shrimp homogenate was studied by Vanderzant and Nickerson *(34)*. They showed that viability was not affected by pH between 6 and 10 during a 2-hr test period. However, rapid inactivation took place at pH 5.0.

Growth Temperatures

The reported optimum temperature for growth varies from 35 to 37 C *(19)*. Given the rapid growth rate of this organism, the difference tends to be insignificant between optimum and sub-optimum temperatures. The minimum and maximum growth temperatures reported are 5-8 and 42-45 C, respectively *(19)*. Although the strains we examined did not grow at temperatures below 10 C, and the generation time was twice as long at 20 C than at 37 C (unpublished data), the minimum growth temperature could be lowered in fish substrate, as shown by some *Salmonella* strains *(20)*.

High Temperatures

V. parahaemolyticus is very heat sensitive and can be inactivated by mild heat. At 48 C, 3 C above its maximum growth temperature, approximately 90% of the cells were inactivated in <1 hr in a broth, and in <1.5 hr in fish homogenate *(7)*. After heating at 60 or 80 C for 15 min, no survivors could be detected in a shrimp homogenate inoculated with 500 cells/ml. Only when the cell concentration was increased to 2×10^6/ml were there detectable survivors after 15 min at 80 C; but no survivors were detected after 1 min at 100 C *(33)*.

The sensitivity of this organism to heat probably has prevented untold numbers of *V. parahaemolyticus* outbreaks in this country. In Japan, where two-thirds of all gastroenteritis during the summer months has been attributed to *V. parahaemolyticus,* the large consumption of seafoods, coupled with the custom of consuming raw seafoods, has been thought as the probable cause.

All reported outbreaks in the United States, however, were due to cooked crustaceans, except a suspected case involving oysters and raw crab used in "poi." It is noteworthy that such seafoods are normally consumed without further cooking.

Another factor that must be considered is that *V. parahaemolyticus* gastroenteritis may not be exclusive to seafoods. Again in Japan, salted cucumbers have been incriminated in a *V. parahaemolyticus* outbreak *(10)*. High protein foods of alkaline pH, such as raw egg and egg products (pH 7.9 to 8.5) had been shown to support the growth of *V. parahaemolyticus (16)*.

Low Temperatures

The cold sensitivity of this organism was recognized early by the reduction of *V. parahaemolyticus* gastroenteritis during winter months *(28)*. The extent of inactivation due to low temperature, however, is far less than that due to heat. Two outbreaks reported in the United States in 1972 involved frozen shrimp, and viable *V. parahaemolyticus* cells were recovered from the frozen samples *(26)*. Conflicting

evidence exists, but in general, the low temperature inactivation of *V. parahaemolyticus* is a negative function of the temperature. Matches et al. *(21)* subjected 13 *V. parahaemolyticus* strains in fish homogenate to 0.6, –18, and –34 C and noted that at 0.6 C, a 2.0 to 6.4 log reduction was obtained in 26 to 48 days. The log reduction values of 2.2 to 6.2 at –18 C were attained in 12 to 19 days, and the same reduction values at –34 C were reached before the 12th day. Vanderzant and Nickerson *(34)* subjected their Gulf Coast isolate (*V. parahaemolyticus strain O*) to 3, 7, 10, and –18 C in whole and homogenized shrimp. In whole shrimp, the initial loss of viability was rapid and resulted in a 2-log reduction within 2 days. After this initial loss, no further reduction was noted during 6 additional days of observation. The loss of viability in shrimp homogenate was not as great as in the whole shrimp, and no more than a 2-log reduction was observed in 8 days. It is also interesting to note that the strain Vanderzant and Nickerson studied was more readily inactivated at 3 than at –18 C. The data, therefore, suggest that refrigeration temperatures may be more detrimental to *V. parahaemolyticus* than freezing. A similar study by Covert and Woodburn *(7)*, however, showed that *V. parahaemolyticus* strain SB04-422 in trypticase soy broth with 6% NaCl was inactivated more readily at –18 than at –5 C, and –5 was more detrimental to this organism than 5 C.

DISINFECTION

Effectiveness of various antibiotics, detergents, disinfectants, and food preservatives against *V. parahaemolyticus* has been thoroughly investigated in Japan *(36)*. Information that may be applicable to food handlers is summarized below.

Among 12 antibiotics tested, chlorotetracycline was the most effective and penicillin the least. The minimum inhibitory concentrations (MIC) were 0.5 μg/ml and 75 μg/ml respectively. The MIC for most other antibiotics were <10 μg/ml.

Among 14 food preservatives, the most effective one was propyl-p-hydroxybenzoate with the MIC of 0.05 to 0.1 mg/ml, and the least effective was potassium sorbate with the MIC of 2.5 to 10.0 mg/ml. Glycerine was shown to be injurious to *V. parahaemolyticus* at 30% concentration, but not at 15% *(6)*.

Sodium hypochlorite (12% available chlorine), diluted in 3% NaCl to 1/3,000th of its original strength, inactivated *V. parahaemolyticus* within 5 min. In 15% methyl or ethyl alcohol, *V. parahaemolyticus* was inactivated within 30 min. Hydrogen peroxide was also lethal to *V. parahaemolyticus;* a 0.5% solution inactivated the organism within 2.5 min.

Some heavy metals such as gold, silver, and copper were found to be bacteriostatic to *V. parahaemolyticus;* but alminium, tungsten, and tin had no effect.

V. PARAHAEMOLYTICUS CONTROL MEASURES

The major natural reservoir of *V. parahaemolyticus* appears to be the sea and the marine animals we harvest. Preventing contamination of raw materials, therefore, would be nearly impossible. To safeguard seafoods, efforts should be directed to preventing contamination of the finished product, especially those foods that are to be consumed without further cooking. Seafood processors must eliminate the time-temperature abuse. This is essential in controlling the organism which can multiply so rapidly.

Although the organism may originate from the sea, it may find the processing plant environment favorable and may establish a secondary source of contamination somewhere in the plant. How such an ecological niche for *V. parahaemolyticus* may be established was shown by Kaneko and Colwell *(15)*. They investigated the seasonal fluctuation of *Vibrio* species, including *V. parahaemolyticus*, in Chesapeake Bay and found the sediment serving as the reservoir. The organisms were released from the sediment, attached themselves to the plankton and multiplied along with the blooming plankton population during the summer, then resettled to the sediment with the dying plankton in winter.

V. parahaemolyticus was not shown to permanently establish itself in human gut *(28)*. Nevertheless, 0.68 to 3.3% of seafood handlers examined in Japan during summer months harbored *V. parahaemolyticus*, in contrast to 0.33% for the control *(11)*.

V. parahaemolyticus is sensitive to heat, disinfectant, low temperatures, low pH, and tap water. However, none of these treatments, except heat, would inactivate *V. parahaemolyticus* to a safe level. To prevent further outbreaks involving seafoods consumed without further cooking, a terminal heating, followed by adequate refrigeration or freezing, is advisable.

ACKNOWLEDGMENTS

This publication was a result of the support by the National Oceanic and Atmospheric Administration (maintained by the U.S. Department of Commerce) Institutional Sea Grant 04-3-158-4.

REFERENCES

1. Aiso, K. 1967. Generation time of *V. parahaemolyticus* and the growth in contaminated foods. p. 345-350. *In* T. Fujino and H. Fukumi (eds) *Vibrio parahaemolyticus* II, Nayashoten, Tokyo, Japan.

2. Anon. 1972. Isolation and identification of *Vibrio parahaemolyticus*. Suppl. to FDA-Bacteriological Analytical

Manual, 2nd Ed. U.S. Dept. of H.E.W., Washington, D.C.

3. Baross, J., and J. Liston. 1970. Occurrence of *Vibrio parahaemolyticus* and related hemolytic vibrios in marine environment of Washington State. Appl. Microbiol. 20:179-186.

4. Bartley, C. H., and L. W. Slanetz. 1971. Occurrence of *Vibrio parahaemolyticus* in estuarine waters and oysters of New Hampshire. Appl. Microbiol. 21:965-966.

5. Buckmire, F. L. A., and R. A. MacLeod. 1965. Nutrition and metabolism of marine bacteria. XIV. On the mechanism of lysis of a marine bacterium. Can. J. Microbiol. 11:677-691.

6. Chun, D., S. Y. Seol, R. Tak, and C. K. Park. 1972. Inhibitory effect of glycerin on *Vibrio parahaemolyticus* and *Salmonella*. Appl. Microbiol. 24:675-678.

7. Covert, D., and M. Woodburn. 1972. Relationships of temperature and sodium chloride concentration to the survival of *Vibrio parahaemolyticus* in broth and fish homogenate. Appl. Microbiol. 23:321-325.

8. Fishbein, M., I. J. Mehlman, and J. Pitcher. 1970. Isolation of *Vibrio parahaemolyticus* from the processed meat of Chesapeake Bay blue crabs. Appl. Microbiol. 20:176-178.

9. Fishbein, M., and J. C. Olson, Jr. 1971. *Vibrio parahaemolyticus*: A real foodborne disease problem. FDA Papers, Sept.: 16-22.

10. Fujino, T. 1964. From *Pasteurella parahaemolytica* to *Vibrio parahaemolyticus*. p. 13-17. *In* T. Fujino and H. Fukumi (eds) *Vibrio parahaemolyticus* I, Isseido, Tokyo, Japan.

11. Fujino, T. 1967. Report of the 1965 food hygiene sub-committee on *Vibrio parahaemolyticus*. p. 673-725. *In* T. Fujino and H. Fukumi (eds) *Vibrio parahaemolyticus* II, Nayashoten, Tokyo, Japan.

12. Hidaka, T., and D. Kakimoto. 1968. Studies on the marine bacteria. IV. On the effect of minerals on the lysis of bacteria in hypotonic medium. Bull. Jap. Soc. Scientific Fisheries 34:72-77.

13. Hidaka, T., and D. Kakimoto. 1968. Studies on the marine bacteria. Comparison of dissolutions of bacteria in hypotonic medium. Bull. Jap. Soc. Scientific Fisheries 34: 706-711.

14. Kampelmacher, E. H., D. A. A. Mossel, L. M. van Noorle Jansen, and H. Vincentie. 1970. A survey on the occurrence of *Vibrio parahaemolyticus* on fish and shellfish marketed in the Netherlands. J. Hyg. Camb. 68:189-196.

15. Kaneko, T., and R. R. Colwell. 1973. Ecology of *Vibrio parahaemolyticus* in Chesapeake Bay. J. Bacteriol. 113: 24-32.

16. Kodama, T. 1967. Growth of *V. parahaemolyticus* in foods and in food processing environment. p. 351-356. *In* T. Fujino and H. Fukumi (eds) *Vibrio parahaemolyticus* II, Nayashoten, Tokyo, Japan.

17. Krantz, G. E., R. R. Colwell, and E. Lovelace. 1969. *Vibrio parahaemolyticus* from the blue crab *Callinectes sapidus* in Chesapeake Bay. Science 164:1286-1287.

18. Lee, J. S. 1972. Inactivation of *Vibrio parahaemolyticus* in distilled water. Appl. Microbiol. 23:166-167.

19. Liston, J., J. R. Matches, and J. Baross. 1969. Survival and growth of pathogenic bacteria in seafoods. *In* Proc. of Tech. Conf. on Fish. Insp. and Qual. Control. Halifax, Canada 15-25/July, F.A.O.

20. Matches, J. R., and J. Liston. 1968. Growth of salmonellae on irradiated and non-irradiated seafoods. J. Food Sci. 33:406-410.

21. Matches, J. R., J. Liston, and L. P. Daneault. 1971. Survival of *Vibrio parahemolyticus* in fish homogenate during storage at low temperatures. Appl. Microbiol. 21:951-952.

22. Miller, A., III, R. A. Scanlan, J. S. Lee, and L. M. Libbey. 1972. Volatile compounds produced in ground muscle tissue of canary rockfish (*Sebastes pinniger*) stored on ice. J. Fish. Res. Board Can. 29:1125-1129.

23. Molenda, J. R., W. G. Johnson, M. Fishbein, B. Wentz, I. J. Hehlman, and T. A. Dadisman, Jr. 1972. *Vibrio parahaemolyticus* gastroenteritis in Maryland: Laboratory aspects. Appl. Microbiol. 24:444-448.

24. Morbidity and Mortality Report. Center for Disease Control, Atlanta, Georgia. Oct. 2, 1971. 20:356.

25. Morbidity and Mortality Report. Center for Disease Control, Atlanta, Georgia. Dec. 22, 1972. 21:430.

26. Morbidity and Mortality Report. Center for Disease Control, Atlanta, Georgia. Oct. 13, 1972. 21:341-342.

27. Nickerson, R., and C. Vanderzant. 1971. *Vibrio parahaemolyticus*—A review. J. Milk Food Technol. 34:447-452.

28. Saito, M. 1967. Clinical study of *V. parahaemolyticus* infection. p. 295-310. *In* T. Fujino and H. Fukumi (eds) *Vibrio parahaemolyticus* II, Nayashoten, Tokyo, Japan.

29. Sakazaki, R. 1969. Halophilic *Vibrio* infections. p. 115--129. *In* H. Riemann (ed), Food-borne infections and intoxications, Academic Press, Inc., New York.

30. Sakazaki, R., S. Iwanami, and H. Fukumi. 1963. Studies on the enteropathogenic, facultatively halophilic bacteria, *Vibrio parahaemolyticus*. I. Morphological, cultural, and biochemical properties and its taxonomical position. Jap. J. Med. Sci. Biol. 16:161-188.

31. Thomson, W. K., and D. A. Trenholm. 1971. The isolation of *Vibrio parahaemolyticus* and related halophilic bacteria from Canadian Atlantic shellfish. Can. J. Microbiol. 17:545-549.

32. Twedt, R. M., P. L. Spaulding and H. E. Hale. 1969. Morphological, cultural biochemical, and serological comparison of Japanese strains of *Vibrio parahaemolyticus* with related cultures isolated in the United States. J. Bacteriol. 98:511-518.

33. Vanderzant, C., R. Nickerson, and J. C. Parker. 1970. Isolation of *Vibrio parahaemolyticus* from Gulf Coast shrimp. J. Milk Food Technol. 33:161-162.

34. Vanderzant, C., and R. Nickelrson. 1972. Survival of *Vibrio parahaemolyticus* in shrimp tissue under various environmental conditions. Appl. Microbiol. 23:34-37.

35. Ward, B. Q. 1968. Isolations of organisms related to *Vibrio parahaemolyticus* from American estuarine sediments. Appl. Microbiol. 16:543-546.

36. Yanagizawa, F. 1967. Growth inhibition of *V. parahaemolyticus* by drugs. p. 357-365. *In* T. Fujino and H. Fukumi (eds) *Vibrio parahaemolyticus* II, Nayashoten, Tokyo, Japan.