Fish. Technol. (1980) 17, 1-6

Vibrio parahaemolyticus and the Seafood Industry

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The role of *Vibrio parahaemolyticus* in food borne gastroenteritis outbreaks associated primarily with the consumption of contaminated seafoods has been well documented. Information pertaining to various aspects of its occurrence in seafoods, procedures for isolation and identification, generation time and inactivation profiles is discussed. Emphasis has been given to the response of *V. parahaemolyticus* to low temperatures, heating and antibacterial agents. The public health hazard posed by the pathogen is outlined and the guidelines for control are reviewed in detail.

Advent of the "blue revolution" to combat protein malnutrition underlines an urgent need to probe into and ascertain the hazards of diseases transmitted primarily through edible marine species. Consequently quality control and microbiological safety of seafoods has of late, attained paramount importance. Partly because of the introduction of feeding programme on an extensive scale, increased consumption of precooked foods, importation of new sorts of feeds and partly because of improved laboratory techniques, outbreakes of food poisoning have burgeoned from a series of sporadic occurrences to epidemic levels (Wilson, 1973).

Vibrio parahaemolyticus has been incriminated since the 1950's, as the vehicle of numerous outbreaks of food borne gastroenteritis in Japan. Subsequent isolation of this halophilic pathogen in many maritime areas outside Japan triggered attempts to tackle the *V. parahaemolyticus* problem on an international basis. Although several research papers have been published on *V. parahaemolyticus,* information pertinent to the actual control of this organism is rather scarce (Lee, 1973). This paper highlights some idiosyncrasies peculiar to *V. parahaemolyticus* (whose rating as a human health hazard has undergone transformation from a mere "potential" to a a grave "real") thereby outlining safety measures for seafood processors in particular and the public in general.

Occurrence

Being a mild halophile, the distribution of *V. parahaemolyticus* is chiefly confined to estuarine, brackish and coastal environs. Numerous workers have documented the occurrence of *V. parahaemolyticus* in various species of fin fish, shrimps, prawns, oysters, mussels, clams, crabs, snails and periwinkles (Krantz *et al.,* 1969; Leistner *et al.* 1971; Thompson & Trenholm, 1971; Kampelmacher *et. al.,* 1972 Chatterjee & Neogy, 1972; Vanderzant *et al.,* 1973; Sutton, 1974: Chun *et al.,* 1974; Sizemore *et al.,* 1975; De *et al.,* 1977; Natarajan *et al.,* 1979 a, b). Suitable growth substrates for *V. parahaemolyticus* are however not limited to fish and shellfish alone as it can also grow on salted extracts of vegetables like cabbage, raddish, lettuce, cucumber, potato and pumkin. Kudoh *et al.,* (1974) in their paper on the epidemiology of food poisoning due to *V. parahaemolyticus* in Tokyo from 1963 to 1972, categorised the implicated food items as raw marine fish and shellfish (38.9%), foods containing fish and shellfish (38.9%), unknown (12%), processed sea products (6.4%) and vegetables and their products (3.6%).

Mariculture

Despite the fact that *Vibrio* spp. have long since been implicated in diseases and mass mortalities in cultured fish and shellfish, *V. parahaemolyticus* was not recognised until recently. Kusuda (1968)

suspected *V. parahaemolyticus,* alongside *V. alginolyticus* and *V. anguillarum,* to be the causative agents of an ulcer-like disease among cultivated fish in Wasaka Bay, Japan. Krantz *et al.,* (1969) observed that the haemolymph of moribund blue crabs, *Callinectes sapidus* consistently harboured higher counts of *V. parahaemolyticus* than their healthy counterparts. Vanderzant and Nickelson (1973) demonstrated that, under certain cultural conditions, *V. parahaemolyticus* caused death of laboratory reared postlarvae and adults of the brown shrimp (*Penaeus azetecus).* Eventhough *V. parahaemolyticus* poses a potential threat to maricultural operations, it must be emphasized that its direct pathogenicity to fish and shellfish is yet to be fully established.

Isolation and identification

Among the numerous isolation and identification schemes formulated for *V. parahaemolyticus* the most commonly employed one is that described in the Bacteriological Analytical Manual for Foods (Food Drug Administration Publication Washington, USA). The method which was evolved after an exhaustive study of the productivity of several Japanese and American techniques is briefly outlined in Tables 1a and 1b.

Table 1a. **Analytical method for V. parahaemolyticus*

Isolation procedure	
Phase	Seafoods
	(Fish, shellfish and crustaceans) 50g + 450 ml 3% NaCl diluent (10° to 10^{-4} dilutions)
Day 1 (Enrichment)	Glucose salt teepol broth (Enumeration by 3-tubes most probable number method)
Day 2 (Isolation)	Thiosulfate citrate bile salts sucrose agar (Bluish-green colonies with darkened centres)
Day 3 (Screening)	Triple sugar iron agar Motility agar Trypticase soy agar Trypticase soy broth
Day 4 (Identification)	Biochemistry Serology

Table 1b. **Identifying characteristics*

Test	Response
Gram stain	—
Morphology	Curved/straight rods
Motility	+
Triple sugar iron agar	K/A. H_2S (–), gas (–)
Hugh-Leifson glucose	Fermentation (+), gas (—)
Cytochrome oxidase	+
Arginine dihydrolase	—
Lysine decarboxylase	+
Gelatin	+
Halophilism (NaCl)	
6% & 8%	+
0% & 10%	—
Growth at 42° C	+
Voges proskauer	—
Indole	+
Cellobiose	—
Sucrose	—
Maltose	+
Mannitol	+
Trehalose	+

* Adapted from FDA By-Lines 4 (2), Consecutive No. 56, September, 1973

Generation time

Generation times ascribed for *V. parahaemolyticus* under optimum conditions are in the range of 8 to 14 min. Katoh (1965) observed that *V. parahaemolyticus* had a generation time of 11 to 13 min at 37°C, both on nutrient and fish extract liquid medium. An astonishingly short generation time of 7.6 min at the logarithmic growth phase was recorded by Aiso (1967) for *V. parahaemolyticus* cultured in brain-heart infusion broth (pH 7.9, supplemented with 1.5% NaCl) at 37°C. Ulitzur (1974) surmised that strains of *V. parahaemolyticus* could be categorized into two groups, one having a short generation time of 12

to 14 min at 37°C and the other with a longer generation time of 20 to 25 min at 37°C.

The extremely short generation time exhibited by *V. parahaemolyticus* under optimum temperatures is of distinct concern when considering its epidemiology. Assuming an average generation time of 10 min, over 10^6 cells could be produced from an initial number of only 10 cells in a matter of 3 hours (Nickelson, 1974). In most outbreaks of *V. parahaemolyticus* gastroenteritis, the incriminated food items were found to harbour relatively low counts of the pathogen originally but were subsequently stored at temperatures favouring proliferation of the bacterium. Such storage prior to consumption would permit *V. parahaemolyticus*, characterised by a short generation time, to attain hazardous levels. Lack of putrefactive appearance, unpalatable flavour or foul odour of fishery products implicated in *V. parahaemolyticus* food poisoning seem to be due to a quantitative rather than a qualitative difference, attributable to the much shorter generation time of *V. parahaemolyticus* as compared to other indigenous microflora and spoilage bacteria (Lee,1973).

Inactivation in distilled water

Studies to determine the kinetics of *V. parahaemolyticus* inactivation in distilled water and other hypotonic solutions were initiated *a posteriori* to cognition of its halophilic nature. Yanagizawa (1964) and Lee (1972) demonstrated that *V. parahaemolyticus* was highly susceptible to suspension in distilled water. In the former report an exposure of less than 10 min yielded a 10^5 fold reduction in viable cells of *V. parahaemolyticus*, while in the latter, the exposure time required to inactivate 90% of the cells was only 0.9 to 4.4 min.

In view of such reports on the osmotic fragility of *V. parahaemolyticus*, it was presumed that washing of seafoods and kitchen utensils with tap water might prove to be an effective preventive measure of *V. parahaemolyticus* infection. However traces of salt and organic substances in freshwater may reduce the efficacy of this measure (Sakazaki, 1973). In fact Hidaka & Kakimoto (1968) showed that the inactivation process was reversible provided the cells were returned to salt solution within 10 min.

Inactivation at elevated temperatures

Susceptibility of *V. parahaemolyticus* to inactivation at elevated temperatures is largely dependent on the cultural procedures used to grow the organism along with the type of menstruum in which the pathogen is harboured during the heat treatment (Beuchat, 1975). Vanderzant & Nickelson (1972) demonstrated that survival of *V. parahaemolyticus* in shrimp homogenate heated to 60 or 80°C for 15 min was determined by its initial population, the two being linked by a positive relationship. In contrast, regardless of the initial population, no survivors were detected in homogenates heated to 100°C for 1 or 5 min. Thermal inactivation times of 30, 15 and 5 min at 60°C have been reported by Liston *et al.* (1971), Sakazaki (1973) and Temmyo (1966) respectively. Further, Temmyo (1966), by simulating heating conditions normally encountered in processing plants, restaurants and homes, showed that *V. parahaemolyticus* could not be recovered from samples categorised as being "well cooked". In cases of *V. parahaemolyticus* gastroenteritis traced to consumption of cooked products, infection may therefore be attributed to insufficient heating or secondary contamination with subsequent storage at temperatures permitting multiplication of the pathogen.

Baab & Johnson (1974) and Goldmintz (1974) observed diphasic (fast followed by slower rates of death) thermal inactivation curves for several strains of *V. parahaemolyticus* heated at 47 to 49°C. A protective function against thermal inactivation of this halophilic bacterium seems to be afforded by the addition of sodium chloride to menstruum heated at 48°C (Covert & Woodburn, 1972; Goldmintz, 1974). Vanderzant *et al.* (1974) correlated the resuscitation efficacy of thermally stressed cells of *V. parahaemolyticus* to the level of sodium chloride in the recovery media.

Inactivation at low temperatures

Optimum temperatures reported for the growth of *V. parahaemolyticus* range from 35 to 37°C. The susceptibility of *V. parahaemolyticus* to low temperatures is well reflected in its seasonal abundance in natural environs during summer and its apparent "disappearance" during winter. While temperatures of 3 to 13°C have been shown to support growth of this organism, the lowest growth temperature in laboratory media was 5°C (Beuchat, 1973).

Matches *et al.* (1971) documented log reduction values of 2.2 to 6.2 in about 12 days for *V. parahaemolyticus* in fish homogenate stored at –18 and –34°C. Vanderzant & Nickelson (1972) reported that after an initial 2 log reduction within two days, the number of *V. parahaemolyticus* in inoculated whole, peeled and deveined shrimp held at 3, 7, 10 and –18°C remained relatively constant over the next six days.

Temmyo (1966), Liston *et al.* (1971) and Johnson *et al.* (1971) reported recovery of *V. parahaemolyticus* from frozen seafoods, thereby emphasizing the need for proper storage and terminal heating prior to consumption. The works of Matches *et al.*. (1971) and Johnson & Liston (1973) indicate that *V. parahaemolyticus* on marine fish and shellfish is more susceptible to the lethal effects of chilling rather than to commercial freezing. However, *V. parahaemolyticus* seems to exhibit some degree of strain variability with regard to the effects of chilling and freezing on the survival of the pathogen in different foodstuffs held at the same temperature. Hence chilling or freezing *per se* cannot be relied upon to protect the consumer from seafoods which are subsequently exposed to conditions permitting outgrowth of this organism to hazardous levels (Liston, 1974).

Response to antibacterial agents

The relative efficiencies of various antibiotics, detergents, disinfectants and food preservatives against *V. parahaemolyticus* was extensively investigated by Yanagizawa (1967). The following is a list of some potent compounds inhibitory to *V. parahaemolyticus* (cited from Lee, 1973). (a) 0.5 μg/ml chlorotetracycline (b) 0.05 to 0.1 mg/ml propyl-p-hydroxybenzoate (c) 30% glycerine (d) 12% commercially available sodium hypochlorite diluted with 3% sodium chloride to 1/3000th of its original concentration (e) 15% methyl or ethyl alcohol and (f) 0.5% hydrogen peroxide. Sanyal *et al.* (1973) tested the drug sensitivity of 111 strains of *V. parahaemolyticus* to several antibacterial agents and reported that all strains were sensitive to common antibiotics like streptomycin, tetracycline, neomycin, chloramphenicol, kanamycin, gentamycin and polymyxin B.

The antibiotic sensitivity pattern of 230 strains of *V. parahaemolyticus* (isolated from cases of gastroenteritis in Calcutta) was evaluated by Sen *et al.* (1977). Their results revealed that gentamycin and chloramphenicol inhibited 99.2% and 92.6% of the strains tested respectively. Doxycycline, neomycin and tetracycline were only moderately effective against the pathogen while ampicillin, kanamycin and streptomycin proved to be ineffective. Based on these findings Sen *et al.* (1977) recommened that chloramphenicol (equally effective against cholera vibrios), rather than the routinely prescribed tetracycline, should be the antibiotic of choice for treatment of *V. parahaemolyticus* induced human gastroenteritis.

Control measures

The ubiquitous distribution of *V. parahaemolyticus* in the marine milieu renders the protection of raw seafoods against contamination with this pathogen virtually out of question. An examination of 635 seafoods, comprising 30 marine species, by scientists of the Food and Drug Administration, U. S. A. (Fishbein *et al.*, 1974) revealed that over 70% of the samples tested harboured *V. parahaemolyticus*. However only 14% of the positive samples contained viable cells of *V. parahaemolyticus* at a level of 10^3/g of seafood. The study indicated that naturally contaminated seafoods normally harbour low counts of 100 cells or less per gram.

V. parahaemolyticus is considered to be the only "causative organism" of seafood-borne illness, the cause as such being mishandling of the incriminated food

product (Nickelson, 1974). Most, if not all, outbreaks of gastroenteritis attributed to *V. parahaemolyticus* can be traced to seafoods with a history of unhygienic production, improper cooking, post-cooking contamination or inadequate refrigeration prior to consumption. Cross contamination through kitchen utensils, chopping boards and knives previously employed for seafoods may be responsible for cases of food poisoning wherein salted vegetables and other nonmarine food items have been implicated.

Under current technology it would seem quite impossible to suggest microbial standards for an organism that can be considered as part of the "natural flora" of freshly harvested seafoods since the high infective dose (10^6 to 10^9) of *V. parahaemolyticus* needed to produce illness would only undermine failure to comply with existing good manufacturing practices (Nickelson, 1974). Simple but strict hygienic measures to check multiplication of the pathogen in seafoods, proper thermal treatment and prevention of secondary contamination from raw seafoods appear to be the most tangible methods of control of infection in man (Sakazaki, 1973). Barrow (1974) aptly sums up the guidelines of control. According to him local culinary habits play a significant role in foodborne illness and food well-cooked shortly before consumption is always preferable. Since established customs die hard, safety is ultimately dependent not-so-much upon arbitrary microbiological standards, but on hygienic production, correct storage, proper distribution and most important of all, on the education of right eating habits.

The authors wish to thank the Indian Council of Agricultural Research and University Grants Commission for financial assistance.

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