

Asian Fisheries Society, Manila, Philippines
Short Communication

Growth and Survival of Kanagawa Positive *Vibrio parahaemolyticus* in Fish and Prawn Preparations Held at Ambient and Elevated Temperature

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Abstract

Vibrio parahaemolyticus is a common cause of gastroenteritis associated with seafood consumption. Though this organism is killed during cooking, there are chances of post process contamination where raw and cooked fish are handled in the same area. The fate of this organism during post-process contamination of cooked and ready to serve fish and prawn preparations stored at ambient temperature as well as elevated temperature (50°C) was investigated. *V. parahaemolyticus* could survive in fish preparations up to 48 h at ambient temperature. In prawn preparations, an increase in counts was seen at ambient temperature. Longer survival period of the same when held at 50°C was observed in prawn in comparison to fish preparations. If contaminated, these foods could support the growth of *V. parahaemolyticus* and cause food borne infection.

Introduction

V. parahaemolyticus, a halophilic marine bacterium has been implicated as the cause of food poisoning upon consumption of contaminated fish and

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fishery products. Its occurrence in estuarine and coastal waters and its association with fish and shellfish is well established (Joseph et al. 1982). Presence of this bacterium in seafoods is of public health concern because of its ability to cause gastroenteritis in man.

In Japan nearly 70% of food borne outbreaks of gastroenteritis during the summer months has been reported to be due to this organism (Joseph et al. 1982). In India, *V. parahaemolyticus* was first isolated from cases of gastroenteritis in Calcutta as early as 1970 (Chatterjee et al. 1970). About 8 to 10% of all cases of acute gastroenteritis reported to the Infectious Diseases Hospital in Calcutta were due to this bacterium (Mazumdar et al. 1977). Involvement of *V. parahaemolyticus* in gastroenteritis among some staff members at the Christian Medical College and Hospital, Vellore following a social get-together has further emphasized the public health hazard caused by this organism in India (Lalitha et al. 1983).

The public health hazard of seafoods due to *V. parahaemolyticus* depends on its actual level in seafoods, the effect of normal handling and processing of this organism, and its capability to multiply to an infective level in the food prior to consumption (Liston 1974). The survival of this organism in seafoods subjected to chilling (Vanderzant and Nickelson 1972, Thomson and Thacker 1973, Liston 1974, Karunasagar et al. 1985), freezing (Matches et al. 1971, Covert and Woodburn 1972, Johnson and Liston 1973), heating (Liston et al. 1971, Vanderzant and Nickelson 1972, Beuchat 1973), drying (Venugopal et al. 1984) and smoking (Alvarez 1982, Karunasagar et al. 1986) has been reported. In South Asian countries, fish is cooked with plenty of spices, some of which may have antibacterial effects.

Cells of *V. parahaemolyticus* are known to be completely destroyed in well cooked food. However, instances of food poisoning due to *V. parahaemolyticus* incriminating seafoods may be due to the habit of consuming raw or semi-cooked foods or to post-process contamination of foods with this organism. Against this background, experiments were designed to study whether *V. parahaemolyticus* could survive in contaminated common fish and prawn preparations stored at ambient temperature as well as at slightly elevated temperature.

Materials and Methods

Bacterial culture

A Kanagawa positive strain of *V. parahaemolyticus*, TY 49, Serotype Δ 03 K57 isolated from a case of gastroenteritis was used for this study.

Fish and prawn preparations

The standard preparation of fish (fish 'curry' and fish 'fry') and prawn (prawn 'curry' and prawn 'fry') were procured from a local restaurant and

brought to the laboratory in a sterile container. A 'curry' is a thick viscous preparation, wherein the fish/prawn is cooked in well ground coconut and a paste of spices including garlic, chilies, ginger and coriander, while a 'fry' is a dry preparation done by shallow frying the fish/prawn in oil.

Survival of *V. parahaemolyticus* in fish and prawn preparations at ambient temperature

Samples of fish and prawn 'curry' were contaminated by adding an 18 h-old culture of *V. parahaemolyticus* grown in trypticase soy broth (TSB, Hi Media, Bombay) at 37°C. The fish and prawn 'fry' were contaminated by dipping them for 1 min in TSB culture of *V. parahaemolyticus*. These recipes were contaminated to get an initial cell count of 10^5 to 10^7 cfu·g⁻¹ of the samples.

The contaminated samples were stored in sterile glass beakers covered with glass lid at ambient temperature ($30 \pm 2^\circ\text{C}$). From these stored samples, 10 g aliquots were drawn at 0, 24 and 48 h time intervals and homogenized with 90 ml saline containing 3% sodium chloride. Appropriate dilutions in 0.1 ml amounts were surface plated on thiosulphate citrate bilesalt sucrose agar (TCBS, Hi Media, Bombay) and incubated at 37°C for 24 h.

Typical bluish green colonies appearing as TCBS agar medium were counted as *V. parahaemolyticus* like organisms and confirmed as *V. parahaemolyticus* after subjecting them to biochemical tests considered as minimum to identify *V. parahaemolyticus* (Hugh and Sakazaki 1972) and expressed as colony forming units per gram (cfu·g⁻¹) of the sample. The experiment was repeated thrice and results of one typical batch has been presented.

Survival of *V. parahaemolyticus* in fish and prawn preparations held at elevated temperature

In this experimental trial, only a standard preparation of fish and prawn 'curry' procured from a local restaurant was used. These preparations were taken in sterile glass beakers, covered with glass lid and placed inside the water bath maintained at $50 \pm 1^\circ\text{C}$. When the temperature of the preparation reached $50 \pm 1^\circ\text{C}$, an 18 h-old TSB culture of *V. parahaemolyticus* was added to get an initial cell count of 10^5 to 10^7 cfu·g⁻¹ of the sample. Samples were drawn at regular time intervals and *V. parahaemolyticus* counts were determined following the procedure outlined above. The experiment was repeated thrice and result of one typical batch has been presented.

Results and Discussion

The survival pattern of *V. parahaemolyticus* in artificially inoculated fish and prawn preparations stored at ambient temperature is shown in

table 1. Both fish and prawn recipes were initially free from *V. parahaemolyticus*. It is evident from table 1 that in fish curry, the counts declined by one log unit in a 24 h storage period while in fish 'fry' the decrease was marginal. However, in a 48 h storage period, this organism could not be detected in the fish preparations. The initial decrease in *V. parahaemolyticus* counts in fish 'curry' could be attributed to the inhibitory effect of several spices used in the preparation of these foods. Studies conducted by Beuchat (1976) have shown the inhibitory activities of several commonly used spices in food preparation on *V. parahaemolyticus*. Failure to detect *V. parahaemolyticus* in 48 h storage time may be due to the over growth of this organism by the native microflora of the fish preparation, as the same was found to be spoilt after 48 h storage.

On the contrary, there was an increase of two log units in the counts of *V. parahaemolyticus* in the case of prawn 'curry' and 'fry' in a 24 h storage period and the same counts were observed even at 48 h storage period. The growth of *V. parahaemolyticus* in both prawn 'curry' and 'fry' is interesting since these preparations also contain the spices used in fish 'curry' and 'fry', but the multiplication could be attributed to the favorable nature of prawn meat constituents for the growth of this organism.

In the above two preparations, there was clear multiplication in 24 h storage period, after which the counts either stabilized as in the prawn 'curry' or showed slight decline as in the case of prawn 'fry'. Even if *V. parahaemolyticus* were killed during cooking there would be chances of post-cooking contamination in the kitchen. The results of this study suggest that

Table 1. Survival of *V. parahaemolyticus* in fish and prawn preparations at ambient temperature.

Recipe	Storage period (h)		
	0	24	48
	Counts of <i>V. parahaemolyticus</i> (cfu·g ⁻¹)		
Fish 'curry'	2.08 x 10 ⁷	3.90 x 10 ⁶	Nil
Fish 'fry'	7.70 x 10 ⁶	1.34 x 10 ⁶	Nil
Prawn 'curry'	4.00 x 10 ⁵	5.60 x 10 ⁷	2.30 x 10 ⁷
Prawn 'fry'	1.51 x 10 ⁶	1.20 x 10 ⁸	1.60 x 10 ⁷

Table 2. Survival of *V. parahaemolyticus* in fish and prawn preparations at 50°C.

Storage period (min)	Preparations	
	Fish 'curry'	Prawn 'curry'
	Count of <i>V. parahaemolyticus</i> (cfu·g ⁻¹)	
0	1.13 x 10 ⁵	3.40 x 10 ⁷
5	1.25 x 10 ³	1.14 x 10 ⁶
10	0.50 x 10 ¹	2.50 x 10 ⁵
20	0.50 x 10 ¹	1.70 x 10 ⁴
40	ND	1.80 x 10 ³
60	ND	7.00 x 10 ²

ND: Not detectable

if such contamination occurs, there are chances that *V. parahaemolyticus* multiply in the prawn preparations despite the addition of spices.

It is common practice that left over fish/prawn preparations are warmed before the next meal or next day consumption. The results presented in table 2 show that *V. parahaemolyticus* survived better in prawn 'curry' held at 50°C, as compared to fish 'curry'. In the prawn preparation, low numbers of this organism was detected after a holding time of 60 min at 50°C, whereas in fish 'curry', the organism could not be detected after 40 min.

The results suggest that conditions for survival of *V. parahaemolyticus* are better in prawn 'curry', as compared to fish 'curry'. Vanderzant and Nickelson (1972) have reported the survival of this bacterium in shrimp tissue cooked at 80°C. A survey conducted to find out the presence of *V. parahaemolyticus* in cooked prawns and shrimps at retail sale point revealed the absence of this organism in all the samples tested, indicating the safety of cooked prawns (Greenwood et al. 1985).

However, the results of the present study indicate that if post cooking contamination occurs, there are chances of multiplication in the recipes and holding the foods at higher temperature or mere warming would fail to eliminate the health hazards due to *V. parahaemolyticus*. Thus, it would be important to heat stored foods to boiling point of water (100°C) just before consumption to kill *V. parahaemolyticus* if there are any, and render the foods safe from this bacterium.

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