

OCCURRENCE OF *VIBRIO PARAHAEMOLYTICUS* IN MARINE PRAWNS AND ENVIRONMENTS

S. VICTOR CHANDRA BOSE & FREDA CHANDRASEKARAN

Office of the Assistant Director of Fisheries, Thanjavur-613007

Qualitative studies on the microflora of slime and guts of prawns and of sea water off Nagapattinam showed the presence of *Vibrio* in the slime and sea water. They were further tested for *Vibrio parahaemolyticus* types and related bio-types. Evidence of its occurrence is given. This points to the need for further studies on the distribution of this organism in terms of public health significance.

INTRODUCTION

In many countries *Vibrio parahaemolyticus* is gaining much importance as a food borne pathogen associated with fish products. It is reported to be a causative agent of gastroenteritis. In Japan, Fujino *et al.* (1953) first reported food poisoning caused by *Vibrio parahaemolyticus* from consumption of fish products. In the United States *Vibrio parahaemolyticus* was first isolated in 1968 (Liston *et al.* 1967; Baross and Liston, 1968). Extensive studies on their occurrence, identification, distribution, pathogenicity and tolerance towards low and high temperatures have been made in the past few years (Baross and Liston, 1970; Matches, Liston and Daneault 1970; Matches and Liston 1971). But the occurrence and isolation of *Vibrio parahaemolyticus* in marine fish or shell fish

has not so far been reported from India. In this communication the presence of *Vibrio parahaemolyticus* in marine prawns and sea water off Nagapattinam is reported.

MATERIALS AND METHODS

Trawling operations were carried out about 5 km. from the shore off Nagapattinam at a depth of 3-4 fathoms. Immediately after hauling, slime and the gut contents of prawns were separately sampled. Sea water from the place of catch was also examined. Plating was carried out within 4 hours on suitably diluted samples using Fischer's media consisting of Bacto peptone 1 g., fish extract 0.5 g., ferric phosphate 0.01 g., agar 1.5 g. and sea water 100 ml. The colonies developed were picked from the plates into test tubes of sea water media. An attempt

was made in the selection of colonies to ensure that preponderant types were fairly represented numerically among the isolates according to their occurrence on the plates. Colonies of unusual appearance were also selected to allow some estimate of the range of bacterial types occurring on the prawns. Each isolate was restreaked repeatedly to obtain pure culture. About 100 cultures thus prepared were studied for their morphological characteristics and bio-chemical properties as per standard methods. They were screened according to a modified method of Simidu and Aiso (1962) and results given in Table I.

The *Vibrio* spp. from slime and sea water samples were streaked onto salt-starch-agar plates (0.5% soluble starch, 0.3% peptone (Bacto), 0.1% yeast extract (Bacto), 5% NaCl., 1.5% agar and distilled water; adjusted to pH 7.5) and incubated anaerobically at 37°C for 24 hours. The halophilic Vibrios from slime

and sea water samples readily grew on this media indicating starch hydrolysis as a halo around the colony. These presumptive positive Vibrios were recovered and maintained in another medium; tryptone 0.5 g., yeast extract 0.5 g., beef extract 0.2 g., sodium acetate 0.2 g., agar 0.4 g., distilled water 500 ml. and sea water 500 ml. pH adjusted to 7.2 and suitably buffered. (Read, 1973).

The Gram negative, oxidase positive pleomorphic rods were then streaked onto human blood agar to examine haemolysis, a presumptive test for *Vibrio parahaemolyticus*. The presumptive positive *Vibrio parahaemolyticus* isolates were then tested for salt tolerance in tripticase broth and subjected to several other tests as listed in Table II (Johnson, Baross and Liston, 1971).

Parallel experiments were conducted with type cultures of *Vibrio parahaemolyticus* (8700 PREI) and *Vibrio alginolyticus*

TABLE I
Distribution of bacterial genera in fresh prawns and sea water
from the offshore region of Nagapattinam

	Genus	Slime	Gut	Sea water
1.	<i>Flavobacterium</i> spp.	15%	46%	27%
2.	<i>Achromobacter</i> spp.	22%	13%	23%
3.	<i>Vibrio</i> spp.	28%	—	20%
4.	<i>Pseudomonas</i> spp.	10%	25%	7%
5.	<i>Photobacterium</i> spp.	10%	8%	3%
6.	<i>Bacillus</i> spp.	10%	8%	10%
7.	<i>Corynebacterium</i> spp.	2.5%	—	10%
8.	<i>Micrococcus</i> spp.	2.5%	—	—

TABLE II
General properties of
Vibrio parahaemolyticus

Test	Reaction
Growth in 1% trypticase broth with 0% NaCl.	—
3% NaCl.	+
7% NaCl.	+
10% NaCl.	—
Glucose (acid)	+
Lactose (acid)	—
Sucrose (acid)	—
Cellobiose (acid)	+
Maltose (acid)	+
Mannitol (acid)	+
Starch (hydrolysis)	+
Chitin digestion	+ (—)
NO ₃ reduction	+
Gelatin liquefaction	+
Hugh-Leifson (anaerobic) acid only	+
Oxidase (positive)	+
Hemolysis of blood	+
Penicillin sensitivity (2-5U)	—
Single polar flagella	+
Gram stain (reaction)	—

(374-V) received from Institute of Food Science and Technology, U. S. A. The bio-types of *Vibrio parahaemolyticus* were differentiated by sucrose fermentation.

RESULTS AND DISCUSSION

Among the bacterial flora of prawns and sea water of the place of catch *Vibrio* spp. formed 28% in slime and

20% in the sea water. Nine isolates from slime and four isolates from sea water hydrolysed starch with the formation of a clear zone. When these starch hydrolysing halophilic Vibrios were tested for haemolysis of human blood 9 from slime and 2 from sea water haemolysed blood. These presumptive positive *Vibrio parahaemolyticus* strains were tested for their various other bio-chemical characteristics along with the U. S. strain. (Table III). Out of the 11 haemolytic vibrio isolates, two isolated from the slime of prawns showed biochemically identical properties of *Vibrio parahaemolyticus*. The rest of the haemolytic Vibrios differed in any one or two of the tests for salt tolerance, sucrose and cellobiose fermentation. The other bio-types *Vibrio alginolyticus* and *Vibrio alginarum* were absent according to the present pattern of identification of haemolytic Vibrios.

The Japanese investigators differentiated *Vibrio parahaemolyticus* and its bio-types by sucrose fermentation reaction. But the American workers recorded that 7 out of 40 Japanese type strains of *Vibrio parahaemolyticus* utilised sucrose. In our study we find both the American strains and Nagapattinam isolates utilise sucrose. Baross and Liston (1970) contended that all haemolytic mesophilic Vibrios meeting the general classification of *Vibrio parahaemolyticus* without regard to sucrose fermentation are to be described as *Vibrio parahaemolyticus*. Our observations also strengthen this conclusion.

The occurrence of *Vibrio parahaemolyticus* in Nagapattinam region suggests that an extensive study of the distribution of this organism and related haemolytic Vibrios in the marine environment of inshore coastal

TABLE III

Bio-chemical characteristics of U. S. strains of *Vibrio parahaemolyticus* and haemolytic Vibrios isolated from Nagapattinam region.

Reaction	U. S. strain	Nagapattinam strain
1. Growth in 1% Trypticase broth with 0% NaCl	—	—
with 3% NaCl	+	+
with 7% NaCl	+	+
with 10% NaCl	—	—
2. Acetyl methyl carbinol	—	—
3. Methyl red	—	—
4. Nitrate reduction	+	+
5. Hemolysis (human blood)	+	+
6. Growth at 40°C	+	+
7. Sucrose (acid only)	pH 7.6 reduced to 5.5 after 20 hours of inoculation	pH 7.6 reduced to 5.5 after 20 hours of inoculation
8. Gelatin liquifaction	+	+
9. Starch hydrolysis	+	+
10. Sensitivity to penicillin (2-5U)	—	—
11. Hugh-Leifson anaerobic (acid only)	+	+
12. Glucose (acid)	+	+
13. Lactose (acid)	—	—
14. Cellobiose (acid)	—	—
15. Maltose (acid)	+	+
16. Mannitol (acid)	+	+
17. Colony morphology	Smooth	Smooth

waters of Tamil Nadu is essential for evaluating the public health hazard from this organism.

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