CHITINOLYTIC BACTERIA IN *PENAEUS INDICUS*

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ABSTRACT

Quantitative distribution of chitinolytic bacteria from the hepatopancreas, stomach and intestine of a commercially important marine prawn *Penaeus indicus* was studied. Twentyfive representative bacteria were isolated from colonies forming well defined clear zones on chitin agar medium and were identified. Eighteen of the isolates were members of the genus *Vibrio* and the rest were members of the genus *Pseudomonas*.

Distribution of chitin has been reported in the exoskeleton of crustacean (ZoBell, 1946), mollusca, coelenterata, protozoa and in cell walls of certain molds and filamentous yeasts (Nabel, 1939) and principally among the Arthropoda-crabs and insects (Karlson, 1963). Abundance of chitin in marine and estuarine environment is well known and chitinolytic role of marine bacteria has been suggested (Aaronson, 1970). The chitinolytic enzyme found in the digestive contents and glands or in the intestinal mucosa in several species of vertebrata is reported to be indigenous to the system instead of being of bacterial origin (Jeuniaux, 1961). Chitinolytic activity has also been reported in the digestive tract of *Lateolabrax japonicus* (Okutani, 1966) and *Mughil cephalus* (Hamid *et al.*, 1979), and bacteria were also found in their alimentary tracts.

So, study was undertaken to identify the bacteria truly involved in chitinolytic activity in the digestive tracts, viz. hepatopancreas, stomach and intestine of a commercially important marine prawn *Penaeus indicus* collected from Narakkal Prawn Culture Laboratory (NPCL), Cochin, India.

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MATERIALS AND METHODS

5 nos. healthy and mature specimens of prawn *Penaeus indicus* were collected from the NPCL ponds, Cochin, India. The prawns were immediately washed with 1% $HgCl_2$ solution for surface sterilization. After repeated washing with sterile distilled water their hepatopancreas, stomach and intestine were dissected from the alimentary tract and put into 100 ml sterile blank saline solution (0.85%) separately for serial dilution. Aseptic condition was maintained throughout the experiment. For enumeration of chitinolytic bacteria, pour plating was carried out on mineral agar medium (Aaronson, 1970) containing 2.5% NaCl with chitin precipitate as the sole source of carbon and energy. Separately sterilized chitin precipitate was supplemented to the melted mineral agar medium until it became turbid. pH of the mineral medium was adjusted to 7.0. Precipitation of chitin has been discussed by Tracey (1955). Enumeration of total aerobic heterotrophic bacteria was carried out by using ZoBell's 2216 E agar medium and chitinolytic bacteria were studied (Tracey, 1955) from 10^5 and 10^6, and 10^4 and 10^5 dilutions respectively, following pour plate technique. The plates were incubated at 30°C for three days in case of total heterotrophic bacteria and for seven days in case of chitinolytic bacteria. Clear zone forming colonies were counted only in case of chitinolytic bacteria. Best incubation temperature were determined by incubating slant cultures at temperatures of 25°, 30° and 37°C. Poor growth was observed at 25° and 37° C but satisfactory growth was noticed at 30°C.

Table 1. Number of chitinolytic bacteria and total viable aerobic heterotrophic bacteria

Prawn specimen number	Region of isolation	Number × 10^5 / g		% of chitinolytic bacteria
		Total bacteria	chitinolytic bacteria	
	S	170	18	10.58
P_1	H	124	22	17.74
	I	46	6	13.04
	S	164	27	16.46
P_2	H	184	37	20.10
	I	120	11	9.16
	S	167	15	8.98
P_3	H	192	27	14.06
	I	98	8	8.16
	S	122	12	9.83
P_4	H	168	24	14.28
	I	64	5	7.81
	S	122	21	17.21
P_5	H	193	37	19.17
	I	87	17	19.54

S—Stomach, H—Hepatopancreas, I—Intestine.

Twenty-five isolates of chitinolytic bacteria were taken from well defined clear zone produced colonies. Cultures were purified by repeated subcultures. Isolates of pure cultures were maintained on nutrient agar slants. Their morphological, cultural and biochemical characters were studied following Bergey's manual (Buchanan and Gibbons, 1974) for identification.

Table 2. Differential biochemical characters of the isolated bacteria

Isolate Nos.	M.O.F. Test	Hydrolysis of starch	Hydrolysis of gelatine	T.S.I.*	V.P. Test	M.R. Test	Nitrate reduction	Ammonia production	Production of Indole	Response to 0/129	Identified as genus
X_1	+	+	+	–	+	–	+	+	–	+	1
X_2	+	+	+	+	+	–	+	+	+	+	1
X_3	+	+	+	–	+	–	+	+	+	+	1
X_4	–	–	–		+	+	+	+	+	–	2
X_5	+	+	–	+	+	–	+	+	–	+	1
X_6	+	–	+	–	+	–	+	+	–	+	1
X_7	–	–	+	–	+	+	–	+	+	–	2
X_8	+	+	–	+	+	–	+	+	–	+	1
X_9	+	+	–		+	–	+	+	–	+	1
X_{10}	+	+	+	–	+	–	+	+	+	+	1
X_{11}	+	–	+	–	+	–	+	+	+	+	1
X_{12}	–	+		–	+	+	+	–	+	–	2
X_{13}	–	+	+		+	+	+	+	+	–	2
X_{14}	+	+	+	–	+	–	+	+	+	+	1
X_{15}	+	+	–	–	+	–	+	+	–	+	1
X_{16}	+	–	+	–	+	–	+	+	–	+	1
X_{17}	+	+	+	+	+	–	+	+	–	+	1
X_{18}	+	+	+	–	+	–	+	+	+	+	1
X_{19}	–	+	–	–	+	+	+	–	–	–	2
X_{20}	+	+	+	+	+	–	+	+	–	+	1
X_{21}	+	+	+	–	+	–	+	+	+	+	1
X_{22}	–	+	+	–	+	–	–	+	–	–	2
X_{23}	+	–	–	–	+		+	+	+	+	1
X_{24}	+	–	+	–	+	–	+	+	+	+	1
X_{25}	–	+	+	+	+	+	+	+	+	–	2

* No isolate produced any gas like CO_2 or H_2S. + indicates positive; – indicates negative. 1 indicates *Vibrio*, 2 indicates *Pseudomonas*. M.O.F. Marine oxidative fermentation. T.S.I. Triple sugar iron; V.P. Voges-Proskauer; M.R. Methyl red; 0/129 2,4 diamino-6,7-diisopropyl pteridine.

RESULTS AND DISCUSSION

The percentage of chitinolytic bacteria in total aerobic heterotrophic viable bacteria in the alimentary tract of the prawn *Penaeus indicus* ranged from 7.81 to 20.10% (Table 1). Their number varied from specimen to specimen but not significantly.

Okutany (1966) reported significantly higher number of chitinolytic bacteria in intestine and stomach of a fish (*Lateolabrax japonicus*) than that in sea water. As the

prawns collected for the present study were cultured in artificial NPCL culture tanks and were fed with artificial feed rich in chitinic material (Ali, 1982), higher accumulation of the chitinolytic bacteria in their hepatopancreas, stomach and intestine than in water is most likely as reported by earlier worker (Okutany, 1966). Naturally the bacteria might have been actively involved in digestion of chitinous material by secreting chitinolytic enzyme as suggested by ZoBell and Rittenberg (1937).

The chitinolytic bacterial population was found in decreasing order in the three regions of alimentary canal—hepatopancreas, stomach and intestine. Higher accumulation of chitinolytes in hepatopancreas followed by stomach and intestine needs further attention.

All the isolated bacteria were Gram negative, asporogenous motile rods, catalase and oxidase positive and showed better growth at 30°C than at 25°C and 37°C on slant culture. Other biochemical behaviour listed in Table 2 indicate the bacteria to be members of the genus *Vibrio* and *Pseudomonas*. So, the results of the study demonstrate that chitin utilizing *Vibrio* and *Pseudomonas* species were present in the digestive tract of the marine crustaceans, viz. *Penaeus indicus*. These two bacteria may aid in digestion of chitinous food items in *Penaeus indicus*.

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