

Gut Microflora of Some Edible Crabs from Porto Novo Coast

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Gut microflora of 5 commercially important crabs, viz. *Charybdis cruciata* (Herbst), *Podophthalmus vigil* (Fabricius) (marine), *Scylla serrata* (Forsk.) (Forsk.), *Portunus pelagicus* (Linnaeus), *P. sanguinolentus* (Herbst) (estuarine), were studied. The midgut harboured highest aerobic and proteolytic bacteria. Luminescent bacterial flora were predominant in the hindgut and on the cuticular membrane of all the crabs examined. High numbers of heterotrophic bacteria were recorded in the midgut of *S. serrata*.

The gut microflora of fishes and prawns¹⁻⁴ have been studied, but information on edible crabs from Indian waters is wanting. Results of a study on total aerobic bacterial population, saprophytic and enteric luminescent bacterial population and proteolytic bacteria of 5 species of edible crabs from marine and mangrove niches of Porto Novo environs are presented and discussed here.

Five species of Portunid crabs, viz. *Charybdis cruciata* (Herbst), *Podophthalmus vigil* (Fabricius), *Portunus pelagicus* (Linnaeus), *P. sanguinolentus* (Herbst) of marine, and *Scylla serrata* (Forsk.) from mangrove were studied. Five male specimens with carapace widths of 46.5-49.5, 111-113, 79.0-86.5, 104-108 and 80-98 mm in each species respectively were caught in fresh condition for study. In addition to the gut and digestive diverticula, the cuticular membrane bordering the body cavity of each individual specimen was aseptically dissected out. That part from mouth to the gizzard was designated as the foregut (stomodeum), from gizzard to the tip of the intestine as the midgut (mesenteron) and from there to the anus as the hindgut (proctodeum). All the dissected portions were homogenised in a sterile homogeniser adding 100 ml of sterile 50% sea water. Samples were plated for the enumeration of total aerobic, proteolytic and bioluminescent bacteria after serial dilutions. ZoBell's 2216e medium⁵ for total aerobic bacterial population, Harrigan and McCance⁶ medium for proteolytic bacteria and complex sea water medium (SWC)⁷ for the bioluminescent bacteria were used. All the isolates were identified^{8,9} and for the identification of bioluminescent bacterial isolates Nealson's¹⁰ procedure was followed.

Food and feeding studies suggested that the tested edible crabs were omnivorous and cannibalistic. Detritus and semidigested materials were also found in the gut contents. Average of various bacterial populations per gram dry weight of the contents of the samples are presented in Table 1. Fifty isolates from

total heterotrophs, 50 from luminescent bacterial populations and 50 strains from proteolytic bacterial populations were picked out for further detailed study.

In all crabs, total aerobic bacterial counts were more in the midgut compared to the fore and hindgut regions. Availability of high amount of nutrients in an easily assimilable form in the midgut, is perhaps conducive for the growth of microorganisms. This proliferation of bacteria could be beneficial to the crabs either in the elaboration of (microbial) enzymes useful in digestion or in the secretion of growth factors and vitamins (by microbes) which are useful for the crabs^{11,12}. Comparatively low bacterial counts in the hindgut indicated destruction of bacterial cells. This finding is in agreement with the previous report by Mary⁴ in fishes. Regarding species composition of the gut and cuticular membrane microflora of crabs, species belonging to Gram positive, *Bacillus*, *Micrococcus*, *Corynebacterium*, and Gram negative, *Pseudomonas*, *Vibrio*, *Flavobacterium* and some members of the family Enterobacteriaceae, have been identified. Of these, *Micrococcus* sp. and *Pseudomonas* sp. were dominant in the gut of all crabs.

Luminous procaryotes were isolated from the marine environment in free-living, saprophytic, commensal, parasitic and symbiotic states¹³⁻¹⁶. Jayabalan *et al.*¹⁷ isolated bioluminescent bacteria from the light organs of Indian leioznathids. From Porto Novo environs, Nair *et al.*¹⁸ isolated free-living, saprophytic bioluminescent bacteria from the gills, gut and external surfaces of marine and estuarine fishes and crustaceans. The present study records the presence of enteric bioluminescent bacteria from the gut, digestive diverticula and the cuticular membrane of all species of crabs (Table 1). The density of enteric luminescent bacterial populations in the hindgut was more than in other regions. Mary⁴ and Nair *et al.*¹⁸ reported that the faecal matter itself served as an ideal medium for the growth of these bacterial population. Also the entry of the bacteria through the anal region

Table 1—Population of Bacterial Count of Some Commercially Important Crabs of Porto Novo

[Values represent No. $\times 10^5$ /g dry wt of contents]

| | <i>Charybdis cruciata</i> | <i>Scylla serrata</i> | <i>Portunus pelagicus</i> | <i>P. sanguinolentus</i> | <i>Podophthalmus vigil</i> |
|---------------------------------|---------------------------|-----------------------|---------------------------|--------------------------|----------------------------|
| Total viable counts | | | | | |
| Fore Gut | 1335.03 | 3047.25 | 1149.43 | 4566.06 | 6594.08 |
| Mid Gut | 2058.48 | 6666.67 | 3344.63 | 9283.83 | 6400.90 |
| Hind Gut | 536.77 | 1113.55 | 2109.71 | 965.145 | 2450.34 |
| Digestive diverticula | 224.17 | 3147.21 | 400.00 | 5895.02 | 3913.49 |
| Cuticular membrane | 267.43 | 2672.07 | 421.46 | 6785.14 | 1928.40 |
| Bioluminescent bacterial counts | | | | | |
| Fore Gut | 6.97 | 292.94 | 19.03 | 40.07 | 4.11 |
| Mid Gut | 85.82 | 24.87 | 93.57 | 412.08 | 124.00 |
| Hind Gut | 122.81 | 1160.59 | 168.47 | 447.68 | 391.35 |
| Digestive diverticula | 14.62 | 452.29 | 68.23 | 60.73 | N.D. |
| Cuticular membrane | 47.76 | 1755.27 | 649.48 | 202.53 | N.D. |
| Proteolytic bacterial counts | | | | | |
| Fore Gut | N.D. | 272.22 | 56.50 | N.D. | N.D. |
| Mid Gut | 124.87 | 417.58 | 277.78 | 295.16 | 2282.51 |
| Hind Gut | 68.32 | 332.69 | N.D. | N.D. | 144.75 |
| Digestive diverticula | N.D. | N.D. | 150.0 | N.D. | N.D. |
| Cuticular membrane | 125.65 | 715.25 | 239.46 | N.D. | N.D. |

N.D. = Not Detected

could not be ruled out. The cuticular membrane also supported a good growth of saprophytic luminescent bacterial population. Among the crabs studied *Scylla serrata* had maximum luminescent bacterial counts. Six species of luminescent bacteria, viz. *Photobacterium fisheri*, *P. leiognathi*, *P. phosphoreum*, *Beneckea harveyi*, *B. splendida* and *Vibrio albensis*, were recorded from the aquatic environment¹⁹. Among them *V. albensis* was not reported so far from the marine environment²⁰ and species of *Photobacterium* were found in symbiotic association with other animals²¹. In contrast, members belonging to the genus *Beneckea* were free-living²². In the present study, 3 species of luminescent bacteria, *P. fisheri*, *P. leiognathi* and *B. harveyi* were isolated from the enteric and cuticular region of crabs. *Photobacterium leiognathi* (52.38%) was found to be most dominant followed by *B. harveyi* (38.10%) and *P. fisheri* (9.52%). Similar observations were made in marine leiognathids²³. Reichelt *et al*¹⁵ found that *P. leiognathi* had a symbiotic host specificity. *Beneckea harveyi* was reported as free-living forms from Porto Novo marine environs¹⁸.

Ability of the marine bacteria to degrade protein macromolecules was reported²⁴⁻²⁶. Cashell *et al.*²⁷ reported widespread occurrence of *Pseudomonas putrefaciens* in fishery products and found that these were able to reduce trimethylamine oxide to trimethylamine. Studies on psychrophillic proteolytic bacteria were reported by McDonald *et al.*²⁸ Leving²⁹ detected that most of the bacteria responsible for spoilage in fish and fishery products are proteolytic, capable of growing at 0°C. Some anaerobic cultures

(*Anaeromonas proteolytica*) were also found to be active in proteolysis³⁰. Kazanas³¹ reported that irradiation eliminated all proteolytic bacteria responsible for spoilage and added that the enzymatic effects were more extensive than expected based on the number of bacteria present.

Results of the present study on proteolytic bacteria of commercially important crabs of Porto Novo are given in Table 1. The midgut in general shows good growth of proteolytic bacteria in all the crabs studied. Bacterial protease, responsible for proteolysis, were reported to be more in the mid gut where digestion is completed. Besides, some growth promoting factors and vitamins are also reported from the mid gut which may thus serve as the ideal medium for the proliferation of microbes^{11,12}. Poor bacterial counts in the digestive diverticula might be due to secretion of digestive enzymes which may act on microbes. In the present investigation, *Micrococcus*, *Corynebacterium* (Gram positive) and *Pseudomonas*, *Vibrio* (Gram negative) were recorded and among these, *Micrococcus* (52%) was found to be dominant followed by *Pseudomonas* (43%). Sizemore and Stevenson³² reported that about 50% of the caseolytic bacteria were Gram positive cocci. *Scylla serrata* (Forsk.) was found to harbour highest number of bacterial counts. This could be due to the fact that this is the only species living in or near the bottom in mangroves whereas the other 4 marine crabs are mostly free swimming forms. The mangrove sediments are known to be very rich in bacteria normally³³.

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