BACTERIAL FLORA ASSOCIATED WITH THE GREEN MUSSEL *PERNA VIRIDIS* (LINNAEUS)

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ENCOURAGING results have been achieved in the culture of the economically important species of the brown mussel (*Perna indica*)[1] and green mussel (*P. viridis*)[2,3] in India. Knowledge regarding the bacterial flora of shellfish is necessary to improve the quality of fishery products as the role of fish food in the transmission of diseases has been recognized[4]. Since *P. viridis* is found in estuarine environment which is influenced by river flow, man-made pollution and other hydrographical factors, any fluctuation in the quality of water is reflected in the bacterial flora of the animal. Hence a preliminary study on the density and types of bacteria associated with this bivalve was undertaken.

The green mussels (45–50 mm in length) were collected from the natural beds in the Uppanar estuary located at Cuddalore (Lat. 11°42'N; Long. 79°46'E). The mussels were immediately transported in sterile plastic bottles to the laboratory where they were scrubbed and washed repeatedly with sterile seawater to remove the epizootics and foreign matter. After opening the shell valves, the mussels were washed with sterile seawater to prevent contamination from mantle fluid.

One gram of mantle tissue was dissected aseptically and homogenized using 50% sterile seawater. It was then transferred into 99 ml of 50% sterile seawater blank. Serial dilutions of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} were made using 9 ml of 50% sterile seawater blank. One ml of aliquot of appropriate dilutions were pipetted out into petriplates having ZoBell's 2216[e] agar medium. The pseudofaeces of the animal were collected under sterile conditions by keeping the mussels in 50% sterile seawater for 4 hr. The discharge of the pseudofaeces was stimulated by varying the pH of the seawater. The discharged pseudofaeces were transferred into 50% sterile seawater and serial dilutions were made and subsequently plated as before. Plates were incubated at 28 ± 2°C for 72 hr. The methods reported earlier[5-7] were followed for identifying the bacterial isolates. Twenty-four hour old cultures were used for gram staining and to study the biochemical and physiological characteristics of the isolates.

Total viable counts were found to be more in pseudofaeces than in the mantle tissue (table 1). Proteolytic forms dominated in the mantle tissue whereas pseudofaeces harboured the maximum number of lipolytic forms (table 2).

Findings of the present study are invariance with those of Rajagopalan and Sivalingam[8] and Thankappan Pillai[9]. *Micrococcus* which was found dominant in the mantle tissue in the present study was not recorded by Rajagopalan and Sivalingam. Though Thankappan Pillai recorded *Micrococcus* in the mantle tissue, it was next to *Pseudomonas* and *Vibrio* in the order of their occurrence. *Pseudomonas* was next in abundance to *Vibrio* in the mantle tissue in the present study, though it was reported as the predominant species in the mantle tissue[9] (table 3).

Table 1 *Total viable bacterial density in Perna viridis*

Material	Bacterial density
Mantle tissue	104.33×10^3 CFU/g
Pseudofaeces	798.33×10^2 CFU/g

CFU: Colony Forming Units

Table 2 *Physiological characteristics*

Material	Physiological character-istics	Number of positive isolates	Number of isolates tested
Mantle tissue	Amylolytic	10	27
	Proteolytic	16	27
	Lipolytic	3	27
Pseudofaeces	Amylolytic	6	17
	Proteolytic	10	17
	Lipolytic	11	17

Table 3 *Generic distribution of various bacteria isolated from the mantle-tissue and pseudofaeces of P. viridis*

Bacterial types	Material: Mantle tissue	Material: Pseudo-faeces
Bacillus	5	3
Micrococcus	15	2
Vibrio	3	5
Pseudomonas	4	4
Enterobacteriaceae	—	3

The microflora such as *Achromobacter*, *Escherichia* and *Neisseria* reported by Rajagopalan and Sivalingam[8] were not encountered in the present study. Such variation in species composition of bacterial flora observed might be due to the effect of environmental conditions and the bacteriological status of water as bivalves respond rapidly to ambient bacterial load. The predominance of the genus *Vibrio* in the pseudofaeces indicates its preference in an enteric habitat. Since pseudofaeces constitute mostly the undigested material, recovery of the genus *Vibrio* in maximum numbers agrees with the findings of Prieur[10] who observed the presence of intact cells of *Vibrio* in the hindgut of *Mytilus edulis*. Thus the preponderance of *Vibrio* recorded from the pseudofaeces can be attributed to the resistance of bacteria to the digestive secretions of the host and their subsequent proliferation in the gut[10].

The presence of bacteria belonging to 'Enterobacteriaceae' group indicates their origin from faecal pollution in the estuary. No reason could be attributed to the occurrence of maximum number of proteolytic forms in the mantle and lipolytic forms in the pseudofaeces. However, the present study is only preliminary and a long term monitoring of the bacterial flora harboured in the animal, surrounding water and sediment will gain paramount importance to understand the impact of environment on the bacteriological quality of *P. viridis*.

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1. Appu Kuttan, R. K., Prabhakaran Nair, T., Mathew Joseph and Thomas, K. T., *CMFRI Bull.*, 1980, **29**, p.30.
2. Kuriakose, P. S. and Nair, K. B., *CMFRI Bull.*, 1980, **29**, p. 33.
3. Rangarajan, K. and Narasimhan, K. A., *CMFRI Bull.*, 1980, **29**, p. 39.
4. W.H.O. *WHO Tech. Rep.*, 1974, **550**, 62.
5. Simidu, U. and Aiso, K., *Bull. Jpn. Soc. Fish.*, 1962, **28**, 1133.
6. Shewan, J. M., Hobbs, G. and Hodgkiss, A., *J. Appl. Bacteriol.*, 1960, **23**, 379.
7. Buchanan, R. E. and Gibbons, N. E., *Bergey's manual of determinative bacteriology*, Eighth edition, Waverly Press Inc., Baltimore, U.S.A., 1974.
8. Rajagopalan, K. and Sivalingam, P. M., *Mal. Appl. Biol.*, 1978, **7**, 43.
9. Thankappan Pillai, C., *CMFRI Bull.*, 1980, **29**, p. 41.
10. Prieur, D., *Kieler Meeresforsch Sondern*, 1981, **5**, p. 376.

UNRECORDED PATHOGEN ON BETELVINE CAUSING ANTHRACNOSE

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BETELVINE (*Piper betle* Linn) is grown in an area of 8221 ha in Karnataka alone and is considered to be a good foreign exchange earner in India. However, this crop suffers from many destructive diseases and one of them is anthracnose caused by *Colletotrichum* spp.

During 1984–85 an intensive survey was made in various districts of Karnataka and the anthracnose incidence was as high as 19%. A close examination of the infected vine indicated depressed lesions on various parts of the vines. On the leaves, the lesions were circular to irregular, light to dark brown surrounded by a yellowish halo, the centre of such spots later turned straw yellow in colour. The spots often coalesced to form bigger patches. On the stems, branches and petioles small, black, irregular specks were seen which occasionally ruptured the cortex underneath. Often the spots grew along the length of the stem in which case the part of the vine above the diseased internode wilted.

Tissue isolations from various parts of the plants yielded a species of *Colletotrichum*. Pathogenicity test was proved and typical symptoms appeared on vines after 4 to 7 days of inoculation. The fungus colony was greyish black and smooth. Conidia were oblong hyaline, non-septate with rounded ends, having oil-globules in the centre, formed in aggregates in culture and measured $8.6\text{–}19.9 \times 3.5 - 6.5\ \mu$. Comparing the descriptions of various species of *Colletotrichum* recorded on betelvine, the fungus under consideration has been identified as *Colletotrichum gloeosporioides* (Penz) Penz and Sacc. The fungus culture has been deposited in C.M.I. herbarium with accession Herb. IMI 286338.