MAHASAGAR
Vol.26, No.1 (June), 1993, pp. 49-53

THE GUT MICROFLORA OF EDIBLE CLAM *MERETRIX CASTA* (CHEMNITZ) FROM THE VELLAR ESTUARY, SOUTHWEST COAST OF INDIA

B. AIYAMPERUMAL, A. VELAMMAL AND K. BALASUBRAHMANYAN
Centre of Advanced Study in Marine Biology
Annamalai University, Parangipettai - 608 502

ABSTRACT

The status of bacterial flora in the digestive tract particularly in the foregut, midgut and hindgut regions of an edible backwater hardclam *Meretrix casta* and the environment (water and sediment) was studied. Sediment and hindgut was found to harbour maximum populations. The predominant genera in the gut regions were *Vibrio* spp., *Pseudomonas* spp. and *Flavobacterium/Cytophaga* sp. The role of bacteria in digestion of food materials is also discussed.

Key-words: Microflora, *Meretrix*, Vellar estuary.

The clams are appreciated as seafood in many countries. *Meretrix casta* is a highly nutritious clam rich in protein, glycogen and mineral components (Balasubrahmanyan, 1984). It is found in abundance in the Vellar estuary (Ajmal Khan, Vivekanandan and Balasubrahmanyan, 1975). The incidence of bacterial flora in the intestinal tract of clams depends upon the food and feeding habits and digestive pattern of the organisms. The gastrointestinal bacteria may play an important role in the nutrition, growth, disease susceptibility of clams and also can be used as an effective bioindicator of bacterial contaminations. In the present study, the total heterotrophic bacteria (THB) and other physiological groups (gelatinolytic, amylolytic and lipolytic bacteria) from the environment and gut regions of clam *M. casta* were studied qualitatively and quantitatively.

Water samples were collected with well cleaned, dried sterile bottles. The sediment samples were collected using a sterile spatula in sterile polythene bags aseptically. Specimens of *M. casta* were collected from the mouth of Vellar estuary in sterile polythene bags. All samples were transported to the laboratory within two hours of collection. The water, sediment and *M. casta* samples were collected fortnightly and analysed for a period of six months. Samples of water (1 ml) and sediment (1 g) were respectively diluted using 99 ml sterile buffered peptone water. Specimens were washed with sterile peptone water, the shells removed and the animals dissected. The alimentary tract of animals was divided into three regions, *viz.*, foregut (oesophagus), anterior part of the intestine and digestive diverticula, midgut

(middle part of the intestine and style sac) and hindgut (posterior region of the intestine and rectum). A known quantity of each was homogenized in 1 ml of peptone water and serially diluted further.

To analyse the total heterotrophic bacteria (THB), pour plate technique was employed using Zobell's marine agar medium. The plates were incubated at room temperature (30° C) for 2-3 days. The results were recorded and expressed as CFU/ml and CFU/g.

Table I – Generic composition of total heterotrophic bacteria (THB), gelatinolytic bacterial population (GBP), amylolytic bacterial population (ABP) and lipolytic bacterial population (LBP) isolated from environmental samples and various gut regions of *M. casta*

Source	*Achromo-bacter*	*Aero-monas*	Entero-bacte-riaceae	Flavo-bacterium/ Cytophaga sp.	*Pseudo-monas*	*Photo-bacterium*	*Vibrio*	*Bacillus*	Cornyne-forms	*Micro-coccus*	Total No. of isolates
THB											
Water	1	-	1	2	2	1	1	-	1	2	11
Sediment	2	1	2	3	2	1	3	1	1	2	18
Foregut	1	1	-	2	2	1	2	-	1	1	11
Midgut	1	-	1	1	2	1	2	-	1	2	11
Hindgut	1	1	-	2	2	-	3	-	1	2	12
Total	6	3	4	10	10	4	11	1	5	9	63
Percentage	9.5	4.8	6.3	15.9	15.9	6.3	17.5	1.6	7.9	14.3	
GBP											
Water	-	1	-	1	2	-	3	-	1	2	10
Sediment	-	1	-	2	2	1	2	-	1	2	11
Foregut	1	1	1	2	2	-	1	-	-	1	9
Midgut	-	1	1	2	1	-	2	-	-	1	8
Hindgut	1	-	1	1	3	-	2	-	-	1	9
Total	2	4	3	8	10	1	10	-	2	7	47
Percentage	4.3	8.5	6.4	17.0	21.3	2.1	21.3	-	4.3	14.9	
ABP											
Water	1	1	1	2	3	-	2	-	-	1	11
Sediment	1	1	1	2	3	-	2	-	1	1	12
Foregut	1	-	-	1	2	-	1	-	1	1	7
Midgut	1	1	-	1	3	1	2	-	1	1	11
Hindgut	-	1	1	1	3	1	2	-	1	1	11
Total	4	4	3	7	14	2	9	-	4	5	52
Percentage	7.7	7.7	5.8	13.5	26.9	3.8	17.3	-	7.7	9.6	
LBP											
Water	1	-	-	2	3	-	3	1	1	1	12
Sediment	1	-	1	3	3	-	2	2	-	1	13
Foregut	1	-	-	1	2	-	2	1	1	1	9
Midgut	1	-	-	1	3	1	3	1	-	1	11
Hindgut	-	-	-	2	2	-	3	1	-	2	10
Total	4	-	1	9	13	1	13	6	2	6	55
Percentage	7.3	-	1.8	16.4	23.6	1.8	23.6	10.9	3.6	10.9	

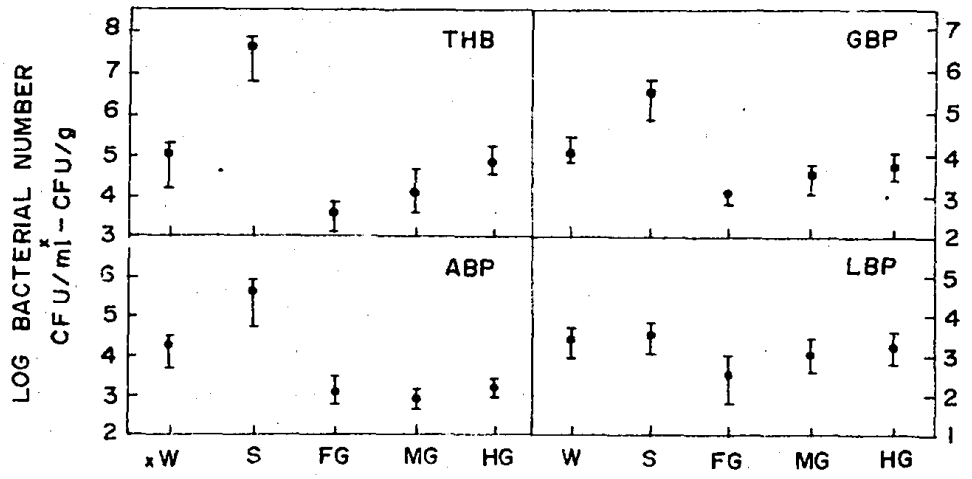

Fig. 1. Incidence of Total heterotrophic bacteria (THB), Gelatinolytic bacterial population (GBP), Amylolytic bacterial population (ABP) and Lipolytic bacterial population (LBP) from water (W), Sediment (S), Foregut (FG), Midgut (MG) and the hindgut (HG) regions of *M. casta*.

For the physiological groupings of bacteria, serial dilutions of water, sediment and gut samples were made to required dilutions. 1 ml was transferred to sterile petriplates. Then, the selective medium of respective physiological group was poured and the plates were incubated for 48 hrs. The total number of positive colonies per gram of the samples were recorded and enumerated. Gelatinolytic bacterial population (GBP) was estimated with Frazier's gelatin agar (Harrigan and Mc Cance, 1972), amylolytic bacterial population (ABP) with Zobell's medium with starch added to it and lipolytic bacterial population (LBP) with Tween agar (Harrigan and Mc Cance, 1972).

The well isolated colonies of total heterotrophs, gelatinolytic, amylolytic and lipolytic bacteria were randomly picked and kept in nutrient agar slants. Identification of bacteria was made following Shewan, Hobbs and Hodgkiss (1960) and Simidu and Aiso (1962). The maximum, minimum and mean values of heterotrophic bacterial population and other physiological groups are presented in Fig. 1. Bacterial flora existing in any animal in the aquatic environment is the function of environment (Sugita, Tanaami, Kobashi and Deguchi, 1981; Chandrasekaran, 1985). In the present study, the bacterial flora in the clam *M. casta* was found to be influenced by the flora present in the environment. High bacterial counts observed in the sediment may be attributed to the enriched nutrient laden freshwater flow through irrigation channels and also land runoffs into the Vellar estuary from adjoining areas (Kanagasabai, 1985). Many authors have observed that bivalves accumulate microorganisms in the gut from the surrounding water (Cabelli and Haffernan, 1970, 1971; Leung, Shortridge, Mortan and Wong, 1973; Aljebouri and Trollope, 1981; Perkins, Haven, Alamo and

Rhodes, 1980; Ledo, Gonzalez, Barja and Toranzo, 1983; Minet, Barbosa, Prieur and Cormier, 1987). The ingested microorganisms are not necessarily digested or killed and may remain viable within the gut. That may be the reason for the isolation of a large number of bacteria in the gut. The foregut harboured lower bacterial population. This could well be due to higher enzymatic activity of the animal in the foregut (Aiyamperumal, 1987). According to Kristensen (1972), the bacterial enzymes help in digestion of high structural polysaccharides indigestable by the animal enzymes. The hindgut harboured higher number of ABP and LBP. The enzymatic activity of the hindgut have been observed to be nil (Aiyamperumal, 1987). The prevalence of lipolytic bacteria in sediment may be due to the lipid content of the surface slime produced by dead algae and diatoms (Gunderson, 1976). The bacterial flora was mainly represented by *Pseudomonas* spp. and *Vibrio* spp. Next in order was *Flavobacterium/Cytophaga* (Table I). Vibrio like bacteria were always more abundant in the bivalve studied than in seawater and frequently more than in sediment. The data obtained lead to the conclusion that such bacteria were a regular component of the bivalve microflora and that molluscs certainly represented an important ecological niche for the bacteria.

ACKNOWLEDGEMENT

The authors (B.A. and A.V.) are grateful to Dr. R. Natarajan (Former Director), Centre of Advanced Study in Marine Biology, Parangipettai, for his constant interest and encouragement in this work.

REFERENCES

Aiyamperumal, B., 1987. *Studies on physiology and microbiology of digestion in Meretrix casta (Chemnitz) from the Vellar estuary, Porto Novo (S. India).* M.Phil. Thesis, Annamalai University (S. India).

Ajmal Khan, S., E. Vivekanandan and K. Balasubrahmanyan, 1975. Botom fauna in two regions of the Vellar estuary. In: *Recent Research in Estuarine Biology*, Hindustan Publishing Corporation, Delhi, 252-272.

Aljebouri, M.M. and D.R. Trollope, 1981. The *Escherichia coli* content of *Mytilus edulis* from analysis of whole tissue and digestive tract. *Journal of Applied Bacteriology*, **51**: 135-142.

Balasubramanyan, K., 1984. *Studies on* Meretrix casta *(Chemnitz) from the Vellar estuary, Porto Novo (S. India).* Ph.D. Thesis, Annamalai University, S. India.

Cabelli, V.J. and W.P. Heffernan, 1970. Elimination of bacteria by the soft shell clam *Mya arenaria. Journal of Fisheries Research Board of Canada*, **27**: 1579-1587.

Cabelli, V.J. and W.P. Heffernan, 1971. The elimination of bacteria by the northern quahaug; variability of the response of individual animals and development of criteria. *Proceedings of National Shellfish Association*, **61**: 102-108.

Chandrasekaran, M., 1985. *Studies on the microbial spoilage of* Penaeus indicus. Ph.D. Thesis, Cochin University, Kerala.

Gunderson, K., 1976. Cultivation of micro-organisms. In: *Marine Ecology*, Vol.3, edited by O. Kinne, John Weley and Sons, London.

Harrigan, W.F. and M.E. McCance, 1972. *Laboratory Methods in Microbiology*, Academic Press, London, 362 pp.

Kanagasabai, R., 1985. *Studies on some neritids (Archaeogastropod: Mollusca) from the Vellar-Coleroon estuarine complex (Southeast coast of India)*. Ph.D. Thesis, Annamalai University, S. India.

Kristensen, J.H., 1972. Carbohydrates of some marine invertebrates with notes on their food and on natural occurrence of carbohydrates studied. *Marine Biology*, **14**: 130-142.

Ledo, A., E. Gonzalez, J.L. Barja and A.E. Toranzo, 1983. Effect of depuration systems on the reduction of bacteriological indicators in cultures mussels (*Mytilus edulis*). *Journal of Shellfish Research*, 3: 59-64.

Leung, C., K.F. Shortridge, B. Morton and P.S. Wong, 1973. The seasonal incidence of faecal bacteria in the tissue of the commercial oyster *Crassostrea gigas* Thunberg 1973 correlated with the hydrology of deep Bay-Hong Kong. In : *Proceedings of Special Symposium on Marine Science*, Hong Kong, Pacific Science Association, p. 114-127.

Minet, J., T. Barbosa, D. Prieur and M. Cormier, 1987. Misevidence du processus de concentration des bacteries par la moule, *Mytilus edulis* (L) C.R. Acad. Sci. Paris Ser. 111. 305, 351-354.

Perkins, F.O., D.S. Haven, R.M. Alamo and M.W. Rhodes, 1980. Uptake and elimination of bacteria in shell fish. *Journal of Food Protection*, **43**: 124-126.

Shewan, J.M., G. Hobbs and W. Hodgkiss, 1960. A determinative scheme for identification of certain general of gram negative bacteria with special reference to the Pseudomonadacea. *Journal of Applied Bacteriology*, **23**: 379-390.

Simidu, U. and Aiso, 1962. Occurrence and distribution of heterotrophic bacteria in sea water from Kanagawa Bay. *Bulletin Japanese Society of Science & Fisheries*, **28**: 1133-1141.

Sugita, H., H. Tanaami, T. Kobashi and Y. Deguchi, 1981. Bacterial flora of coastal bibalves. *Bulletin Japanese Society of Science & Fisheries*, **47**: 655-661.