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ARYLSULFATASE PRODUCING BACTERIA ASSOCIATED WITH *VILLORITA CYPRINOIDES*

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ABSTRACT

Villorita cyprinoides was selected to isolate total heterotrophic bacteria (THB) and arylsulfatase producing bacteria (APB). Both THB and APB colonised more in the visceral mass of the shellfish during pre-monsoon season. Physico-chemical parameters do not exert any significant influence over the distribution of THB and APB. *Micrococcus* was the predominent genus among THB and *Moraxella* among APB. Higher percentage of bacteria showed more affinity towards carbohydrates. The microbial participation in the breakdown of conjugated sulfuric esters and their importance in the physiology and nutrition of host organisms were discussed in the light of available literature.

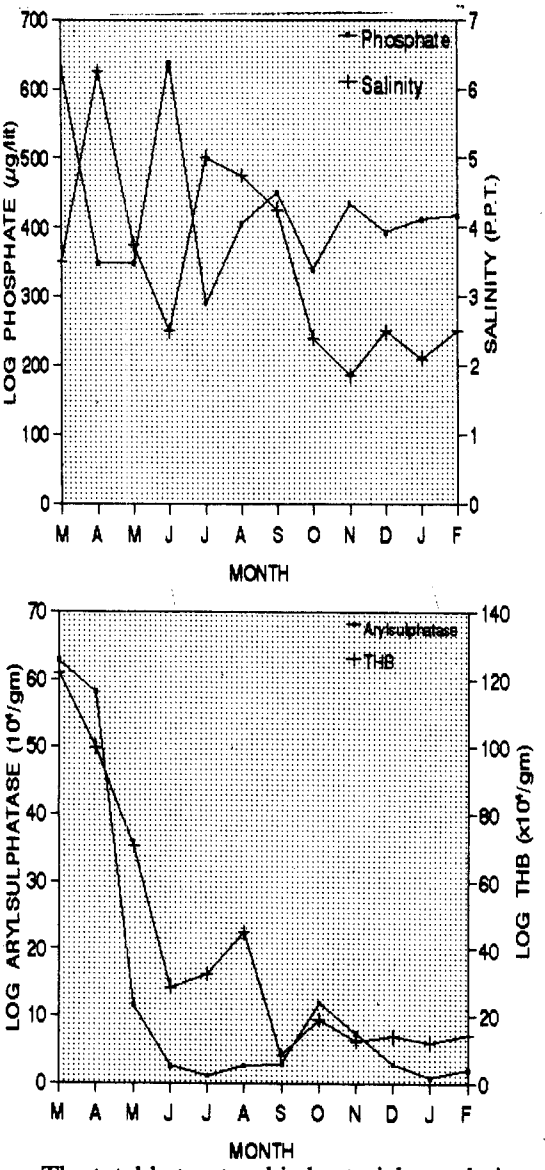
INTRODUCTION

FISH and shellfish are considered to be the delicious sea food. They contribute significantly to the human diet as a high quality protein with low cost (Larkin and Hunt, 1982). Extensive literatures are available on the role of microorganisms in the digestion of complex organic food of host, selectivity of nutrients, diseases and spoilage of sea food. No pertinent literature is available about the nature and association of arylsulfatase producing bacteria (APB) in the alimentary tract of shellfish. The occurrence of arylsulfatase was first demonstrated by Derrien (1911) in the extract of *Murex trunculus*. Dodgson and Spencer (1956) reported various types of arylsulfatase in mammals, birds, amphibians and marine molluscs. Corner *et al.* (1960) observed elevated level of arylsulfatase in *Pecten maximus*. Dhevendaran *et al.* (1980) reported this enzyme activity in different marine gastropods. The inhibiting effect of DDT on arylsulfatase producing bacteria in the primary film and foulers were also reported (Dhevendaran, 1984; Dhevendaran *et al.*, 1986). Maya (1990) made an attempt on the significance of arylsulfatase in fish and shellfish of Veli Lake. With all these reports an effort has been made to note the presence of this enzyme producing microbes in *Villorita cyprinoides*.

MATERIAL AND METHODS

The samples were collected every month from March 1987 to February 1988. Water sample was collected with the help of sterile glass water sampler. *Villorita cyprinoides* was collected through hand picking from the mud. The salinity, phosphate, sulfate and ammonia of the water sample were analysed. Salinity and phosphate of the water were determined following the method of Strickland and Parson (1968), and Murphy and Riley (1962) respectively. The sulfate in water column was estimated gravimetrically (Hassler, 1955). The ammonia content of water was estimated by

Nesslerisation method as adopted by Selvakumar *et al.* (1977).



The total heterotrophic bacterial population (THB) and the arylsulfatase producing bacteria (APB) were estimated by the method adapted by Dhevendaran *et al.* (1985). Identification of pure cultures upto generic level was also undertaken (Simidu and Aiso, 1962). The physiological groupings such as hydrolysis of starch, protein and lipid were also carried out (Harringon and Mc Cance, 1972). Statistical analysis were carried out by the method of Snedecor (1956).

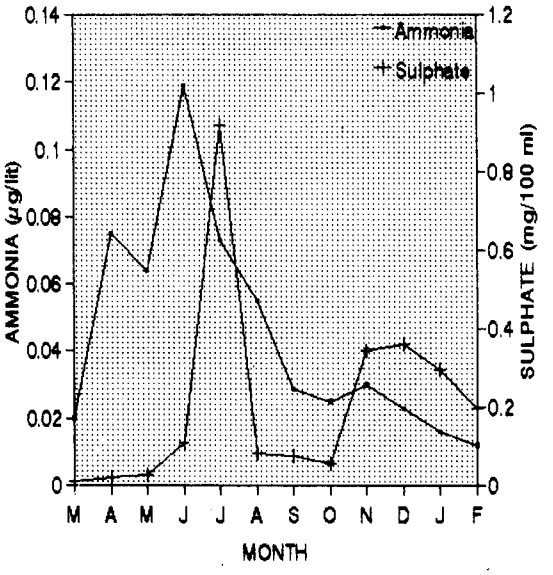


Fig. 1. Arylsulfatase producing bacteria in the visceral mass of *Villorita cyprinoides* in relation to physico-chemical and biological parameters.

Results and Discussion

The observed monthly values for various factors are represented graphically in Fig. 1.

The highest salinity was recorded during monsoon (4.13‰) and lowest during postmonsoon (2.2‰). The highest salinity during monsoon is because of the direct influence of sea water by opening the sand bar. Similar pattern of observation have already been made by Dhevendaran *et al.* (1987) and Alex (1988) from the same area. The observed correlation coefficient value (r) between salinity and other parameters was not significant. Inorganic phosphate content of water did not show much variation. The highest phosphate content was during monsoon (455.45 µg/1). This may be due to the influence of sea water and land drainage. Our report confirmed the earlier observation made by Rajendran and Venugopalan (1973). The 'r' value between phosphate and biotic as well as abiotic factors was not significant (Table 1). The results indicated that higher concentration of adsorbed sulfate was in monsoon (0.295 mg/100 ml).

TABLE 1. *Correlation coefficient values (r) of various parameters*

Parameters			Correlation coefficient
Salinity	VS	THB	0.5480
Salinity	VS	APB	0.4320
Ammonia	VS	THB	0.2140
Ammonia	VS	APB	0.00902
Phosphate	VS	THB	0.2300
Phosphate	VS	APB	0.2600
Sulfate	VS	THB	0.3700
Sulfate	VS	APB	0.4120

We have observed it for the first time in Veli Lake and therefore it is not possible to compare with any literature from west coast. No significant correlation was obtained between sulfate and biotic as well as abiotic factors. (Table 1). Variations in the ammonia concentrations were observed, for a period of one year. The highest ammonia content (0.068 µg/1) was found in monsoon season and lowest (0.023 µg/1) during post-monsoon season. Unnithan *et al.* (1977) reported the incidence of mortality of fish *Ambassis gymnocephalus* due to industrial pollution containing heavy load of ammonia. But for this no other pertinent literature is available to discuss with our findings on the west coast of India. The correlation coefficient of ammonia with other biological and abiological factors was not significant.

Variations in THB was noticed in different seasons (Fig. 1). Highest population was recorded during pre-monsoon (76.72×10^4/g) and lowest during post-monsoon (14.31×10^4/g) from the visceral mass of *V. cyprinoides*. Vanajakumar (1980) observed highest number of microbes in the mantle of molluscs with a few exception like *Anadara rhombea* and *Crassostrea madrasensis* in which shell surface and gut harboured maximum population. Similarly noted visceral mass of foulers such as *Balanus* and *Modiolus* harboured more bacterial population than their exoskeleton (Dhevendaran *et al.*, 1986).

The observed results of APB indicated that maximum population was in pre-monsoon (33.65×10^4/g) (Fig. 1). APB were not significantly correlated with both biotic as well as abiotic factors. Analysis of variance (Table 2) shows that the variation was not significant between THB and

TABLE 2. *ANOVA showing the significance in arylsulfatase and total heterotrophic bacteria of* V. cyprinoides *and between months*

Source		df	MS	F
Total	17.33	23		
Between months	08.514	11	0.774	03.748^{NS}
Between THB & APB	06.544	01	6.544	31.69^{**}
Error	02.272	11	0.2065	

** = P < 0.01, NS = Not significant

APB. This clearly indicates that the changes may be due to nutritions of shellfish.

350 colony forming units of THB were isolated from the visceral mass of *V. cyprinoides*. Randomly selected isolates belonged to eight genera (Table 3). *Micrococcus* (31.42%) showed maximum percentage followed by *Bacillus* (17.14%). Velammal (1987) observed higher percentage of *Micrococcus* in *Melampus ceylonicus*. However, Sreekumari and Lakshmanaperumalsamy (1986) found that *Pseudomonas* was the dominant group followed by *Vibrio* in *V. cyprinoides* of Cochin Backwater. Thus it is premature to state the dominance of a particular group without making the statistical analysis with large samples and also by the analysis of food composition of shellfish.

TABLE 3. *Generic composition of THB and APB from visceral mass of* V. cyprinoides

Bacteria	Total isolates	*Pseudo-monas*	*Bacillus*	*Vibrio*	*Micro-coccus*	*Coryne-bacterium*	*Aeromonas*	*Moraxella*	*Flavo-bacterium*
THB	350	2.86	17.74	14.28	31.42	11.43	8.57	11.43	2.86
APB	180	–	27.77	16.67	16.67	5.56	–	33.33	–

The distribution of APB in the visceral mass of *V. cyprinoides* is given in Table 3. Of the 180 bacterial strains selected five genera such as *Bacillus, Vibrio, Micrococcus, Corynebacterium* and *Moraxella* were obtained. *Moraxella* was the predominant genus (33.33%) followed by *Bacillus* (27.77%). Maya *et al.* (1989) observed that *Therapon jarbua* harboured wide diversity of APB in the gut whereas *V. cyprinoides* harboured reduced number denoting the nature of diet. We have already stated that the arylsulfatase activity in the gut as well as visceral mass of fish and shellfish may be due to the presence of bacterial population (Dodgson *et al.*, 1954). Dhevendaran *et al.* (1986) observed that *Bacillus* and *Vibrio* were the predominant group of APB in primary film and foulers in Cochin Backwater. But for this no other nutrient literature is available to discuss the diversity of APB among shellfish. It is presumed that the abundant and readily harvested molluscs such as *V. cyprinoides* and other related organisms may be the suitable commercial sources for arylsulfatase enzyme.

Table 4 shows the physiological characteristics of the THB and APB. A total of 350 isolates from THB and 180 strains from APB were selected for physiological activities. In both the groups maximum percentage (71.43% in THB and 88.88% in APB) showed more affinity towards carbohydrates. Bacterial flora existing in any animal in the aquative environment is the fluctuation of the environment. In the present study the physiological groups of bacteria of the bivalve were found to be influenced by the environmental flora. To support our findings Sreekumari and Lakshmanaperumalsamy (1986) already stated that lipase producers were more in association with *V. cyprinoides* in Cochin Backwater. Alex (1989) observed the maximum percentage of amylase producers from *V. cyprinoides* in Veli Lake. The predominance of a particular physiological group and cesses percentage of other group of gut microflora clearly indicate inverse relation between enzyme

TABLE 4. *Physiological characteristics of selected isolates of THB and APB from* V. cyprinoides

Bacteria	Total isolates	Amylase producers (%)	Protease producers (%)	Lipase producers (%)
THB	350	71.43	48.57	14.20
APB	180	88.88	50.00	38.00

activity of gut and particular physiological group of bacteria as suggested by Kanakasabai (1985).

It is understood from the investigation that the microbes associated with shellfish play a significant role in the physiology and metabolism of host organism. It has been speculated that arylsulfatase is an important enzyme in the breakdown of sulfuric acid esters of aromatic compounds present in the feed of the shellfish.

REFERENCES

ALEX EAPEN 1988. *Studies on phosphatase bacteria associated with fish and shellfish in Trivandrum Coast.* M.Sc. dissertation, Kerala University.

CORNER, E. D. S., Y. A. LEON AND R. D. BULBROOK 1960. Steroid sulfatase, arylsulfatase and p-glucosidase in marine bacteria. *J. Mar. Biol. Assoc. U.K.*, **39** : 51-61.

DERRIN, M. 1911. Arylsulfatase in *Murex trunculus. Bull. Soc. Chim. Biol.*, **9** : 110.

DHEVENDRAN, K., T. KANNUPANDI AND R. NATARAJAN 1980. Arylsulfatase activity in marine gastropods. *Mahasagar*, 13 : 173-178.

——— 1984. Arylsulafatase activity in marine polychaetes. *Fish. Technol.*, **21** : 57-61.

———, D. CHANDRAMOHAN AND R. NATARAJAN 1985. Arylsulfatase producing bacteria in marine sediments. *Ibid.*, **22** : 121-125.

———, M. O. VALSA JOSEPH AND N. JALAJA 1986. Bacteria associated with primary film and foulers. *Proc. National Seminar on 'Mussel Watch'. Cochin University*, pp. 150-156.

DODGSON, K. S., T. H. MELVILLE, B. SPENCER AND K. WILLIAMS 1954. The arylsulfatase of a strain of *Alcaligenes metalcaligenes* isolated from intertidal mud. *Biochem. J.*, **58** : 182-187.

———, ——— AND ——— 1956. Characteristics of *Alcaligenes metalcaligenes. Ibid.*, **62** : 30.

———, K. MAYA AND P. NATARAJAN 1987. Studies on microbial ecology of Southwest coast of India. *Proc. National Seminar of Estuarine management, Trivandrum.* pp. 425-432.

HARRINGAN, W. F. AND MCCANCE 1992. *Laboratory methods in microbiology.* Academic Press, London. pp. 362.

HASSLER, F. R. (Ed.) 1955. Sulfate, gravimetric method. *Standard methods for the examination of water, sewage and industrial wastes.* American Public Health Association, New York. pp. 196.

KANAKASABAI, R. 1985. *Studies on some neritids (Archeogastropoda : Mollusca) from Vellar Coleroon estuarine complex (Southwest Coast of India).* Ph.D. Thesis, Annamalai University, pp. 158.

LARKIN, E. P. AND D. A. HUNT 1982. Bivalve molluscs : Control of microbiological contaminants. *Bio. Sci.*, **32** : 3.

MAYA, K. 1990. *Arylsulfatase enzyme activity of fish, shellfish and associated bacteria of Veli Lake.* Ph.D. Thesis, Kerala University, pp. 123.

———, K. DHEVENDRAN AND P. NATARAJAN 1989. Arylsulfatase producing bacteria associated with the gut of *Therapon jarbua.* Colloquium on poikilotherms. Elsvier Publishers, London. pp. 203-210.

MURPHY, J. AND J. P. RILLEY 1962. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chem. Acta.*, **26** : 31-36.

RAJENDRAN, A. AND V. K. VENUGOPALAN 1973. Distribution of dissolved particulated and mud phosphorus in Vellar Estuary. *Indian J. Mar. Sci.*, **2** : 13-18.

SELVAKUMAR, N., D. CHANDRAMOHAN AND R. NATARAJAN 1977. α-asparaginase activity in marine sediments. *Curr. Sci.*, **46** : 287-291.

SIMIDU, U. AND K. AISO 1962. Occurrence and distribution of heterotrophic bacteria in sea water from Kamogawa Bay. *Bull. Jpn. Soc. Fish.*, **28** : 1133-1136.

SNEDECOR, G. W. (Ed.) 1956. *Statistical methods.* Iowa State College Press, Ames, Iowa, USA. pp. 1-534.

SREE KUMARI, K. R. AND P. LAKSHMANAPERUMALSAMY 1986. Effect of organo-phosphorus pesticide on the bacteria associated with *Villorita cyprinoides. Proc. National Seminar on Mussel Watch*, **1** : 105-109.

STRICKLAND, J. D. H. AND T. R. PARSON 1968. A practical handbook of sea water analysis. *Bul. Fish. Res. Bd. Canada*, **167** : 311-313.

UNNITHAN, R. V., M. VIJAYAN, E. V. RADHAKRISHNAN AND K. N. RAMANI 1977. Incidence of fish mortality from industrial pollution in Cochin Backwater. *Indian J. Mar. Sci.*, **6** : 81-83.

VELAMMAL, A. 1987. *Studies on the gut microflora of some gastropods from three different environments.* M.Phil. dissertation, Annamalai University, 42.