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34. A STUDY ON BIOCHEMICAL GENETICS ON *CHASSOSTREA MADRASENSIS* OF COCHIN

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ABSTRACT

To determine genetic differences between geographic populations of *Crassostrea madrasensis,* studies on the protein band pattern in four tissues namely, adductor muscle, gills mant'e and digestive divert'cula was determined by polyacrylamide gel electrophoresis. The interpopulation differences observed in protein expression are discussed in relation to biochemical genetic characterisation of C. *madrasensis.*

INTRODUCTION

Many marine bivalve species are known to exhibit genetic polymorphism at a number of loci (Wilkins 1975; Ahmad et al 1977, Skibinski et al 1978, Beaumont & Beveridge 1984). In oysters, the presence of genetically variable enzymes has been indicated by the studies of Wilkins and Mathers (197''), Mathers et al (1974), Schaal and Anderson (1974), Buroker et al (1979 a, b) and Buroker (1933). Most of the above studies have been carried out on specific enzymes. Attempts to identify polymorphic loci from general protein zymograms have been carried out only on a limited number of bivalve species eg., *Ostrea lurida* (Johnson *et a\ }972), Saxidomus giganteus* (Johnson and Utter 1973), *Crassostrea gigas* (Buroker et al *^975) and Chlamys opercularis* (Beaumont and Gruffydd 1975, Beaumont 1982 a, b).

A biochemical genetic study has been recently initiated to identify polymorphic loci in *the \n6\an ed\b\e oyster Crassostrea madrasensis,* by which genetically distinct stocks of oyster can be identified from different locations along Indian coasts. This report describes the result of preliminary investigations carried out to determine the basic protein zymogram of adductor muscle, mantle, gills and digestive diverticula. The presence of two polymorphic loci have also been shown.

MATERIAL AND METHODS

Specimens of adult *C. madrasensis* (shell height 41-70mm) used in the electrophoretic analysis were collected from the Vypeen bar mouth jetty at Cochin. Live oyster were maintained in the laboratory without feeding for a maximum period of one week. The oysters were dissected for the specific tissues required in the analysis. The tissue samples were homogenized in double distilled water, centrifuged at 10,000 rpm for 15 minutes. Disc electrophoresis as described by Davis (1964) was followed using 10% acrylamide and 5% bisacrylamide. The gels were stained for general protein using 0.25% Kenacid. Very faint bands and those bands not observed in all the gels were excluded from the general pherogram pattern The relative mobility (rf) of each band to that of the marker dye front was calculated and the general protein pherogram was drawn on the basis of mean rf values of gels from a minimum of 12 individuals for each tissue. For determiniing the presence of polymorphic loci, electrophoretic analysis of the adductor muscle of 20 individuals were carried out. From each individual, three samples were analysed. The most intensely stained bands were marked 4x and the remaining bands comparatively graded as 3x, 2x and 1x.

RESULTS

Tissues specific variations in the general protein pherogram were observed with regard to ^^^ """^^er of bands, their relative position, thickness and staining intensity (Fig. 1, Table 1). In the adductor muscle, 9 bands were observed in all individuals. Compared to the other tissues, in the adductor muscle there were less variations between individuals with regard to staining intensity and thickness of bajnd*. The

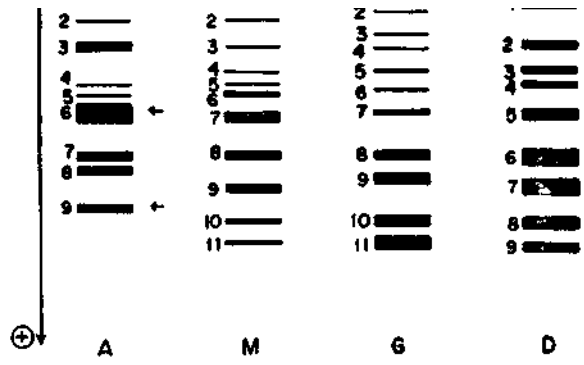

Fig. 1. Electropherogram in the adductor muscle (A), mantle (M), gonad (G) and digestive diverticula (D) in *Crassostrea madrasensis*. →+ indicates the direction of protein migration. Arrows at 6th 9th band in adductor muscle indicate polymorphic loci. O indicates the origin.

11 bands observed in the mantle tissue had more or less the same *rf* values in all individuals, but there were variations in staining intensity and thickness. These variations were marked for bands 4, 5 and 6. In the gill and digestive diverticula there were 11 and 9 bands respectively. In both tissues there were variations in staining intensities between individuals, but not in the thickness of bands.

(A, B and C) monomeric system. The most common type (BB) was a single band with *rf* value of 40.6 ± 1.45. Two other two banded phenotypes (AB, BC) were observed at this position (Fig. 2). The staining intensities of the 2 distinct bands in AB and BC system were half of BB band indicating that BB is the homozygous state and the other two phenotypes (AB and BC) are the heterozygous state. The homozygous AA and CC bands were not observed. Of the 20 individuals, AB phenotype was

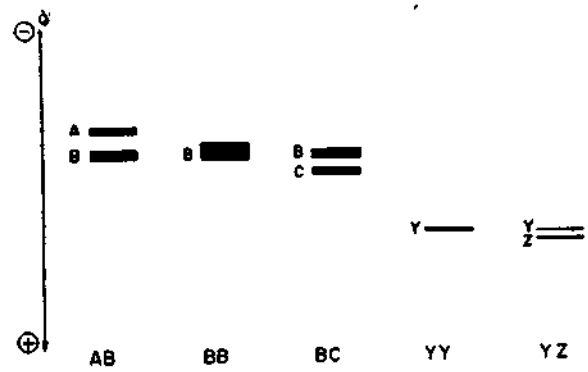

Fig. 2. The phenotypes observed at band 6 (AB, BB, BC) and band 9 (YY, YZ) in the adductor muscle of *Crassostrea madrasensis*. (→+ indicates direction of protein migration. O indicates the origin.)

TABLE 1. *Staining intensities (X), thickness (T in mm) and relative mobilities (rf) of the protein bands in different tissues of* Crassostrea madrasensis.

Band No.	TISSUES											
	Adductor muscle			Mantle			Gills			Digestive diverticula		
	X	T	rf	X	T	rf	X	T	rf	X	T	rf
1.	3	+	7.4	3	+	7.2	2	+	7.2	1	+	11.3
2.	2	+	13.7	2	+	13.9	1	+	12.1	3	0.5	21.7
3.	3	1	21.4	3	+	20.8	1	+	18.2	2	0.5	28.4
4.	1	+	30.8	2	+	27.8	1	+	21.8	2	0.5	32 2
5.	2	+	33.8	1	+	31.2	4	1	27.4	3	1	40.5
6.	4	2	40.6	2	0.5	34.2	1	+	32.9	2	2	52.9
7.	3	1	51.2	2	1	40.6	2	+	38.4	2	2	61.1
8.	1	1	55.1	2	1	51.2	2	1	51.2	2	1	69.4
9.	1	0.5	64.3	2	1	59.7	2	1	57.5	2	1	75.7
10.					1	68.2	1	1	68.6			
					1	1	1	1	74.5			

4 x — Dark
3 x — Medium
2 x — Light
1 x — Faint
+ bands less than 0.25 mm thick

observed in two individuals and BC in one individual indicating that B and C alleles occur at low frequencies in the population. The tissues from the AB and BC individuals were again electrophoresed with that of the common type to confirm the observed band patterns. At band 9 with rf values of 64.3 ± 1 26, in 50% of the samples two banded phenotype (YZ) was observed. But, unlike band 6 which in its homozygous state (BB) is 2 mm thick with a staining intensity of 4x, band 9 as a single band is 0.5 mm thick with a staining intensity of Ix. Further the interspace between the two bands y and z is small. Therefore, the presence of polymorphic system at band 9 cannot be said with certainty as indicated for band 6.

DISCUSSION

Of the four tissues tested, adductor muscle is the best tissue for detecting polymorphic loci since the variations between individuals with respect to staining intensity and thickness is minimum. Of the remaining tissues, the mantle tissue is the least suitable. In all tissues other than band 1 and 2 the coefficient of variation in rf values was less than 6% indicating that the general protein pherogram observed in the present study is repeatable under identical electrophoretic conditions. The differences in staining intensities between individuals could be partly due to the differences in the duration of starvation between individuals- The electrophoretic variants observed in adductor muscle protein of other molluscs have been either due to geographic variation (Johnson and Utter 1973, Beaumont 1982a) or due to differences in size classes tested (Beaumont 1982b). Due t& the low frequencies of alleles A and C and the small sample size, the effect of size class cannot be tested in this study. The present study, besides giving the tissue specific protein pherogram has indicated the presence of polymorphic loci. The actual allelic frequencies at those loci and the presence of other polymorphic loci can be substantiated by examining a larger sample size.

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REFERENCES

AHMAD, M., D. 0. F. SKIBINSKY AND J. A. BEARDMORE. 1977. An estimate of the amount of genetic variation in the common mussel *Mytilus edulis. Biochem. Genet* , 1 5 : 833-845.

BEAUMONT, A. R. 1982 a. Geographic variation in allele frequencies at three loci in *Chlamys opercularis* from Norway to the Brittany coast. *J. Mar. bio/. Ass-* 6/./f, 62:243-261.

BEAUMONT, A. R. 1982 b Variations in heterozygosity at two loci between year classes of a population of *Chlamys opercularis* (L.) from a Scottish sea-loch. *Mar. Biol. Lett.* 3 : 25-33.

BEAUMONT A. R. AND L. I. D. GRUFFYDD. 1975. A polymorphic system in the sarcoplasm of *Chlamys opercularis. J. Cons. int. Explor. Mer.,* 36(2) :190-192.

BEAUMONT, A R. C. M, BEVERIDGE 1984. Electrophoretic survey of genetic variation in *Pecten maximus, Chlamys opercularis, C. varia,* and C. *distorta* from the Irish sea. *Mar. Biol,* 81 : 299-306.

BUROKER, N. E. 1983. Population genetics of the American oyster *Crassostrea virglnica* along the Atlantic coast and the gulf of Mexico, *Mar. Biol.,* 75 : 99-912.

BUROKER, N. E.,W. K., HERSHBERGER AND K. K. CHEW. 1975. Genetic variation in the Pacific oyster *Crassostrea gigas J. Fish. Res. Board Can.,* 32:2471-2477.

BUROKER, N. E , W. K. HERSHBERGER AND K. K., CHEW. 1979 a. Population genetics of the family Ostreidae. I, Intraspecific studies of *Crassostrea gigas* and *Saccostrea commercialis. Mar. Biol.,* 54: 157-169.

BUROKER. N. E., W. K., HERSHBERGER AND K. K. CHEW. 1979 b. Population

genetics of the family Ostreidae. II. Interspecific studies of the genera *Crassostrea* and *Saccostrea. Mar. Biol.* 54 : 171-184.

DAVIS, B.J. 1g64. Disc Electrophoresis II Method and application to human serum proteins. *Annals New York Academy of Sciencies*- 404 427.

JOHNSON, AG , F.M. UTTER AND K. NIGGOL. 1972. Electrophoretic variants of aspartate amino transferase and adductor muscle proteins in the native oyster *(Ostrea lurlda). Anim. Blood Grps biochem. Genat..* 3 : 109-113

JOHNSON, A. G. F. M. UTTER 1973 Electrophoretic variation of adductor muscle proteins and tetrazoluim oxidase in the smooth Washington clam, *Saxidomus giganteus* (Deshayes, 1839) *Anim. Blood Grps biochem. Genet.,* 4.147-152.

MATHERS, N. S., N. P. WILKINS AND P. R. WALNE. 1974- Phosphoglucose isomerase and esterase phenotypes in *Crassostrea angulate* and *C. gigas Biochem. Syst. Ecoi,* 2 : 93-96

SCHAAL, B. A., W.W.I974. An outline of techniques for starch-gel electrophoresis of enzymes from the American oyster *Crassostrea virginica* G me I in. *Mar. Sci cent Tech. Rep. Ser.* No. 74 : 3 19.

SKIBINSKI, D. 0. F., A. R. MENEE, AND J. A. BEARDMORE. 1978. Protein variation in the marine bivalve *Scrobicularia plana. Anim. Blood Grps. biochem. Genet.,* 9 : 223 228.

WILKINS, N. P. 1975. Phosphoglucose isomerase in marine molluscs. *In :* Isozymes. IV. Genetics and Evolution (C. L. Markert Ed.) pp 931-943. Academic press N. Y.

WILKINS, N. P. N. F. 1973. Enzyme polymorphism in the European oyster, *Ostrea edulis* L. *Anim. Blood Grps. biochem. Genet.,* 4 : 41-47.