Biochemical Polymorphism in the Pacific Oyster—II Variants in Tetrazolium Oxidase and Leucine Aminopeptidase^{*1}

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With the same objectives as those of the previous report on myogen and esterase variants, the genetic variations in tetrazolium oxidase and leucine aminopeptidase from digestive diverticula of the Pacific oyster, *Crassostrea gigas*, sampled from Iwate Prefecture and Miyagi Prefecture were examined and compared with the results obtained by other authors on an oyster population from Puget Sound, U.S.A.

Described were two loci in tetrazolium oxidase, indicating monomorphic F locus and three phenotypes determined by two codominant alleles in S locus with dimeric structure. At least two loci were suggested in leucine aminopeptidase, demonstrating eleven bands, among which combinations of three bands expressed six phenotypes with the frequencies of occurrence expected under Hardy-Weinberg's genetic equilibrium. Three alleles were proposed for this locus. No association was observed between the above loci and the loci of either myogen or esterases reported before.

FUJINO and NAGAYA¹⁾ described variants in myogen from adductor muscle and esterase S zone from digestive diverticula in the Pacific oyster, *Crassostrea gigas*, sampled from the Pacific coast of Japan and compared the results with those reported by other authors. The present paper describes the results of analyses of variants in tetrazolium oxidase and leucine aminopeptidase from digestive diverticula of the Pacific oyster, *C. gigas*, sampled from Iwate Prefecture and Miyagi Prefecture and compares those with the results reported by BUROKER, HERSHBERGER, and CHEW²⁾.

Materials and Methods

Materials One hundred specimens of the Pacific oyster, *C. gigas*, were sampled from wild populations from the Bays of Ofunato, Hirota and Okkirai, Iwate Prefecture. Additional 120 specimens were sampled from cultured populations, of which the seeds were transplanted from the Bay of Mangoku, Miyagi Prefecture. In addition, 50 unidentified specimens were collected from the Bay of Okkirai.

Tissue Homogenates and Electrophoresis Tissue homogenates of digestive diverticula were prepared by following FUJINO *et al.*¹⁾ Horizontal starch gel electrophoresis was performed under the same conditions as FUJINO *et al.*¹⁾, except for buffer solutions for bridge and gel and solutions for staining and destaining, which were prepared according

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to BAUR *et al.*⁸⁾ as follows.

Tetrazolium oxidase (1) Gel buffer: A solution containing 0.1 M Tris (hydroxymethyl) aminomethane, 0.00325 M ethylenediamine tetraacetate (disodium salt) and 0.015 M H_3BO_3 (pH 8.7). (2) Bridge buffer: A solution (pH 8.8) containing 0.3 M Na_3BO_3 . (3) Staining mixture: 100 ml of 0.1 M Tris-HCl buffer (pH 8.5) containing 15 mg of nitroblue tetrazolium, 15 mg of phenazine methosulfate, and 20 mg of MgCl_2 . (4) Procedures: Electrophoresis was carried out for 6 to 8 h at approximately 5 to 6 V per linear cm of gel. Gel was soaked in a solution (pH 8.5) containing 0.3 M Na_3BO_3 for 3 min at 4°C and was incubated in the staining mixture for 2 to 3 h at 37°C. Then the stained gel was washed in a mixture of ethanol, acetic acid, and distilled water (5: 1: 5) for 2 min and placed in a solution of 15% glycerol and 2% acetic acid for storage.

Leucine aminopeptidase (1) Gel buffer: A solution containing 0.0144 M Tris, 0.0027 M citric acid, 0.0076 M H_3BO_3 , and 0.002 M LiOH (pH 7.6). (2) Bridge buffer: A solution (pH 8.7) containing 0.38 M H_3BO_3 and 0.1 M LiOH. (3) Staining mixture: 100 ml of solution containing 0.012 M monobasic potassium phosphate and 0.003 M dibasic sodium phosphate. To this add 30 mg of L-leucyl- β -naphthyl-amido-HCl and 60 mg of Fast Black K salt immediately before use. (4) Procedures: Electrophoresis was carried out for 3 to 4 h at approximately 7 to 8 V per linear cm of gel. Gel was stained for 30 min at 37°C. The stained gel was submerged in destaining solution for 2 to 3 h and placed in a storage solution.

Results

Tetrazolium oxidase (TO) TO activities were observed as the anodically migrating

Fig. 1. Diagrammatic representation of variants in TO from digestive diverticula in the Pacific oyster.

two achromatic zones, F and S, but not in the cathodic direction in zymogram. In the F zone, a single band occurred commonly in all specimens examined without variation. In the S zone observed were two bands, 1 and 2, and their hybrid band in between, indicating dimeric structure (Fig. 1). Three phenotypes are possible to occur as combinations of these bands. Frequencies of occurrence of the phenotypes are summarized in Table 1. Results of Hardy-Weinberg analyses for the figures suggested that the variations were determined by a set of two co-dominant alleles. No geographical difference

Table 1. Phenotype and allele frequencies of TO variants in the Pacific oyster

No. of lots		Phenotypes			Total	Alleles	
		1	1.2	2		TO ¹	TO ²
Ofunato 1	Obs.	50	0	0	50	1.000	0.000
	Exp.	50.0	0.0	0.0			
Hirota 2	Obs.	25	0	0	25	1.000	0.000
	Exp.	25.0	0.0	0.0			
Cultured 4	Obs.	38	2	0	40	0.975	0.025
	Exp.	38.0	2.0	0.0			
5	Obs.	37	0	0	37	1.000	0.000
	Exp.	37.0	0.0	0.0			
6	Obs.	22	1	0	23	0.978	0.022
	Exp.	22.0	1.0	0.0			
7	Obs.	20	0	0	20	1.000	0.000
	Exp.	20.0	0.0	0.0			
Okkirai 8	Obs.	25	0	0	25	1.000	0.000
	Exp.	25.0	0.0	0.0

Fig. 2. Diagrammatic representation of variants in leucine aminopeptidase from digestive diverticula in the Pacific oyster.

was seen in frequencies of the alleles between the four populations sampled. Zymogram of 50 unidentified specimens, collected from the Bay of Okkirai, revealed the distinct pattern as shown in Fig. 1, supporting our earlier work¹⁾ that they were different from *C. gigas*. No association was observed between locus of TO and loci of either myogen or esterases.¹⁾

Leucine aminopeptidase (LAP) Anodically migrating 11 bands were observed in zymogram of LAP. They were numbered from 1 through 11 in the descending order of their mobilities (Fig. 2). Bands 1, 5 and 7 were stained lighter than the other eight bands. All 11 bands revealed individual variations in the occurrence. Phenotypes as expressed by combinations of the bands 6, 8 and 10 were in a good agreement between their observed and expected frequencies under the Hardy-Weinberg's equilibrium with three codominant alleles (Table 2). The zymogram indicated that LAP in this locus was monomeric.

Table 2. Phenotype and allele frequencies of LAP variants in the Pacific oyster

No. of lots		Phenotypes						Total	Alleles		
		6	6.8	8	8.10	10	6.10		Lap ⁶	Lap ⁸	Lap ¹⁰
Ofunato 1	Obs.	0	3	35	9	0	0	47	0.032	0.872	0.096
	Exp.	0.1	2.6	35.7	7.9	0.4	0.3				
Hirota 2	Obs.	0	1	22	2	0	0	25	0.020	0.940	0.040
	Exp.	0.0	0.9	22.2	1.9	0.0	0.0				
Cultured 6	Obs.	0	1	17	5	0	0	23	0.022	0.870	0.108
	Exp.	0.0	0.9	17.3	4.4	0.3	0.1				
7	Obs.	0	0	14	6	0	0	20	0.000	0.850	0.150
	Exp.	0.0	0.0	14.4	5.1	0.5	0.0				
Okkirai 8	Obs.	0	2	21	2	0	0	25	0.040	0.920	0.040
	Exp.	0.0	1.8	21.3	1.8	0.0	0.1				

Differences in the degree of staining noted above suggested that at least two separate loci were involved in LAP. Insufficient data, however, did not permit us to make a genetic interpretation for the other bands. No significant difference was seen in frequencies of the alleles between the four populations sampled so far (Table 2). Zymogram of 50 unidentified specimens collected from the Bay of Okkirai, revealed much more simple zymogram pattern than those of *C. gigas* as shown in Fig. 2. This observation further supports our view that they were different from *C. gigas*. No association was observed between locus of LAP and all other loci examined by us.

Discussions

TO BUROKER *et al.*²⁾ recognized two loci in TO without individual variation in *C. gigas*. Our present results indicated no individual variation in the fast migrating (F) zone but variations in the slowly migrating (S) zone. The difference between the work of BUROKER *et al.*²⁾ and ours seems due to scantiness of specimens examined.

LAP BUROKER *et al.*²⁾ reported two zones (I and II) determined by three loci Lap-1a, Lap-1b, and Lap-2, expressing distinct 15 phenotypes as combinations of five bands

A, B, C, D, and E in Lap-1b locus. Frequency of allele C was highest (0.73 ± 0.032) among the five alleles for the population sampled from Puget Sound. Our work indicated that the highest frequency of occurrence appeared in allele Lap⁸ among three alleles (0.890 ± 0.076), which is significantly higher than those obtained for the population from Puget Sound. The above facts suggest necessity of (1) comparison test of zymogram for identifying phenotypes and loci and (2) further accumulation of population data.

References

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