ELECTROPHORETIC STUDIES ON TUBERCULATED OYSTERS FROM RANONG PROVINCE, THAILAND

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ABSTRACT

The electrophoretic patterns of protein in the adductor muscles of three species of tuberculated oysters from Ranong Province, Thailand were studied. Starch gel electrophoresis was used in order to differentiate the genetic characteristics, which could confirm the species identifications. Five loci showed suitable resolution, namely isocitrate dehydrogenase (*IDH**), malate dehydrogenase (*MDH**), phosphoglucomutase (*PGM**), leucine aminopeptidase (*LAP**) and mannose-phosphate isomerase (*MPI**). The *IDH** locus showed three different band patterns for *Striostrea* (*Parastriostrea*) *mytiloides*, *Saccostrea cucullata* and *Saccostrea forskali*. The *MDH** locus could differentiate only *Striostrea* (*Parastriostrea*) *mytiloides* from the others. These two enzyme loci were found to be genetic markers for tuberculated oysters from Ranong Province, Thailand.

INTRODUCTION

Species of oysters are very common in tropical areas including Thailand. However, the taxonomic status of Thai oysters remain uncertain, especially regarding tuberculated oysters, which are distributed throughout the country. Their habitats are rocky substratum, trunks and stilt roots of mangrove plants, cement poles and other substrata. It has been known that tuberculated oysters could display ecomorphology (Tack *et al.* 1992), because of the effect of environmental influences on the shell shape. These phenomena have produced an exceedingly confusing classification.

A variety of techniques have been applied such as morphometric analyses and studies of genetic variation in order to answer the question about species identification. Allozyme characters have been used to clarify the taxonomy of bivalve molluscs (McDonald *et al.* 1991; Beaumont *et al.* 1991). Torigoe (1975) examined 4 species of Japanese oysters using disc-electrophoresis and found that the electrophoretic patterns of adductor muscle extracts from each species had species characterizing patterns. A few papers reports on genetic variation in Pacific oyster (Buroker *et al.* 1975) and American oyster (Groue & Lester 1982; Buroker 1983). This study was undertaken in order to find the allozyme characters of each species, which could be used for taxonomic study of tuberculated oysters from Ranong Province.

MATERIALS AND METHODS

Sample collections

Samples of tuberculated oysters were collected from Ranong Province, west coast of Thailand, facing Andaman Sea (Long. 98°16' E, Lat. 9°13' N) during January 1999. Five collections were made at the following stations (Figure 1):

Station 1. Mangrove area, around the coastal water at the mouths of Kamphuan canal.

The oysters were attached to the trunk of

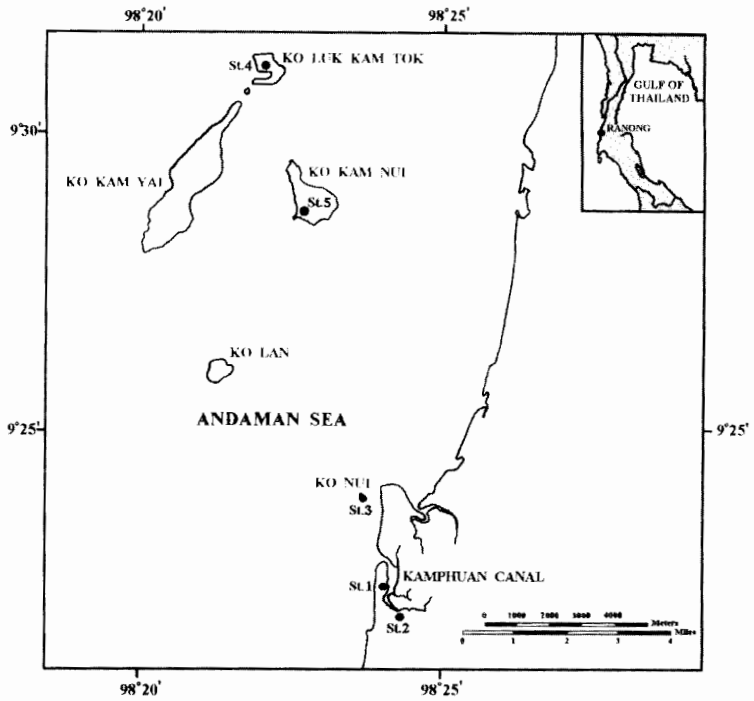

Figure 1.
Map of sampling stations at Ranong Province, Thailand.

mangrove plants.

Station 2. Mangrove area, along Kamphuan canal. The oysters were attached to the stilt roots of mangrove plants.

Station 3. Ko Nui, small offshore island about 500 meters from the coastline. The oysters were found on rocky substratum.

Station 4. Ko Luk Kam Tok; offshore island about 9 kilometers from the shore. The oysters were found on rocky substratum.

Station 5. Ko Kum Nui; offshore island about 6.7 kilometers from the shore. The oysters were found on rocky substratum.

Electrophoresis

Oyster samples were taken alive to the laboratory and placed at -40 °C. Preparation of the samples and electrophoretic technique were performed by methods described by Hara & Na-Nakorn (1996). Electrophoresis

Table 1 Enzymes studied, buffer and tissues, number of loci resolved in electrophoretic study. T.C. E = Tris citrate EDTA pH 7.0, T.C. = Tris citrate pH 8.0, Add.M = Adductor muscle, Poly. = polymorphic, Mono. = monomorphic.

Enzyme system	Enzyme No.	Buffer	Tissue	Locus	Number of loci	
					Poly.	Mono.
Isocitrate dehydrogenase	1.1.1.4.2	T.C.E	Add.M	IDH*	0	1
Malate dehydrogenase	1.1.1.37	T.C.E	Add.M	MDH*	1	0
Phosphoglucomutase	5.4.2.2	T.C.E	Add.M	PGM*	1	0
Leucine aminopeptidase	3.4.11.1	T.C	Add.M	LAP*	1	0
Mannose-6-phophate isomerase	5.3.1.8	T.C	Add.M	MPI*	1	0

Table 2. Allele frequencies at 5 loci for the 3 species of tuberculated oysters collected from Ranong Province, Thailand. STM = *Striostrea (Parastriostrea) mytiloides*, SF = *Saccostrea forskali*, SC = *Saccostrea cucullata*

Locus	Alleles	STM	SF			SC
		Station 1	Station 2	Station 3	Station 4	Station 5
		N=50	N=49	N=39	N=45	N=50
IDH*	*350	**1.000**	0.000	0.000	0.000	0.000
	*100	0.000	**1.000**	**1.000**	**1.000**	0.000
	*-125	0.000	0.000	0.000	0.000	**1.000**
		N=50	N=49	N=39	N=47	N=50
MDH*	*129	0.000	**0.133**	**0.321**	0.000	0.000
	*106	**0.410**	0.000	0.000	0.000	0.000
	*100	0.000	**0.867**	**0.679**	**0.862**	**0.970**
	*76	**0.590**	0.000	0.000	0.000	0.000
	*71	0.000	0.000	0.000	**0.138**	0.000
	*65	0.000	0.000	0.000	0.000	**0.020**
	*35	0.000	0.000	0.000	0.000	**0.010**
		N=50	N=48	N=37	N=46	N=50
MPI*	*160	0.000	0.000	0.000	0.000	**0.100**
	*135	0.000	0.000	0.000	0.000	**0.320**
	*125	0.000	0.000	0.000	**0.120**	**0.420**
	*120	**0.080**	0.000	0.000	0.000	0.000
	*115	0.000	**0.229**	**0.257**	0.000	0.000
	*110	0.000	0.000	0.000	0.000	**0.060**
	*100	**0.920**	**0.771**	**0.743**	**0.695**	**0.100**
	*75	0.000	0.000	0.000	**0.185**	0.000
		N=49	N=42	N=37	N=47	N=50
PGM*	*131	0.000	**0.274**	0.000	0.000	0.000
	*123	0.000	0.000	**0.108**	0.000	**0.020**
	*115	**0.143**	**0.262**	0.000	0.000	**0.540**
	*108	0.000	**0.202**	**0.405**	**0.074**	**0.110**
	*104	0.000	0.000	0.000	**0.383**	0.000
	*100	**0.469**	**0.155**	**0.459**	0.000	0.000
	*96	0.000	0.000	0.000	0.000	**0.310**
	*88	0.000	**0.107**	0.000	**0.106**	0.000
	*85	**0.368**	0.000	0.000	**0.436**	0.000
	*77	0.000	0.000	**0.028**	0.000	**0.020**
	*62	**0.020**	0.000	0.000	0.000	0.000

Table 2 (continued) Allele frequencies at 5 loci for the 3 species of tuberculated oysters collected from Ranong Province, Thailand. STM = *Striostrea (Parastriostrea) mytiloides*, SF = *Saccostrea forskali*, SC = *Saccostrea cucullata*.

Genetic data Locus	Alleles	STM	SF			SC
		Station 1	Station 2	Station 3	Station 4	Station 5
		N=48	N=43	N=38	N=46	N=50
LAP*	*130	0.000	0.000	0.000	0.000	**0.670**
	*117	0.000	0.000	0.000	0.000	**0.040**
	*109	**0.052**	0.000	0.000	0.000	**0.280**
	*100	**0.552**	0.000	0.000	0.000	**0.010**
	*96	0.000	0.000	0.000	**0.359**	0.000
	*91	**0.396**	0.000	0.000	0.000	0.000
	*87	0.000	0.000	0.000	**0.250**	0.000
	*83	0.000	0.000	0.000	**0.325**	0.000
	*74	0.000	**0.220**	**0.579**	**0.033**	0.000
	*65	0.000	**0.198**	**0.368**	0.000	0.000
	*61	0.000	0.000	0.000	**0.033**	0.000
	*57	0.000	0.000	**0.053**	0.000	0.000
	*52	0.000	**0.232**	0.000	0.000	0.000
	*35	0.000	**0.349**	0.000	0.000	0.000

was carried out on horizontal gels of 11 % starch (Sigma laboratory). Pretest for screening of few individuals for up to 23 enzyme systems were made and 5 enzyme loci were found to give suitable resolution for investigation in all samples. The enzyme system, E.C. number, buffer systems, tissue source, and number of loci are summarized in Table 1.

RESULTS AND DISCUSSION

Oyster identification

According to shell morphological characters, the collected samples from each station could be identified as follows

Station 1. *Striostrea (Parastriostrea) mytiloides* (Lamarck, 1819)

Station 2, 3, and 4. *Saccostrea forskali* (Gmelin, 1791)

Station 5. *Saccostrea cucullata* (Born, 1778)

Allozyme banding patterns

Five loci were found from five enzyme systems with one locus for each enzyme. Among these loci, one was found to be monomorphic while the other four loci were polymorphic. Allozyme frequencies for each locus are presented in Table 2.

The *IDH** locus proved to be monomorphic in all collections and showed only one allele for each species: at **350* for *Striostrea (Parastriostrea) mytiloides*, at **-125* for *Saccostrea cucullata* and **100* for *Saccostrea forskali*. The *IDH** locus had a marker allele, which could be used as genetic marker for the three species of tuberculated oysters from Thailand (Table 2).

The *MDH** locus was polymorphic in all collections, there were two alleles **106* and **76* which could be used to differentiate *Striostrea (Parastriostrea) mytiloides* from other species (Table 2).

The other three loci *MPI**, *PGM** and *LAP** did not show any marker allele, which could be used to differentiate tuberculated oysters.

Among the five collections, the oysters from stations 1 and station 5 had unique shell characteristics and could be clearly identified as *Striostrea (Parastriostrea) mytiloides* and *Saccostrea cucullata* respectively. But the oysters from stations 2, 3, and 4 displayed pronounced variations of shell morphology, including shell size, shell shape, depth of umbonal cavity and commissural plications. However, they all had the same character of the adductor muscle scar and the crenulation of left valve. These oysters were identified as *Saccostrea forskali*. These morphological variations may due to environmental differences because the habitats and environmental factors of station 2, 3 and 4 are different with respect to salinity, wave action, turbidity, and substratum.

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