UNIVERSITI PUTRA MALAYSIA

GENETIC POLYMORPHISMS IN SEA CUCUMBER (HOLOTHUROIDEA) USING RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD)

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By

NORAZILA BT KASSIM SHAARI

Thesis Submitted in Fulfilment of the Requirement for the Degree of Master of Science in the Faculty of Medicine and Health Sciences Universiti Putra Malaysia

November 2000

UPM

A SPECIAL DEDICATION.....

For my parents

Kassim Shaari and Zaini

For my sister and brother

Norin, Atan, Anja and Yan

and my friends

Azizah, Wan, Asma, Samsiah and Erin

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science.

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Chairman: **Associate Professor Patimah Ismail, Ph.D.**

Faculty: **Medicine and Health Sciences.**

Random Amplified Polymorphic DNA (RAPD) fingerprinting was used to identify DNA-based genetic polymorphism and estimate genetic variability among 13 species of sea cucumber. It offers a rapid and efficient method for generating a new series of DNA markers in sea cucumber. Seventeen single arbitrary primers (9,10 and 12 nucleotides) with G+C contents 50%-80% were chosen for their utility in identifying genetic polymorphism in this study. The numbers of amplification fragments were 3-7 bands per primer that ranged in size from 80 to 1750 nucleotide base pair. Bands of the amplified DNA was scored and analyzed using the RAPDistance Package and NTSYS Program. The presence or absence of bands on the gel detects a DNA polymorphism for inter- and intra-locality studies. There were 839 types of genotype profiles from 4 locations of sea cucumber with an average of 43 in Sabah, 44 in Terengganu, 47 in Perak and 49 in Kedah. For each of the primers, 3 types of genotypes (42%) are the most frequently observed followed by 38% for 2 genotypes, 12% for 4 genotypes and 8% for 1 genotype. A total of

2494 bands were amplified with an average of 147 bands per primer. In Sabah a total of 893 RAPD markers were scored followed by 1074 in Terengganu, 124 in Perak and 303 in Kedah. Two to nine markers per primer were detected from each location with a total of 306 monomorphic bands and 2188 polymorphic bands. Perak samples showed the most variability, having an average of 98.5% of it bands polymorphic followed by Kedah (94.5%), Sabah (85.9%) and Terengganu being the least variable (only 85.5%). The overall polymorphism was high among species within a locality for sea cucumbers. The individuals from each of the four locations are tightly clustered with one another with mean genetic distances between 0.160 and 0.750. A study on interlocality and intralocality genetic distances showed no association with geographic distances between the localities. Species from Sabah were found to be closer to Terengganu compared to Perak and Kedah. However, for the interpopulation study of *Stichopus variegatus* Semper the analysis showed this association.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains.

KAJIAN POLIMORFISMA GENETIK POPULASI TIMUN LAUT (HOLOTHUROIDEA) DENGAN TEKNIK RAPD

Oleh

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Pengerusi: **Profesor Madya Patimah Ismail, Ph.D.**

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Random Amplified Polymorphic DNA (RAPD) fingerprinting digunakan bagi mengenalpasti dan menentukan kepelbagaian genetik bagi 13 spesies timun laut. Ia merupakan teknik rawak yang berkesan di dalam membentuk penanda DNA bagi organisma ini. Sejumlah 17 primer rawak (9,10 dan 12 rantaian) dengan kandungan G+C di antara 50%-80% telah digunakan dalam pencirian kepelbagaian genetik bagi kajian inter dan intra lokasi. Jalur amplifikasi yang terhasil bagi setiap primer(pencetus) adalah di antara 3 hingga 7 yang berada dalam julat 80-1750 rantaian base pair. Jalur-jalur yang terhasil dikira dan dianalisis dengan program RAPDistance dan NTSYS. Sejumlah 839 profil genotip terhasil daripada kesemua lokasi dengan purata 43 bagi Sabah, 44 bagi Terengganu, 47 bagi Perak dan 49 bagi Kedah. Bagi setiap primer, 3 genotip (42%) merupakan kekerapan tertinggi diikuti dengan 38% bagi 2 genotip, 12% bagi 4 genotip dan 8% bagi 1 genotip. Sejumlah 2494 jalur terhasil dengan purata 147 jalur bagi setiap primer. Sabah menghasilkan 893 penanda RAPD diikuti dengan 1074 bagi Terengganu, 124 bagi Perak and 303

bagi Kedah. Secara purata penanda yang terhasil bagi setiap primer adalah di antara 2-9 penanda dengan 306 jalur yang monomorfik dan 2188 jalur yang polimorfik. Perak menunjukkan peratus jalur polimorfik tertinggi dengan 98.5%, Kedah (94.5%), Sabah (85.9%) dan Terengganu dengan peratus terendah (85.5%). Secara keseluruhan, polimorfisma adalah tinggi di antara spesies timun laut. Setiap individu daripada kesemua lokasi, dicluster bersama yang terkandung di antara jarak genetik pada nilai 0.160 dan 0.750. Kajian intra dan inter lokasi tidak menunjukkan sebarang perhubungan antara jarak geografi dengan kumpulan cluster yang terbentuk. Spesies dari Sabah didapati adalah rapat dari segi genetik dengan spesies dari Terengganu berbanding dengan Perak dan Kedah. Namun bagi kajian populasi *Stichopus variegatus* Semper, terdapat perhubungan di antara jarak geografi dengan jarak genetik di mana populasi Terenganu di dapati lebih rapat dengan populasi daripada Perak berbanding populasi daripada Sabah.

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TABLE OF CONTENTS

		Page
DEDICATION		ii
ABSTRACT		iii
ABSTRAK		v
ACKNOWLEDGEMENTS		vii
APPROVAL SHEETS		viii
DECLARATION FORM		x
LIST OF TABLES		xiii
LIST OF FIGURES		xv
LIST OF PLATES		xviii
LIST OF ABBREVIATIONS		xx
CHAPTER		
I	INTRODUCTION	1
II	LITERATURE REVIEW	
	Biology of Sea Cucumber	7
	Population Studies	11
	Genetic Markers	13
	Polymerase Chain Reaction (PCR)	16
	RAPD Markers	20
	Polyacrylamide Gel Electrophoresis (PAGE) and Silver Staining	24
	Polymorphisms	25
III	MATERIALS AND METHODS	
	Collection of Samples	28
	Extraction of Genomic DNA	35
	Analysis of DNA by gel electrophoresis	36
	Quantification and Purity of DNA Samples	37
	Polymerase Chain Reaction (PCR) Amplification	37
	Polymorphism and Data Analysis	39
IV	RESULTS	
	Extraction of Genomic DNA	41
	RAPD Amplification	42
	Polymorphism	44
	Inter-locality Study	56

Intra-locality Study 60
Population of *Stichopus variegatus* Semper 66

V DISCUSSION 67
Extraction of Genomic DNA 71
Optimization of PCR Assay 73
RAPD Amplification 76
Inter-locality Study 79
Intra-locality Study 82
Population of *Stichopus variegatus* Semper 84

VI CONCLUSION AND RECOMMENDATION 85

REFERENCES 87

APPENDICES 96

VITA 160

LIST OF TABLES

Tables		Page
1	Properties of systems for generating RAPD markers (Rafalski and Tingey, 1993)	23
2	Sea cucumber samples from the different geographical regions	28
3	Silver staining protocol (Herring *et. al.,* 1982)	36
4	List of the primers that are used in the PCR amplification	38
5	The number (range) of bands and the range of size (bp) of the amplification products	42
6	Number of genotypes in various species of sea cucumber (Holothuroidea) from Sabah, Terengganu, Perak and Kedah	48
7	Number of bands in species of sea cucumber (Holothuroidea) from Sabah, Terengganu, Perak and Kedah	49
8	Number of monomorphic bands in various species of sea cucumber (Holothuroidea) from Sabah, Terengganu, Perak and Kedah	50
9	Number of polymorphic bands in various species of sea cucumber (Holothuroidea) from Sabah, Terengganu, Perak and Kedah	51
10	Number of bands(T), polymorphic bands(P) and proportion of polymorphic bands(Pm) in various species of sea cucumber (Holothuroidea) from Sabah, Terengganu, Perak and Kedah	52
11	Genetic distances in 19 species of sea cucumber from Sabah, Terengganu, Perak and Kedah based on Lynch's (1991) band sharing similarity indices	59

12	Genetic distances in 8 species of sea cucumber from Sabah based on Lynch's (1991) band sharing similarity indices	64
13	Genetic distances in 8 species of sea cucumber from Terengganu based on Lynch's (1991) band sharing similarity indices	65
14	Genetic distances in species of *Stichopus variegatus* from Sabah, Perak and Terengganu based on Lynch's (1991) band sharing similarity indices	66
15	Genetic distances in 65 individual sea cucumbers from Sabah based on Lynch's (1991) band sharing similarity indices	136
16	Genetic distances in 75 individual sea cucumbers from Terengganu based on Lynch's (1991) band sharing similarity Indices	142
17	Genetic distances in 95 individual sea cucumbers from Sabah, Terengganu, Perak and Kedah based on Lynch's (1991) band sharing similarity indices	150

LIST OF FIGURES

Figures		Page
1	Anotomy of sea cucumber (Nichols, 1969)	9
2	Life cycle of sea cucumber	10
3	Diagram illustrating the use of PCR to amplify DNA molecule *in vitro* (Klug and Cummings, 1999)	19
4	Location map of Sea Cucumber (Holothuroidea) sampling sites	29
5	UPGMA cluster analysis based on distances generated from Nei and Li's indices for 95 individual sea cucumbers from Sabah, Terengganu, Perak and Kedah	57
6	UPGMA cluster analysis based on distances generated from Nei and Li's indices for 19 species of sea cucumbers from Sabah(S), Terengganu(T), Perak(P) and Kedah(K)	58
7	UPGMA cluster analysis based on distances generated from Nei and Li's indices for 65 individual sea cucumbers from Sabah	61
8	UPGMA cluster analysis based on distances generated from Nei and Li's indices for 75 individual sea cucumbers from Terengganu	63
9	UPGMA cluster analysis based on distances generated from Nei and Li's indices for 8 species of sea cucumbers from Sabah	64
10	UPGMA cluster analysis based on distances generated from Nei and Li's indices for 8 species of sea cucumbers from Terengganu	65
11	UPGMA cluster analysis based on distances generated from Nei and Li's indices for *Stichopus variegatus* from Sabah, Perak and Terengganu	66

12 Schematic diagram of genotypes observed in *Holothuria edulis* from Sabah 100

13 Schematic diagram of genotypes observed in *Stichopus variegatus* from Sabah 102

14 Schematic diagram of genotypes observed in *Stichopus badionotus* from Sabah 104

15 Schematic diagram of genotypes observed in *Bohadschia marmorata vitiensis* from Sabah 106

16 Schematic diagram of genotypes observed in *Holothuria atra* from Sabah 108

17 Schematic diagram of genotypes observed in *Holothuria tubulosa* from Sabah 110

18 Schematic diagram of genotypes observed in *Holothuria impatiensis* from Sabah 112

19 Schematic diagram of genotypes observed in *Holothuria scabra* from Sabah 114

20 Schematic diagram of genotypes observed in *Holothuria edulis* from Terengganu 116

21 Schematic diagram of genotypes observed in *Stichopus badionotus* from Terengganu 118

22 Schematic diagram of genotypes observed in *Bohadschia marmorata vitiensis* from Terengganu 120

23	Schematic diagram of genotypes observed in *Holothuria atra* from Terengganu	122
24	Schematic diagram of genotypes observed in *Stichopus horrens* from Terengganu	124
25	Schematic diagram of genotypes observed in *Stichopus chloronotus* from Terengganu	126
26	Schematic diagram of genotypes observed in *Stichopus variegatus* from Terengganu	128
27	Schematic diagram of genotypes observed in *Bohadschia marmorata marmorata* from Terengganu	130
28	Schematic diagram of genotypes observed in *Stichopus variegatus* from Perak	132
29	Schematic diagram of genotypes observed in *Stichopus* Sp_1 from Kedah	134
30	Schematic diagram of genotypes observed in *Stichopus* Sp_2 from Kedah	135

LIST OF PLATES

Plates		Page
1	*Holothuria atra* (Lollyfish)(Jaeger)	30
2	*Holothuria edulis* (Pinkfish)(Lesson 1830)	30
3	*Holothuria tubulosa* (Gmelin 1877)	31
4	*Holothuria impatiens* (Forskal 1773)	31
5	*Holothuria scabra* (Sandfish)(Jager 1833)	32
6	*Stichopus variegatus* (Curryfish)(Semper 1868)	32
7	*Stichopus badionotus* (Selenka 1867)	33
8	*Stichopus Sp1*	33
9	*Stichopus chloronotus* (Greenfish)(Brandt 1935)	34
10	*Bohadschia marmorata vitiensis* (Chalkfish)(Jaeger 1833)	34
11	Sea cucumber genomic DNA run on 10% nondenaturing polyacrylamide gel (dPAGE)	41
12	DNA fingerprints generated by primer NUSZG3 in 19 species of sea cucumber	43
13	DNA fingerprints generated by primer NUSZG8 in 19 species of sea cucumber	44

14	DNA fingerprints generated by primer NUSZG2 in Sabah and Terengganu samples	53
15	DNA fingerprints generated by primer NUSZG4 in Sabah and Terengganu samples	53
16	DNA fingerprints generated by primer GEN 1 in 14 species of sea cucumber	54
17	DNA fingerprints generated by a primer GEN 6 in 14 species of sea cucumber	54
18	DNA fingerprints generated by primer GEN 9 in 14 species of sea cucumber	55
19	DNA fingerprints generated by primer NUSZG 8 in 14 species of sea cucumber	55

LIST OF ABBREVIATIONS

Chemicals

EDTA	Ethylenediamine tetraacetate
KCl	Potassium chloride
$MgCl_2$	Magnesium chloride
SDS	Sodium dodecyl sulphate
TBE	Tris-borate-EDTA buffer

Units

bp	base pair
°C	Celcius
hr	hour
kb	kilobase
min	minute
mM	millimolar
ng	nanogram
nmole	nanomole
OD	optical density
pmole	picomole
sec	second
ug	microgram
ul	microliter
V	volt

CHAPTER I

INTRODUCTION

Sea cucumber or holothurian is one of the five classes belonging to the marine phylum of echinoderms which also includes sea urchins, sea stars, sea-lilies and brittle stars. Sea cucumbers are locally known as 'bat', 'balat' or 'trepang' among the ethnic groups of Sabah. In Peninsular Malaysia, they are referred to as 'gamat'and are widely used in the traditional treatment of asthma, hypertension and injuries (Ridzwan *et al*., 1995).

The class holothuroidea is composed of approximately 1200 species with the greatest degree of diversity in the tropics (Rowe, 1969). There are close to 300 species of sea cucumbers in the Indo-West Pacific region but probably only a dozen or more are known to be of commercial value. Ridzwan (1993) reported that 23 species of holothurians were recorded in Sabah and fourteen of them are edible being taken either raw or after simple preparation.

According to the FAO's annual statistics (FAO, 1996), the sea cucumber harvest had increased steadily in volume from 102,705 tones in 1987 to 127,149 in 1995. In 1996 the total world production of sea cucumber is about 117,915 metric

tonnes with Japan, Indonesia , Philippines and United States of America (USA) being the top producers. The beche-de-mer and its associated industries are economically important and give good income for most Asian countries.

In the Malaysian Annual Fisheries Statistic Report for 1995, sea cucumbers are classified into three categories of commodity; live, fresh and chilled; frozen and others. Sea cucumber production in 1995 was 155 metric tonnes with average retail prices ranging from RM 80 to RM 120 for each kilogram. The import value of this product had decreased from 5.96 tonnes with a value RM 33,574 in 1991 to 0.50 tonnes with a value RM 4,743 in 1995. The export value also decreased from 77.75 tonnes (RM 1205,34) in 1991 to 77.20 tonnes (RM 1176,186) in 1996.

The cured products are termed beche-de-mer (meaning seaworm), trepang (meaning sea slug) and hoi-sum in Chinese. According to Chan and Liew (1985), sea cucumbers are believed to have some aphrodisiac qualities as well as curing effects on a variety of ailments. In traditional Malay medicine, 'air gamat' is used in post-natal treatment and 'minyak gamat' is used for healing open wounds. Among the Chinese, it is considered as a delicacy, normally taken during festive occasions. Sea cucumber is known to contain a toxin called holothurin that could inhibit hemolytic neuromuscular and cytotoxic activities.

The preparation procedure involves boiling, evisceration, a second boiling, smoke drying and sun curing. Grading of products is first done by species, followed by size, appearance and dirt content. The largest markets for beche-de-mer is

Hong Kong and Singapore. The first grade products can fetch as much as RM 30 per kilogram. In Malaysia, only Sabah produces some beche-de-mer although edible species are known to occur in Peninsular Malaysia as well.

The domestication of species used in aquaculture will not be completed until we have control over all aspect of their biology including genetics. For virtually all echinoderms, we at present do not have that control but significant advances have been made in the past few years towards understanding many aspects of their genetics. Genetics is a broad discipline covering any field, which deals with inheritance. The various fields of genetics such as cytogenetics, population genetics and Mendelian genetics are often used as tools in selective breeding. Breeding here means we are concerned with the genetic control, manipulation and improvement of traits of commercial interest.

The use of population genetics data offers the geneticist a means of identifying the level of genetic differentiation in a species and, therefore, a rational basis for the selection of subpopulations to develop better cultured strains. According to Martinez *et al.*, 1997, molecular techniques allowing differentiation between strains, genotypes or individuals had become indispensable tools in population genetic surveys. Some of the approaches used in holothurian genetics have limited potential for making immediate practical contributions, but may be useful in the future when aquaculture breeding becomes more sophisticated.

One of the ongoing enigmas that faces evolutionary biologists is the problem of ascertaining key characters that can reveal the relationships at higher level taxa; i.e., phylum, class and order. Polymerase chain reaction (PCR) amplification, DNA cloning and sequencing have been used to examine the relationships of the brittle star (class: Ophiuroidea) and sea cucumbers (class: Holothuroidea) to the sea star and sea urchins (Smith *et al*., 1993). In Malaysia, recent studies on the biology and genetics of sea cucumbers are still limited.

DNA analyses and fingerprinting are assuming important roles in aquaculture and will become standard techniques for population identification, monitoring inbreeding levels, pedigree studies and genetic broodstock management (Hallerman and Beckmann, 1988). The general lacks of visible genetic markers make the use of biochemical markers essential.

In some countries, genetic markers have been widely used in fisheries management (Ferguson *et al*., 1995) but this approach is still new in Malaysia. Not much work has been done on the genetic characterization of local marine species through the use of molecular markers. Instead, emphasis had been placed on environmentally influenced characters such as morphological characters for evaluating variation among populations.

Several types of molecular markers have been developed to assist in genetic analysis. Random amplified polymorphic DNA (RAPD) marker, is particularly well suited to high-throughput systems required for plant breeding because of the