

STUDY ON GENETIC VARIABILITY OF *Caecotrypa* sp.
USING RAPD-PCR TECHNIQUE

MCHD. SUNDHARJAN BIN IKMAL

FACULTY AGRIKULTUR DAN TEKNOLOGI

UNIVERSITI SAINS MALAYSIA

2005

STUDY ON GENETIC VARIABILITY OF *Saccostrea* sp. USING RAPD – PCR
TECHNIQUE

By

Mohd. Syahrilhezrian Bin Zainal

Research Report submitted in partial fulfillment of
the requirements for the degree of
Bachelor of Science (Biological Sciences)

Department of Biological Sciences
Faculty of Science and Technology
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA
2005

This project should be cited as:

Syahril, Z. 2005. Study on genetic variability of *Saccostrea* sp. using RAPD – PCR technique. Undergraduate thesis, Bachelor of Science in Biological Sciences, Faculty of Science and Technology, Kolej Universiti Sains dan Teknologi Malaysia, Terengganu. 74p.

No part of this project report may be produced by any mechanical, photographic, or electronic process, or in the form of phonographic recording, nor may it be stored in a retrieval system, transmitted, or otherwise copied for public or private use, without written permission from the author and the supervisor(s) of the project.



**JABATAN SAINS BIOLOGI
FAKULTI SAINS DAN TEKNOLOGI
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA**

**PENGAKUAN DAN PENGESAHAN LAPORAN
PROJEK PENYELIDIKAN I DAN II**

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: STUDY ON GENETIC VARIABILITY OF *Saccostrea* sp. USING RAPD – PCR TECHNIQUE oleh MOHD. SYAHRILHIZRIAN BIN ZAINAL No. Matrik UK 6534 telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperoleh Ijazah Sarjana Muda Sains-Sains Biologi Fakulti Sains dan Teknologi, Kolej Universiti Sains dan Teknologi Malaysia.

Disahkan oleh:

Penyelia Utama

WAN BAYANI WAN OMAR

Nama:

PENSYARAH

Cop Rasmi:

Jabatan Sains Biologi
Fakulti Sains & Teknologi
Kolej Universiti Sains dan Teknologi Malaysia
21030 Kuala Terengganu, Terengganu.

Tarikh:

7/4/2005

Penyelia Kedua (jika ada)

Nama:

Dr. Zaleha Binti Kassim

Cop Rasmi

Pensyarah
Jabatan Sains Samudera
Fakulti Sains dan Teknologi
Kolej Universiti Sains dan Teknologi Malaysia
21030 Kuala Terengganu.

Tarikh:

6/4/05

Ketua Jabatan Sains Biologi

Nama:

PROF. MADYA DR. NAKISAH BT. MAT AMIN
Ketua

Cop Rasmi:

Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Kolej Universiti Sains dan Teknologi Malaysia
(KUSTEM)
21030 Kuala Terengganu.

Tarikh:

9/4/05

ACKNOWLEDGEMENT

First of all, with the completion of this project, I like to express my grateful to God for His blessing that assist me throughout the project.

Secondly, I would like to extend my gratitude and my appreciation to my first supervisor, Miss Wan Bayani binti Wan Omar and my second supervisor, Dr. Zaleha Binti Kassim, whose help, guidance, advice and patience help rendered my progress throughout my project. I am also would like to thank to the library of Universiti Kebangsaan Malaysia, Universiti Putra Malaysia and Universiti Malaya for helping me providing resources of my project. My thanks also go to the all staffs of Department of Biology, KUSTEM, for their assistant in one way or another.

My most thanks must go to my entire friend, Hasbullah, Norin, Roslina, Rizal, Diah, Anitha and Devikee for their help along the project. I am also grateful to all my housemate and course mate for being so supportive. Last but not least, I would like to thank to my parents and relative for making this reality and success in my life.

Thanks to everyone.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
LIST OF TABLES	v
LIST OF FIGURES	vi
LIST OF APPENDICES	viii
LIST OF ABBREVIATIONS	ix
ABSTRACT	xi
ABSTRAK	xii
CHAPTER	
1. INTRODUCTION	1
2. LITERATURE REVIEW	
2.1 Taxonomy and Morphology	4
2.2 Habitat and Distribution	8
2.3 Feeding	8
2.4 Reproduction and Growth	9
2.5 Molecular Genetic Marker	10
2.6 Rapid Amplification Polymorphism DNA – Polymerase Chain Reaction	11
2.7 Electrophoresis and Staining	14
2.8 Genetic Variation	15
2.9 Polymorphism	16

3.	MATERIALS AND METHODS	
3.1	Sample Collections	17
3.2	DNA Extraction	19
	Kit Wizard™ Genomic DNA Purification (Promega)	
3.3	Agarose Gel Electrophoresis	20
3.4	Measurement of DNA purity and Quality	20
3.5	Screening of RAPD Primer	21
3.6	Data Analysis	23
4.	RESULT	
4.1	Purity and Quantity of DNA	25
4.2	Screening of RAPD Primers	30
4.3	RAPD Profiles	33
4.5	Dendrogram Analysis	42
5.	DISCUSSION	
5.1	Purity and Quantity of DNA	49
5.2	Screening of RAPD Primers	51
5.3	RAPD Profiles.	52
5.4	Dendrogram Analysis	53
6.	CONCLUSION	55
	REFERENCES	57
	APPENDICES	62
	CURRICULUM VITAE	74

LIST OF TABLE

Table		Page
3.1	Code, sequence, nucleotide length and G+C content of primers used in Random Amplified Polymorphic DNA analysis	22
4.1	Observed density (OD) of DNA purity and quantity of DNA from Pulau Che Him area.	29
4.2	Observed density (OD) of purity and quantity of DNA from Pulau Semut area.	29
4.3	Number of fragments, size of fragments, total number of fragments, number of polymorphic fragments and percentage of polymorphic of <i>Saccostrea</i> sp. generated from OPA 15, Opa 19 and OPA 20 for Pulau Che Him.	34
4.4	Number of fragments, size of fragments, total number of fragments, number of polymorphic fragments and percentage of polymorphic of <i>Saccostrea</i> sp. generated from OPA 15, OPA 19 and OPA20 for Pulau Semut.	34
4.5	Number of fragments, size of fragments, total number of fragments, number of polymorphic fragments and percentage of polymorphic of <i>Saccostrea</i> sp. from Pulau Che Him and Pulau Semut.	35
4.6	Similarity Index of <i>Saccostrea</i> sp. from Pulau Che Him	44
4.7	Similarity Index of <i>Saccostrea</i> sp. from Pulau Semut	44

LIST OF FIGURES

Figure		Page
2.1	The classification of <i>Saccostrea</i> sp.	6
2.2	Picture of <i>Saccostrea</i> sp.	7
2.3	Picture of <i>Saccostrea</i> sp.	7
3.1	Sampling area in Setiu Wetland, Terengganu	18
3.2	<i>Saccostrea</i> sp. attach on mangrove trunks	18
4.1	Genomic Extracted using Wizard Genomic DNA Purification Kit (Promega) on 0.8% agarose gel and stained with 0.5 µg/ml ethidium bromide from Pulau Che Him.	27
4.2	Genomic Extracted using Wizard Genomic DNA Purification Kit (Promega) on 0.8% agarose gel and stained with 0.5 µg/ml ethidium bromide from Pulau Semut	28
4.3	RAPD banding pattern for screening of Operon Technology Kit A primers, OPA 01 to OPA 10	31
4.4	RAPD banding pattern for screening of Operon Technology Kit A primers, OPA 11 to OPA 20	32
4.5	Banding patterns of RAPD fragments of <i>Saccostrea</i> sp. from Pulau Che Him using primer OPA 15	36
4.6	Banding patterns of RAPD fragments of <i>Saccostrea</i> sp. from Pulau Che Him using primer OPA 19	37
4.7	Banding patterns of RAPD fragments of <i>Saccostrea</i> sp. from Pulau Che Him using primer OPA 20	38
4.8	Banding patterns of RAPD fragments of <i>Saccostrea</i> sp. from Pulau Semut using primer OPA 15	39
4.9	Banding patterns of RAPD fragments of <i>Saccostrea</i> sp. from Pulau Semut using primer OPA 19	40
4.10	Banding patterns of RAPD fragments of <i>Saccostrea</i> sp. from Pulau Semut using primer OPA 20	41

4.11	UPGMA cluster analysis based on the genetic distance generated from Nei and Li's indices <i>Saccostrea</i> sp. from Pulau Che Him	45
4.12	UPGMA cluster analysis based on the genetic distance generated from Nei and Li's indices of <i>Saccostrea</i> sp. from Pulau Semut	46
4.13	UPGMA cluster analysis based on the genetic distance generated from Nei and Li's indices of different <i>Saccostrea</i> sp. populations.	47
4.14	Dendrogram showing genetic relationship between 12 individuals between Pulau Che Him and Pulau Semut	48

LIST OF APPENDICES

		Page
Table A1	Length, width and body weight of <i>Saccostrea</i> sp from Pulau Che Him	63
Table A2	Length, width and body weight of <i>Saccostrea</i> sp from Pulau Semut	63
Table B1	Presence and absence of DNA bands of <i>Saccostrea</i> sp. from Pulau Che Him using OPA 15	64
Table B2	Presence and absence of DNA bands of <i>Saccostrea</i> sp. from Pulau Che Him using OPA 19	65
Table B3	Presence and absence of DNA bands of <i>Saccostrea</i> sp. from Pulau Che Him using OPA 20	66
Table B4	Presence and absence of DNA bands of <i>Saccostrea</i> sp. from Pulau Semut using OPA 15	67
Table B5	Presence and absence of DNA bands of <i>Saccostrea</i> sp. from Pulau Semut using OPA 19	68
Table B6	Presence and absence of DNA bands of <i>Saccostrea</i> sp. from Pulau Semut using OPA 20	69
Table C	Similarity Index of <i>Saccostrea</i> sp. from both populations (Pulau Che Him and Pulau Semut).	70
Figure D1	Image Master VDS Machine	71
Figure D2	Eppendorf Marter Cyclor	71
Figure D3	UV Transluminator	72
Figure D4	Eppendorf Centrifuge	72
Figure D5	Kit Wizard™ Genomic DNA Purification (Promega) and Eppendorf Micropipette.	73

LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celsius
1X	One Time
A	Adenosine
bp	Base pair
C	Cytosine
cm	Centimeter
dH ₂ O	Distilled water
DNA	Deoxyribonucleic acid
dNTP mix	Deoxyribonucleotides mixture
EDTA	Ethylenediaminetetracetic acid
g	Gram
G	Guanocine
M	Molarity
µg	Microgram
µL	Microlitre
µM	Micromolar
mg	Miligram
mL	Mililitre

mM	Milimolar
min	Minutes
ng	Nanogram
OD	Optical density
PCR	Polymerase Chain Reaction
Pmole	Picomole
Ppt	Part per trillion
RAPD	Random Amplified Polymorphic DNA
rpm	Rotation per minute
sec	Seconds
T	Thymine
TBE	Tris-borate-EDTA buffer
TE	10mM Tris Cl, 1 mM EDTA
Tris-HCL	Tris [Hydroxymethyl] aminomethane hydrochloride
UV	Ultra violet
V	Volt
VDS	Video Documentation System
v/v	volume/volume
w/v	weight/volume

ABSTRACT

The genetic variability and relationship among individuals between populations of oysters (*Saccostrea* sp.) from Setiu Wetland, Terengganu Darul Iman were examined using the random amplified polymorphic DNA (RAPD) technique. The genomic DNA was extracted from the oysters tissues using Kit Wizard™ Genomic DNA Purification (Promega). The results produced by the machine produced clear RAPD banding pattern. Twenty oligonucleotide primers were screened and three primers (OPA 15, OPA 19 and OPA 20) were selected to amplify DNA from twelve samples of *Saccostrea* sp. from Pulau Che Him and Pulau Semut, Setiu Wetland, Terengganu Darul Iman. A total of 80 RAPD fragments (RAPDs) with 64 polymorphic fragments (80%) with size ranging from 350 – 3000 bp were scored from the population. The highest level of polymorphisms were detected from Pulau Che Him (82.1 %) followed by Pulau Semut (78.1 %). Genetic distance for both populations ranges from 0.222 to 0.504.

KAJIAN MENGENAI KEPELBAGAIAN GENETIK *Saccostrea* sp. DENGAN MENGGUNAKAN TEKNIK RAPD- PCR

ABSTRAK

Kepelbagaian dan perhubungan genetik individu – individu di antara populasi tiram (*Saccostrea* sp.) dari Setiu Wetland, Terengganu, telah dikaji dengan menggunakan kaedah , Amplifikasi Rawak DNA Polimorfik (RAPD). Genomik DNA telah diekstrak daripada tisu dengan menggunakan Kit Wizard™ Genomic DNA Purification (Promega). Hasil keputusan yang diperolehi daripada mesin menghasilkan jalur – jalur RAPD yang jelas. Dua puluh pencetus telah diuji dan tiga daripada pencetus tersebut (OPA 15, OPA 19 dan OPA 20) telah dipilih untuk mengamplifikasikan DNA daripada dua belas sampel yang dipilih daripada Pulau Che Him dan Pulau Semut, Setiu Wetland, Terengganu Darul Iman. Sejumlah 80 jalur RAPD dan 64 jalur RAPD yang polimorfik (80%) yang bersaiz diantara 350 – 3000 bp telah dihasilkan dan dikenalpasti. Tahap polimorfik yang tertinggi dikenalpasti daripada Pulau Che Him (82.1 %) dan diikuti sampel daripada Pulau Semut (78.1 %). Paras jarak perbezaan genetik antara kedua – dua populasi adalah daripada 0.222 sehingga 0.504.