

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

ROSLINA BINTI HAF MAZD

FAKULTI SAINS DAN TEKNOLOGI
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA
2005

1100036831

LP 38 FST 1 2005



1100036831

Study on genetic variability of nerita sp. using RAPD-PCR technique / Roslina Mat Yazid.



PERPUSTAKAAN
KOLEJ UNIVERSITI SAINS & TEKNOLOGI MALAYSIA
21030 KUALA TERENGGANU

1100036831		

Lihat sebelah

HAK MILIK
PERPUSTAKAAN KUSTEM

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

By

Roslina binti Mat Yazid

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:

Roslina, M.Y. 2005. Study on genetic variability of (*Nerita* sp.) using RAPD-PCR technique. Undergraduate thesis, Bachelor of Science in Biological Sciences, Faculty of Science and Technology, Kolej Univesiti Sains dan Teknologi Malaysia, Terengganu. 66p.

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 *Nerita* sp. USING RAPD-PCR TECHNIQUE oleh ROSLINA BINTI MAT YAZID, No. Matrik UK6692 telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperolehi 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

Jabatan Sains Biologi

Fakulti Sains & Teknologi

Cop Rasmi:

**Kolej Universiti Sains dan Teknologi Malaysia
21030 Kuala Terengganu, Terengganu.**

Tarikh:

7/4/2005

.....

Penyelia Kedua (jika ada)

Nama:

Dr. Zaleha Binti Kassim

Pensyarah

Jabatan Sains Samudera

Fakulti Sains dan Teknologi

Cop Rasmi

**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

Jabatan Sains Biologi

Fakulti Sains dan Teknologi

Cop Rasmi:

**Kolej Universiti Sains dan Teknologi Malaysia
(KUSTEM)
21030 Kuala Terengganu.**

Tarikh:

7/4/05

ACKNOWLEDGEMENTS

First of all, I would like to express my sincere appreciation to my supervisor, Cik Wan Bayani Wan Omar and also my co-supervisor Dr. Zaleha Kassim from Department of Biological Science and Marine Science, Faculty of Science and Technology for their guidance, advice, encouragement and understanding. Without their cooperation, patience and full support, this project will not survive.

I also wish to convey my appreciation to my family for their moral support and understanding.

My heartiest gratitude and big thank you goes especially to my project partners, who always willing to give their fully support during the process of completing this project. Thanks for your encouragement, caring, understanding and patience.

Lastly, I would like to dedicate my appreciation to all my friends and coursemates who had comfort me during my sad time in this project. Thanks for your concern very much. Thank you.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
LIST OF TABLES	vi
LIST OF FIGURES	vii
LIST OF APPENDICES	ix
LIST OF ABBREVIATIONS	xi
ABSTRACT	xii
ABSTRAK	xiii
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	
2.1 Taxonomy and Morphology	4
2.2 Habitat and Distribution	6
2.3 Feeding and Importance	7
2.4 Reproduction and Growth	8
2.5 Genetic Variability	8

2.6	DNA Polymorphism	9
2.7	Molecular Genetic Marker	10
2.8	Polymerase Chain Reaction (PCR)	11
2.9	Random Amplified Polymorphic DNA (RAPD)	14
2.10	Agarose Gel Electrophoresis	16
3	MATERIALS AND METHODS	
3.1	Collection of sample, <i>Nerita</i> sp.	18
3.2	DNA Extraction	
3.2.1	Wizard Genomic DNA Purification Kit (Promega)	19
3.3	Agarose Gel Electrophoresis	20
3.4	Measurement of DNA Purity and Quantity	21
3.5	Screening of RAPD Primers	21
3.6	DNA Amplification of RAPD Selected Primers	22
3.7	Data Analysis	23
4	RESULT	
4.1	Purity and Quantity of DNA	25
4.2	Screening of RAPD Primer	28
4.3	DNA Amplification of RAPD Selected Primer	28
4.4	Dendrogram Analysis	37
5	DISCUSSION	
5.1	Purity and Quantity of DNA	44
5.2	Screening of RAPD Primer	45

5.3	DNA Amplification of RAPD Selected Primer	45
5.4	Dendrogram Analysis	46
6	CONCLUSION AND RECOMMENDATION	49
	REFERENCES	51
	APPENDICES	57
	CURRICULUM VITAE	66

LIST OF TABLES

Table		Page
3.1	Code, sequence, nucleotide length and G+C content of primers used in RAPD analysis	22
4.1	DNA Purity and Quantity of <i>Nerita</i> sp. at Pulau Che Him	27
4.2	DNA Purity and Quantity of <i>Nerita</i> sp. at Pulau Semut	27
4.3	Fragment number and length of <i>Nerita</i> sp. DNA after amplified using various primers	36
4.4	Total number of fragments, polymorphic fragments and proportion of polymorphism and size range of bands of <i>Nerita</i> sp. at two populations	36
4.5	The range of similarity index among individuals within population of two populations of <i>Nerita</i> sp.	38
4.6	Matrix of similarity indices of <i>Nerita</i> sp. from Pulau Chek Him population	39
4.7	Matrix of similarity indices of <i>Nerita</i> sp. from Pulau Semut population	39

LIST OF FIGURES

Figure		Page
2.1	The classification of <i>Nerita</i> sp.	5
3.1	The morphology of <i>Nerita</i> sp.	19
4.1	Genomic DNA extracted by Wizard Genomic DNA Purification Kit at Pulau Che Him	26
4.2	Genomic DNA extracted by Wizard Genomic DNA Purification Kit at Pulau Semut	26
4.3	RAPD banding pattern for screening primer of <i>Nerita</i> sp. using OPA 01-OPA 10 from one sample	29
4.4	RAPD banding pattern for screening primer of <i>Nerita</i> sp. using OPA 11-OPA 20 from one sample	29
4.5	Banding pattern of RAPD fragments of <i>Nerita</i> sp. using OPA 02 from Pulau Che Him	30
4.6	Banding pattern of RAPD fragments of <i>Nerita</i> sp. using OPA 08 from Pulau Che Him	31
4.7	Banding pattern of RAPD fragments of <i>Nerita</i> sp. using OPA 11 from Pulau Che Him	32
4.8	Banding pattern of RAPD fragments of <i>Nerita</i> sp. using OPA 02 from Pulau Semut	33
4.9	Banding pattern of RAPD fragments of <i>Nerita</i> sp. using OPA 08 from Pulau Semut	34
4.10	Banding pattern of RAPD fragments of <i>Nerita</i> sp. using OPA 11 from Pulau Semut	35
4.11	Dendrogram showing genetic relationship between individuals of the <i>Nerita</i> sp. population collected in Pulau Che Hi	40

4.12	Dendrogram showing genetic relationship between individuals of the <i>Nerita</i> sp. population collected in Pulau Semut	41
4.13	Dendrogram showing genetic relationship between 12 individuals of the <i>Nerita</i> sp. population collected in Pulau Semut and Pulau Che Him	42
4.14	UPGMA cluster analysis based on the genetic distance generated from Nei and Li's indices of different <i>Nerita</i> sp. population	43

LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celsius
1×	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	Tris-EDTA buffer
Tris-HCL	Tris [Hydroxymethyl] aminomethane hydrochloride
UV	Ultra violet
V	Volt
VDS	Video Documentation System
v/v	volume/volume
w/v	weight/volume

LIST OF APPENDICES

Appendices		Page
Appendix A	Length, wide and body weight of <i>Nerita</i> sp. from two populations.	58
Appendix B	List of different primers for two populations	59
Appendix C	Matrix similarity indices of <i>Nerita</i> sp. in two populations	62
Appendix D	Apparatus used in this study	63

ABSTRACT

The main objectives of this study are to assess the degree of polymorphism of *Nerita* sp. by using RAPD-PCR technique and to establish the genetic database on the genetic variability of *Nerita* sp. The Random Amplified Polymorphic DNA (RAPD) in association with Polymerase Chain Reaction (PCR) was used to examine the genetic variability and relationship among individuals within and between populations of *Nerita* sp. from Pulau Che Him and Pulau Semut, Setiu Wetland, Terengganu. The genomic DNA was extracted from the snail tissues by using Wizard Genomic DNA Purification Kit method. Twenty oligonucleotide primers were screened and only three primers were selected to amplify DNA from twelve samples of *Nerita* sp. from the two populations. A total of 68 RAPD fragments with 55 polymorphic fragments (80.88%) with the size ranging between 200 to 1500 bp were obtained. Genetic variability of *Nerita* sp. from Pulau Che Him population is lower than Pulau Semut population. The polymorphism detected in sample from Pulau Che Him is 78% and from Pulau Semut is 83%. From the dendrogram analysis, samples from Pulau Che Him and Pulau Semut population come from same ancestor and have a closer genetic relationship to each other.

KAJIAN MENGENAI KEPELBAGAIAN GENETIK *Nerita* sp. MENGUNAKAN TEKNIK RAPD-PCR

ABSTRAK

Tujuan penyelidikan ini ialah untuk menentukan darjah polimorfisme bagi siput *Nerita* sp. menggunakan teknik RAPD-PCR dan untuk menubuhkan pangkalan data kepelbagaian genetik bagi siput ini. Polimorfisme DNA rawak teramplifikasi (RAPD) bersama dengan tindak balas berantai polimerase (PCR) telah digunakan bagi menentukan kepelbagaian dan pertalian genetik di antara individu-individu di dalam dan di antara populasi-populasi siput *Nerita* sp. dari Pulau Che Him dan Pulau Semut, Setiu Wetland, Terengganu. Pengekstrakan DNA daripada tisu siput dijalankan dengan menggunakan kaedah 'Wizard Genomic DNA Purification Kit'. Dua puluh pencetus telah diuji dan hanya tiga pencetus telah dipilih untuk mengamplifikasi DNA daripada dua belas sampel yang mewakili dua populasi siput. Sejumlah 68 fragmen RAPD dengan 55 jalur segmen yang polimorfik (80.88%) dan saiz di antara 200 hingga 1500 bp telah diperolehi. Kepelbagaian genetik *Nerita* sp. dari Pulau Che Him lebih rendah berbanding Pulau Semut. Polimorfisme yang didapati dalam sampel dari Pulau Che Him ialah 78% dan dari Pulau Semut ialah 83%. Daripada analisis dendrogram yang dijalankan, sampel dari populasi Pulau Che Him dan populasi Pulau Semut adalah datang daripada leluhur yang sama dan mempunyai hubungan genetik yang rapat antara satu sama lain.