

EFFECTS OF DRYING METHODS AND DMSO EXTRACTION
METHODS FOR THE STUDY OF *SACCOSTREMA* SP.
CONTAINED IN BSI: AN LITERATURE STUDY

AINNA NADHIAH B. MOHAMMAD

FAKULTI SAINS DAN TEKNOLOGI
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA

2006

DIFFERENT PRESERVATIVES AND DNA EXTRACTION METHODS FOR TISSUE
OF *SACCOSTREA* SP. (OYSTER) IN PCR AMPLIFICATION STUDY

By

Ainna Nadhiah A. Wahab

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
2006

1100046004

This project should be cited as:

Ainna Nadhiah, A.W. 2006. Different preservatives and DNA extraction methods for tissue of *Saccostrea* sp. (Oyster) in PCR amplification study. Undergraduate thesis, Bachelor of Science in Biological Sciences, Faculty of Science and Technology, Kolej Universiti Sains dan Teknologi Malaysia, Terengganu. 51p.

No part of this 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.

HT
1
FS
3
2006



**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: DIFFERENT PRESERVATIVES AND DNA EXTRACTION METHODS FOR TISSUE OF SACCOSTREA SP. IN PCR AMPLIFICATION STUDY oleh Ainna Nadhiah A. Wahab, No. Matrik UK 7921 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 dan Teknologi
Kolej Universiti Sains dan Teknologi Malaysia
21030 Kuala Terengganu, Terengganu.**

Tarikh: 30/4/2006

Penyelia Kedua (jika ada) **Dr. Zaleha Binti Kassim,**

Nama: **Pensyarah**
Cop Rasmi: **Jabatan Sains Samudera
Fakulti Sains dan Teknologi
Kolej Universiti Sains dan Teknologi Malaysia
21030 Kuala Terengganu**

Tarikh: 30/04/06

Ketua Jabatan Sains Biologi

Nama: **PROF. MADYA DR. NAKISAH BT. MAT AMIN**

Cop Rasmi: **Ketua
Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Kolej Universiti Sains dan Teknologi Malaysia
(KUSTEM)
21030 Kuala Terengganu.**

Tarikh: 30/04/06

ACKNOWLEDGEMENTS

First of all, I would like to thank to Allah the Merciful for His bless in providing me sufficient time, excellent health and ideas in successfully complete my final year project and the thesis. The thesis would not be possible without the work and support of many individuals. The completion of this work has been made possible with assistance of my supervisor, co-supervisor, friends and families. I am deeply indebted to Ms. Wan Bayani Wan Omar, my supervisor for her helps and thoughtful advices throughout the project. I also greatly appreciate the assistance of my co-supervisor, Dr. Zaleha Kassim. I also express my gratitude to my friends especially my housemates for their helps and considerations. I formally acknowledge the contribution of them and appreciation to all those who have helped to make the thesis reality. Finally, I would like to acknowledge and thank my families. They are the most important parts of my life. Their support and encouragement did not go unnoticed and I express my love to them.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
LIST OF TABLE	vi
LIST OF FIGURES	vii
LIST OF ABBREVIATIONS	viii
LIST OF APPENDICES	x
ABSTRACT	xi
ABSTRAK	xii
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	
2.1 Taxonomy and Morphology	4
2.2 Habitat and Distribution	9
2.3 Reproduction and Growth	9
2.4 Feeding	10
2.5 Preservation	11
2.6 DNA Extraction	12
2.7 Gel Electrophoresis	15
2.8 Purity and Quality of DNA	16
2.9 RAPD-PCR	17

3	MATERIALS AND METHODS	
3.1	Sampling	21
3.2	Preservation	21
3.3	DNA Extraction	
3.3.1	Wizard Genomic DNA Purification Kit	21
3.3.2	Phenol-Choloroform Method	22
3.4	Analysis	
3.4.1	Analysis of DNA Quality by Gel Electrophoresis	23
3.4.2	Measurement of DNA Purity and Quality	24
3.4.3	DNA Amplification and Screening of RAPD Primer	24
4	RESULTS	
4.1	Purity and Quantity of DNA	26
4.2	Primer Screening	27
5	DISCUSSION	
5.1	Purity and Quantity of DNA	34
5.2	DNA Extraction Method	35
5.3	Screening of RAPD Primer	36

6 CONCLUSION AND RECOMMENDATION	39
REFERENCES	41
APPENDICES	47
CURRICULUM VITAE	51

LIST OF TABLES

Table		Page
4.1	The purity and quantity of extracted genomic DNA of <i>Saccostrea</i> sp. from Phenol-Chloroform method.	28
4.2	The purity and quantity of extracted genomic DNA of <i>Saccostrea</i> sp. from Wizard Genomic DNA Purification Kit method.	28
4.3	Numbers of bands present and absent for <i>Saccostrea</i> sp. in TNES-Urea buffer and 95% Ethanol by using Phenol-Chloroform method with OPA 01 to OPA 10.	33

LIST OF FIGURES

Figure		Page
2.1	The sample of <i>Saccostrea</i> sp. (external)	7
2.2	The sample of <i>Saccostrea</i> sp. (internal)	7
2.3	The classification of <i>Saccostrea</i> sp.	8
4.1	Genomic DNA extracted by Phenol-Chloroform method.	29
4.2	Genomic DNA extracted by Wizard Genomic DNA Purification Kit (Promega) method.	30
4.3	RAPD banding pattern from primer screening of <i>Saccostrea</i> sp. in TNES-Urea buffer by using Phenol-Chloroform method OPA 01 to OPA 10.	31
4.4	RAPD banding pattern from primer screening of <i>Saccostrea</i> sp. in 95% Ethanol by using Phenol-Chloroform Isoamyl method with OPA 01 to OPA 10.	32

LIST OF ABBREVIATIONS

IX	One time
A	Adenosite
Bp	Base pair
C	Cytosine
Cm	Centimeter
dH ₂ O	Distilled water
DNA	Deoxyribonucleic acid
dNTP mix	Deoxyribonucleotides mixture
EDTA	Ethylenediaminetetacetic 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
RAPD	Random Amplified Polymorphic DNA
rpm	Rotation per minute
sec	Seconds
T	Thymine
TBE	Tris-borate-EDTA buffer
TE	10mM Tris Cl, 1mM EDTA
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

Appendix		Page
Appendix 1	Constitution and concentration of TNES-Urea Buffer modification from Asahida <i>et al.</i> (1996).	47
Appendix 2	Code, sequence, molecular weigh, melting point and G + C content of primers used in the Random Amplified Polymorphic DNA(RAPD) analysis.	48
Appendix 3	PCR Mix Buffer for screening primers OPA 01- OPA 10	49
Appendix 4	Table of absence (0) and presence (1) of the bands for primers OPA 01- OPA 10 for samples in TNES-Urea Buffer and 95% Ethanol using Phenol-Chloroform method.	50

ABSTRACT

Saccostrea sp. is an oyster which belongs to family Ostreidae. This edible oyster is very popular for its cultivation. In order to reach the objectives of the study, Random Amplified DNA Polymorphism (RAPD) based on Polymerase Chain Reaction (PCR) technique was used. The technique was used to amplify and detect genomic DNA from tissues which have been preserved in TNES-Urea buffer and 95% Ethanol. The genomic DNA of the species has been extracted by using two different methods which were Phenol-Chloroform extraction method and Wizard Genomic DNA Purification method. TNES-Urea buffer was found to be the most suitable preservative for the sample as the tissues preserved in the preservatives were all produced clear and sharp bands extracted using Phenol-Chloroform method. The best DNA extraction technique for *Saccostrea* sp. was Phenol-Chloroform method as the bands produced were all better than the bands produced by using Wizard Genomic DNA Purification method. The purity of genomic DNA from Phenol-Chloroform method was estimated from the ratio of reading absorbance at 260nm and 280nm (OD_{260} / OD_{280}) using a UV-Spectrophotometer. The purity of genomic DNA from Phenol-Chloroform method was ranged between 0.708 (OD_{260}/OD_{280}) to 1.53 (OD_{260}/OD_{280}) while the quantity was in the range of 252.0 ng / μ l to 1512.5 ng / μ l. Out of 10 primers, OPA 01, OPA 02, OPA 03, OPA 05, OPA 07, OPA 08, OPA 09 and OPA 10 or about 80% of primers were able to amplify fragments.

PENGAWET DAN KAEDAH PENGEKSTRAKAN DNA YANG BERBEZA UNTUK TISU *SACCOSTREA* SP. (TIRAM) DALAM KAJIAN AMPLIFIKASI PCR.

ABSTRAK

Saccostrea sp. merupakan sejenis tiram yang berasal dari Famili Ostreidae. Tiram ini bukan sahaja oleh dimakan tetapi juga terkenal untuk penternakan. Bagi mencapai objektif kajian, teknik Polimorfisma DNA Rawak Teramplifikasi (RAPD) yang berdasarkan Tindakbalas Rantaian Polymerase (PCR) telah digunakan. Teknik ini telah digunakan untuk mengamplifikasi dan mengenalpasti genomik DNA bagi tisu *Saccostrea* sp. yang telah diawet dalam pengawet penimbal TNES-Urea buffer dan Etanol 95%. Genomik DNA bagi spesis ini telah diekstrak dengan menggunakan dua kaedah yang berbeza iaitu kaedah Pengekstrakan Fenol-Kloroform dan kaedah Kit Wizard Genomic DNA Purification. Pengawet Penimbal TNES-Urea didapati merupakan pengawet yang terbaik bagi spesis ini memandangkan semua jalur yang terhasil dari kaedah Pengekstrakan Fenol-Kloroform adalah jelas. Kaedah Pengekstrakan Fenol-Kloroform adalah kaedah yang terbaik bagi pengekstrakan DNA *Saccostrea* sp. kerana secara keseluruhannya jalur yang terhasil adalah lebih baik dari jalur dari kaedah pengekstrakan Kit Wizard Genomic DNA Purification. Ketulenan genomik DNA yang telah diperolehi dari kaedah Fenol-Kloroform pada nisbah bacaan penyerapan pada 260nm dan 280nm (OD_{260} / OD_{280}) dengan menggunakan UV-Spectrophotometer ialah 0.708 to 1.53. Manakala kuantiti genomik DNA spesis ini ialah di antara julat 252.0 ng / μ l to 1520.0 ng / μ l. Lapan primer primer dari 10 primer (OPA 01, OPA 02, OPA 03, OPA 05, OPA 07, OPA 08, OPA 09 dan OPA 10) atau sebanyak 80% didapati berupaya menghasilkan segmen.