DIFFERENT PRESERVATIVES AND DNA EXTRACTION METHODS FOR TISSUES OF *LITTORINA* SPP. FOR IDENTIFICATION

CHE ROSLAILIY CHE PA

2006

Lihat sebelah

DIFFERENT PRESERVATIVES AND DNA EXTRACTION METHODS FOR TISSUES OF *LITTORINA* SP. IN PCR AMPLIFICATION

By

Che Roslailiy bt. Che Pa

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

This project should be cited as:

Che Roslailiy, C.P. (2006) Different Preservatives and DNA Extraction Methods for Tissues of *Littorina* sp. in PCR Amplification. Undergraduate thesis, Bachelor of Science in Biological Sciences, Faculty of Science and Technology, Kolej Universiti Sains dan Teknologi Malaysia, Terengganu. 55p.

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 TISSUES OF *LITTORINA* SP. IN PCR AMPLIFICATION oleh Che Roslailiy Binti Che Pa, no. matrik: UK 8502 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

Nama: WAN BAYANI WAN OMAR

Cop Rasmi: PENSYARAH
Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Kolej Universiti Sains dan Teknologi Malaysia
21030 Kuala Terengganu, Terengganu.

Tarikh: 30/4/2006

.....

Penyelia Kedua

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: 30/4/2006

.....

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: 30/4/06

ACKNOWLEDGEMENTS

A very high gratitude I express to the Almighty to Allah for his blessing for me to finish my final year project. I would like to give my thanks especially to my supervisor and my co-supervisor, Miss Wan Bayani Wan Omar and Dr. Zaleha Kassim, for their advice, patience and conduct this project.

Also, this acknowledges goes to my project partners who always give their support and share their knowledge during the process of completing this project. I'm also would like to thank all staff of Department of Biology, KUSTEM for their commitment.

Special thanks to my lovely housemates, Aisyah Syairah, Nurul Hazwani, Melati and Fatmawati for their support and understanding in the production this report. Lastly, I would like to thank for my mother and relative for giving me a spirit to finish my project and my study in KUSTEM.

Thank you.

TABLE OF CONTENT

LIST OF TABLE

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celcius
1 X	One Time
A	Adenosine
AP-PCR	arbitrary primed PCR
bp	Base pair
C	Cytosine
DAF	DNA amplification fingerprinting
dH_20	Distilled water
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleoyides
EDTA	Ethylenediaminetetracetic acid
EtBr	Ethidium Bromide
g	Gram
G	Guanocine
kb	Kilobase
M	Molarity
µg	Microgram
µL	Microlitre
µM	Micromolar

MAAP	multiple arbitrary amplicon profiling
mg	Miligram
mL	Mililitre
mM	Milimolar
min	Minutes
NaOh	Sodium Hidroxide
NaCl	Natrium Chlorida
ng	Nanogram
OD	Optical Density
PCR	Polymerase Chain Reaction
RAPD	Random Amplification Polymorphic DNA
Rpm	Rotation per minute
Sec	Seconds
SDS	Sodium Dodecyl Sulphate
T	Thymine
TBE	Tris-borate-EDTA buffer
TE	10mM Tris Cl, 1 mM EDTA
TNES-urea	Tris-Natrium Chloride-EDTA-SDS-Urea
Tris-HCl	Tris (Hydroxymethyl) aminomethane hydrochloride
UV	Ultra violet
V	Volt
VDS	Video Documentation System
v/v	volume/volume

w/v	weight/volume

ABSTRACT

Littorina sp. is from phylum mollusk that belonging to the class of gastropoda and their family is Littorinidae. The common names of the genus Littorina are periwinkle. In this study, two DNA extraction methods were applied to gain the purity and quantity of *Littorina* sp., there were Phenol Chloroform method and Wizard Genomic DNA Purification Kit (Promega). Besides that, ethanol 95% and TNES-urea buffer were used as preservative agents for *Littorina* sp. The phenol chloroform method was found to be most reliable method compared to Wizard Genomic DNA Purification Kit (Promega). The genomic DNA from phenol chloroform method showed the clearer band from Wizard Genomic DNA Purification Kit (Promega). For preservation, the results showed the tissue preserved in TNES-urea buffer were give clearer band compared to ethanol 95% which is degraded. The purity of genomic DNA was measured with ratio of absorbance 260nm and 280nm using UV Spectrophotometer. The purity of genomic DNA obtained from different preservatives was as follows: 0.99 to 1.1354 (TNES-urea buffer), 1.1619 to 1.4629 (ethanol 95%). The ratio was ranged from 0.99 to 1.2302 for Wizard Genomic DNA Purification Kit (Promega) method and 1.0794 to 1.475 for Phenol Chloroform method.

PENGAWET DAN KAEDAH PENGEKSTRAKAN DNA YANG BERBEZA UNTUK TISU-TISU *LITTORINA* SP. DALAM KAJIAN AMPLIFIKASI PCR

ABSTRAK

Littorina sp. adalah daripada filum moluska yang termasuk dalam kelas gastropoda dan famili Littorinidae. Nama panggilan bagi genus ini ialah periwinkle. Dalam kajian ini, dua kaedah pengekstrakkan DNA yang berlainan telah digunakan untuk mendapatkan ketulenan dan kuantiti bagi *Littorina* sp., iaitu kaedah Kit Wizard DNA Purification Kit (Promega) dan kaedah Fenol Klorofom. Di samping itu, 95 % ethanol dan TNES-urea buffer telah digunakan sebagai agen pengawet bagi *Littorina* sp. Kaedah Fenol Klorofom menunjukkan keputusan yang lebih baik berbanding dengan kaedah Kit Wizard Genomic DNA Purification (Promega). Genomik DNA daripada kaedah Phenol Klorofom menunjukkan kehadiran band yang lebih jelas berbanding dengan kaedah Kit Wizard Genomic DNA Purification (Promega). Bagi pengawet yang digunakan pula, keputusan menunjukkan bahawa tisu yang telah diawet di dalam TNES-urea buffer menunjukkan kehadiran band yang lebih jelas berbanding dengan 95% ethanol. Ketulinan genomik DNA dapat di cerap daripada nisbah antara 260nm dan 280nm dengan menggunakan UV Spectrophotometer. Ketulinan genomik DNA yang dicerap daripada pengawet yang berlainan adalah seperti berikut: 0.99 hingga 1.1354 (TNES-urea buffer), 1.1619 hingga 1.4629 (95% ethanol). Nisbah yang diperolehi adalah berjulat di antara 0.99 hingga 1.2302 untuk kaedah Kit Wizard Genomic DNA Purification (Promega) dan 1.0794 hingga 1.475 bagi Kaedah Fenol Klorofom.