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

By

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Research Report submitted in partial fulfillment of
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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 memperolehi Ijazah Sarjana Muda Sains- Sains Biologi, Fakulti Sains dan Teknologi, Kolej Universiti Sains dan Teknologi Malaysia.

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LIST OF ABBREVIATIONS

1X	One time
A	Adenosite
Bp	Base pair
C	Cytosine
Cm	Centimeter
dH_2o	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

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ABSTRACT

Saccostrea sp. is an oyster which is 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 /µl to 1512.5 ng /µl. 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 ojektif 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 /µl to 1520.0 ng /µl. Lapan primer primer dari 10 primer (OPA 01, 0PA 02, OPA 03, OPA 05, OPA 07, OPA 08, OPA 09 dan OPA 10) atau sebanyak 80% didapati berupaya menghasilkan segmen.