

THE USE OF PRESERVATIVES AND
THE EXCRETION PATTERNS OF *CLOSTRIDIUM*
CLOSTRIDIUM AND *C. difficile* IN PIGS
IN THE GROWTH STAGES

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THE USE OF TWO DIFFERENT PRESERVATIVES AND DNA EXTRACTION
METHODS OF *CLAUSINELLA CHLOROTICA* (CLAM) TISSUES IN
PCR AMPLIFICATION STUDY

By

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Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: THE USE OF TWO DIFFERENT PRESERVATIVES AND DNA EXTRACTION METHODS OF *CLAUSINELLA CHLOROTICA* (CLAM) TISSUES IN PCR AMPLIFICATION STUDY oleh Ting Shy Lang, no. matrik: UK 7817 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 SYMBOLS

bp	base pairs
C	Cytosine
DNA	Deoxyribonucleic Acid
dNTP	2' –deoxynucleoside- 5'-triphosphate
EDTA	Ethylene Diamide Tetra-Acetate
G	Guanine
NaCl	Sodium Chloride
OD	Optical Density
PCR	Polymerase Chain Reaction
RAPD	Random Amplified Polymorphic DNA
rpm	revolution per minute
SDS	Sodium Dodecyl Sulphate
TBE	Tris-Borate-EDTA buffer
TE	10mM Tris-HCl, 1 mM EDTA
TNES	Tris-NaCl-EDTA-SDS
VDS	Video Documentation System

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ABSTRACT

The aims of this study were to measure the purity and quantity of DNA of *Clausinella chlorotica* from TNES-urea buffer and 95%ethanol and to compare the efficiency of Wizard Genomic Purification Kit and Phenol-chloroform method for DNA extraction. The clams, *Clausinella chlorotica* were collected from Setiu Wetland, Terengganu and follow by tissue preservation in 95% ethanol and TNES-urea buffer respectively. Then DNA was extracted using Wizard Genomic Purification Kit and Phenol-Chloroform extraction methods. Quality and quantity was assessed by agarose gel electrophoresis and UV spectrophotometer and followed by PCR amplification. In this study, Phenol-Chloroform extraction method produced more satisfactory results in DNA extraction for both preservatives. The overall purity of genomic DNA for both preservative was in the range of 1.181 to 1.742 and the quantity of DNA was in the range of 245 to 892.5 µg/mL. The purity and quantity of genomic DNA for samples that preserved in TNES-urea buffer were ranged from 1.181 to 1.208 and 245 to 290 µg/mL. So, TNES-urea buffer was found to be most efficient and could prevent degradation of DNA for at least three months.

**PENGGUNAAN DUA JENIS PENGAWET DAN KAEDEAH
PENGEKSTRAKAN DNA YANG BERBEZA DARIPADA TISU-TISU
CLAUSINELLA CHLOROTICA (KEPAH) DALAM KAJIAN AMPLIFIKASI
PCR**

ABSTRAK

Tujuan kajian ini adalah untuk mengukur ketulenan dan kuantiti DNA daripada TNES-urea buffer dan 95% etanol dan membandingkan keberkesanan Wizard Genomic Purification Kit dan kaedah fenol-kloroform bagi pengekstrakan DNA. Kepah *Clausinella chlorotica* dikutip dari Setiu Wetland, Terengganu dan diikuti dengan pengawetan tisu dalam 95% etanol dan TNES-urea buffer. Kemudian DNA diekstrak dengan menggunakan kaedah Wizard Genomic Purification Kit dan fenol-klorofom. Kualiti dan kuantiti akan ditafsirkan dengan menggunakan elektroforasi gel agarose dan UV Spektrofotometer diikuti dengan PCR amplifikasi. Dalam kajian ini, kaedah fenol-klorofom menghasilkan keputusan yang lebih memuaskan dalam pengekstrakan DNA untuk kedua-dua jenis bahan pengawet. Ketulenan keseluruhan genomik DNA untuk kedua-dua pengawet adalah antara 1.181-1.742 dan kuantiti DNA adalah antara 245-892.5 μ g/mL. Manakala ketulenan dan kuantiti genomik DNA untuk tisu yang diawet dalam TNES-urea buffer adalah antara 1.181-1.208 dan 245-290 μ g/mL. TNES-urea buffer didapati merupakan bahan pengawet yang paling sesuai dan ia boleh mencegah DNA daripada degradasi sekurang-kurangnya tiga bulan.