EVALUATION AND OPTIMIZATION OF EXTRACTION AND PURIFICATION METHODS FOR OBTAINING PCR – AMPLIFIABLE DNA FROM MANGROVE SOIL FOR STUDIES IN MICROBIAL ECOLOGY

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THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

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"In the creation of the heavens and the earth, the alternation of night and day, the ships that roam the ocean for the benefit of the people, the water that GOD sends down from the sky to revive dead land and to spread in it all kinds of creatures, the manipulation of the winds, and the clouds that are placed between the sky and the earth, there are sufficient proofs for people who understand."

Holy Qur'an (Al-Baqarah: 164)

I dedicate this work to my family for their love, support, and for their understanding of the times that school and research took precedence over them.
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In the name of Allah, Most Gracious, Most Merciful. Praise be to Allah, “Rabb” of the universe and peace be upon the Prophet Muhammad S.A.W., his family, and his followers.

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Kuala Lumpur, Monday, 29 March 2004
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<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Wentworth Grade Scale</td>
<td>2</td>
</tr>
<tr>
<td>2.1</td>
<td>List of equipment used in this study</td>
<td>1</td>
</tr>
<tr>
<td>2.2</td>
<td>List of TE used in this study</td>
<td>10</td>
</tr>
<tr>
<td>2.3</td>
<td>DNA extraction buffer</td>
<td>1</td>
</tr>
<tr>
<td>2.4</td>
<td>Choice of DNA extraction buffer for evaluation and optimization of DNA extraction procedures</td>
<td>22</td>
</tr>
<tr>
<td>3.1</td>
<td>Analysis of eight published direct lysis method</td>
<td>3</td>
</tr>
<tr>
<td>3.2</td>
<td>Experiment of selected published direct lysis method</td>
<td>3</td>
</tr>
<tr>
<td>3.3</td>
<td>Reported of results from published direct lysis methods</td>
<td>40</td>
</tr>
<tr>
<td>3.4</td>
<td>Comparison of DNA extraction from mangrove soil using eight different DNA extraction buffers</td>
<td>4</td>
</tr>
<tr>
<td>3.5</td>
<td>Comparison of DNA extraction using different mechanical cell lysis and period treatment</td>
<td>4</td>
</tr>
<tr>
<td>3.6</td>
<td>Comparison of DNA extraction using different chemical and enzymatic cell lysis for treatment</td>
<td>4</td>
</tr>
<tr>
<td>3.7</td>
<td>Comparison of DNA extraction using removal of cell fragment</td>
<td>4</td>
</tr>
<tr>
<td>3.8</td>
<td>Recovery of crude soil microbial DNA from mangrove soil by the Soil DNA Direct Lysis Method</td>
<td>4</td>
</tr>
<tr>
<td>3.9</td>
<td>Analysis of five published DNA purification methods</td>
<td>4</td>
</tr>
<tr>
<td>3.10</td>
<td>Experiment of selected published DNA purification methods</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Comparison of DNA purification using different protein precipitation</td>
<td>5</td>
</tr>
</tbody>
</table>
Table 3.11: Comparison of DNA purification using different humic acid precipitation

Table 3.12: Comparison of DNA purification using different nucleic acid precipitation

Table 3.13: Recovery of pure DNA by the Soil DNA Purification Method

Table 3.14: Recovery of pure DNA by the Soil DNA Purification Method

Table 4.1: Analysis of PCR protocols used in this study

Table 4.2: Characterization of primers used for PCR amplification used in this study

Table 5.1: Analysis of processing time and amount of samples used

Table 5.2: Analysis of processing time and amount of samples used using Soil DNA Direct Lysis Method

Table 5.3: Analysis of processing time of samples used using five published purification method

Table 5.4: Analysis of processing time of samples used using Soil DNA Purification Method

Table 5.5: Analysis of processing time of samples used using selected published PCR protocols
LIST OF FIGURES

Figure 2.1  Type of mechanical cell lysis for DNA extraction from mangrove soil................................................................. 2

Figure 2.2  Calculation of the concentration of dsDNA.......................................................... 3

Figure 3.1 :  Agarose gel electrophoresis of crude microbial DNA from mangrove soil using eight published direct lysis method ................................................................. 4

Figure 3.2 :  Steps involved during DNA extraction from soil samples ................................................................. 4

Figure 3.3 :  Steps involved during DNA purification from soil samples ................................................................. 5

Figure 3.4 :  Agarose gel electrophoresis of crude soil microbial DNA from mangrove soil................................................................. 5

Figure 3.5 :  Agarose gel electrophoresis of crude DNA from other soil ................................................................. 5

Figure 4.1 :  Agarose gel electrophoresis of PCR products employing DNA extracts of laboratory culture................................................................. 6

Figure 4.2 :  Agarose gel electrophoresis of PCR products employing crude DNA that was subjected to five purification methods ................................................................. 6

Figure 4.3 :  Agarose gel electrophoresis of PCR products using 12 sets of primers ................................................................. 6
EVALUATION AND OPTIMIZATION OF EXTRACTION AND PURIFICATION METHODS FOR OBTAINING PCR – AMPLIFIABLE DNA FROM MANGROVE SOIL FOR STUDIES IN MICROBIAL ECOLOGY

ABSTRACT

Recovery of microbial DNA from environmental samples such as mangrove soil is a significant challenge. In this study, combinations of DNA extraction and purification methods were compared based on DNA yield, humic acid and protein contamination and PCR amplifiability.

Several approaches were analysed to obtain optimum DNA yield. The effects of different treatments of DNA extraction such as extraction buffer, chemical and enzymatic cell lysis (sodium dodecyl sulfate, lysozyme and proteinase K), mechanical cell lysis (rotex, vortex and sonicate) and removal of cell fragment were compared and evaluated. A method for the extraction of microbial DNA from mangrove soil was developed during this study and it is called the Soil DNA Direct Lysis Method. The advantages of this method are that it is simple, robust, rapid and may be used to analyse many samples simultaneously.

Several reagents were used to reduce protein and humic acid contaminations. Such reagents include sodium chloride, ammonium acetate, potassium acetate and sodium acetate for removal of proteins contaminants and polyvinylpolypyrrolidone and cymethylammonium bromide for removal of humic acid. Nucleic acid precipitation using ethanol, isopropanol or polyethlene glycol were also compared and evaluated. A method for the purification of microbial DNA
from mangrove soil was developed during this study and it is called the Soil DNA Purification Method. The advantages of this method are that it is rapid cleanup of DNA, removing inhibitors to produce PCR-ready DNA.

PCRs were successful in amplifying 16S rRNA gene fragments using the extracted and purified microbial DNA from mangrove soil.
PENILAIAN DAN PENGOPTIMAAN KAEDAH PENGEKSTRAKAN DAN PENULINAN DNA BAGI MENDAPATKAN AMPLIFIKASI PCR DARIPADA TANAH PAYA BAKAU UNTUK KAJIAN EKOLOGI MIKROB

ABSTRAK

Mendapatkan DNA mikrob daripada persekitaran seperti tanah paya adalah sesuatu yang sangat mencabar. Dalam kajian ini, gabungan kaedah pengekstrakan dan penulinan DNA telah dibandingkan berdasarkan kepada hasil DNA, pencemaran asid humik, protein dan amplifikasi PCR.

Beberapa langkah telah diambil untuk menghasilkan DNA yang optima. Ini termasuklah menganalisa kesan perbezaan rawatan seperti rawatan kimia dan enzim (sodium dodecyl sulfate, lysozyme dan proteinase K), rawatan mekanikal (rotex, vortex dan sonicate) dan pengasingan fragmen sel. Semua keadaan ini dibandingkan dan dinilai.

Satu kaedah baru telah dibuat untuk pengekstakan DNA mikrob daripada tanah dan ia dikenali sebagai Kaedah Lisis DNA Tanah Secara Terus. Kebaikan kaedah ini ialah ianya mudah, cepat dan banyak sampel tanah boleh dianalisa dalam masa yang sama.

Beberapa bahan kimia digunakan untuk mengurangkan pencemaran protein dan asid humik dalam ekstrak DNA. Bahan ini adalah seperti natrium klorida, ammonium asitat, potassium asitat and sodium asitat digunakan untuk mengurangkan pencemaran protein. Manakala bahan kimia seperti polyvinylpolypyrrolidone dan cylumethylammonium bromide digunakan untuk
mengurangkan pencemaran asid humik. Perbandingan dan penilaian juga dilakukan ke atas pengikatan asid nukleik seperti ethanol, isopropanol dan polyetheleneglicol. Satu kaedah baru dibuat untuk penulihan DNA mikrob daripada tanah dan ia dikenali sebagai Kaedah Penulihan DNA Tanah. Kebaikan kaedah ini ialah ia cepat membersihkan dan menulikan DNA daripada bahan perencat dan DNA ini sedia untuk amplifikasi PCR.

Semua PCR berjaya mengamplifikasi fragmen gen 16S rDNA dalam ekstrak DNA daripada tanah paya bakau menggunakan kaedah penulihan Yeates et al. (1997) dan Kaedah Penulihan DNA Tanah.
TABLE OF CONTENTS

DEDICATION ........................................................................................................... i
ACKNOWLEDGEMENTS ..................................................................................... ii
LIST OF TABLES .................................................................................................. iii
LIST OF FIGURES ................................................................................................. v
ABSTRACT ............................................................................................................. vi
ABSTRAK ............................................................................................................... viii
TABLE OF CONTENTS .......................................................................................... x
ABBREVIATIONS .................................................................................................. xvi

Chapter 1 : General Introduction

1.1 Microbial ecology ......................................................................................... 1
1.2 Soil ............................................................................................................... 1
1.3 Mangrove soil .............................................................................................. 3
1.4 Diversity of soil microorganism ................................................................. 3
1.5 Molecular microbial ecology .................................................................... 5
  1.5.1 Polymerase Chain Reaction .............................................................. 5
  1.5.2 Adaptation of DNA probe technology for use in soil samples ....... 7
1.6 Comparison of molecular and culture techniques .................................... 7
1.7 Recovering bacterial cells from soil ......................................................... 10
1.9 Recovering nucleic acids from environment ......................................... 11
1.9 Objectives of the study ............................................................................. 13
Chapter 2: Materials & Methods

2.1 Bacterial strain (DNA template) ........................................... 14
2.2 Microbiological media ..................................................... 14
2.3 Maintenance and growth of the bacterial strains ..................... 14
2.4 Chemical and reagents ..................................................... 15
2.5 Equipment and applications ............................................. 15
2.6 Buffer ........................................................................... 16
   2.6.1 TAE buffer (50X) .................................................. 16
   2.6.2 TE buffer .............................................................. 16
   2.6.3 Electrophoresis loading dye buffer ............................. 16
   2.6.4 DNA extraction buffer ........................................... 17
2.7 Agarose gel electrophoresis ............................................. 17
2.8 Soils sampling and characterization .................................... 18
2.9 Extraction of DNA (direct lysis method) .............................. 18
   2.9.1 Method 1 (Selenska and Klingmuller, 1991) .................. 18
   2.9.2 Method 2 (Tsai and Olson, 1991) ............................... 19
   2.9.3 Method 3 (Tebbe and Vahjan, 1993) ........................... 20
   2.9.4 Method 4 (Yeates et al., 1997) ................................. 21
   2.9.5 Method 5 (Picard et al., 1992) .................................. 21
   2.9.6 Method 6 (Zhou et al., 1996) ................................... 22
   2.9.7 Method 7 (Cho et al., 1996) .................................... 23
   2.9.8 Method 8 (McDonald et al., 1999) ............................ 23
2.10 Evaluation and optimization of DNA extraction procedures.................................................. 25
  2.10.1 Selection of reagents for extraction buffer........................................................................ 25
  2.10.2 Selection of mechanical cell lysis..................................................................................... 26
  2.10.3 Selection of reagents for chemical and enzymatic cell lysis............................................. 27
  2.10.4 Selection of reagents for removal of cell fragment.......................................................... 27

2.11 Purification of DNA............................................................................................................... 28
  2.11.1 Method 1 (Steffan and Atlas, 1988).................................................................................. 28
  2.11.3 Method 2 (Yeates et al., 1997)....................................................................................... 29
  2.11.2 Method 3 (Großkopf et al., 1998).................................................................................. 29
  2.11.5 Method 4 (Miller et al., 1999).......................................................................................... 29

2.12 Evaluation and optimization of DNA purification procedures.............................................. 31
  2.12.1 Selection of reagents for protein precipitation................................................................. 31
  2.12.2 Selection of reagents for humic acid precipitation......................................................... 31
  2.12.3 Selection of reagents for nucleic acid precipitation.......................................................... 31

2.13 Determination of purity and yield of DNA........................................................................... 32

2.14 PCR amplification................................................................................................................... 33
  2.14.1 DNA extraction for PCR positive control........................................................................ 33
  2.14.2 PCR amplification of extracted crude DNA.................................................................... 33
    2.14.2.1 Method 1 (McDonald et al., 1999)........................................................................... 33
    2.14.2.2 Method 2 (Kuske et al., 1998).................................................................................. 34
    2.14.2.3 Method 3 (LaMontagne et al., 2002)........................................................................ 34
    2.14.2.4 Method 4 (Hurt et al. 2001)...................................................................................... 34
    2.14.2.5 Method 5 (Standard Method).................................................................................... 34
Chapter 3: Extraction, purification & Characterization of Soil Microbial DNA from Mangrove Soil

3.1 Objective ............................................................................................................ 35
3.2 Results .................................................................................................................. 36
  3.2.1 DNA extraction method ............................................................................... 36
    3.2.1.1 Analysis of selected published direct lysis methods .............................. 36
    3.2.1.2 Experiments performed based on selected published direct lysis methods .......................................................... 38
    3.2.1.3 Evaluation and optimization of DNA extraction procedures ............. 42
      3.2.1.3.1 Selection of reagents for extraction buffer .................................. 43
      3.2.1.3.2 Selection of mechanical cell lysis.................................................. 44
      3.2.1.3.3 Selection of reagents for chemical and enzymatic cell lysis .......... 45
      3.2.1.3.4 Selection of reagents for removal of cell fragment .................... 46
  3.2.1.4 Development of a simple direct lysis method (Soil DNA Direct Lysis Method) ................................................................. 47
3.2.2 DNA purification method ........................................................................................................... 48

3.2.2.1 Analysis of selected published DNA purification methods ......................................................... 48

3.2.2.2 Experiment performed based on selected published DNA purification methods ...................... 49

3.2.2.3 Evaluation and optimization of DNA purification procedures ................................................. 51

3.2.2.3.1 Selection of reagents for protein precipitation ................................................................. 52

3.2.2.3.2 Selection of reagents for humic acid precipitation ............................................................ 53

3.2.2.3.3 Selection of reagents for nucleic acid precipitation ............................................................ 54

3.2.2.4 Development of a DNA purification method (Soil DNA Purification Method) ......................... 55

3.2.3 Electrophoresis of extracted and purified microbial DNA ......................................................... 57

3.2.3.1 Electrophoresis of extracted and purified microbial DNA from mangrove soil using Soil DNA Direct Lysis Method and Soil DNA Purification Method ......................................................... 57

3.2.3.2 Electrophoresis of extracted and purified microbial DNA from other soil type ........................ 58
Chapter 4: PCR Amplification of Extracted Mangrove Soil Microbial DNA

4.1 Objective.................................................................................................................. 59
4.2 Introduction............................................................................................................... 59
4.3 Results......................................................................................................................... 60
  4.3.1 Analysis of five PCR protocols ........................................................................... 60
  4.3.2 PCR amplification using five different protocols ............................................... 62
  4.3.3 PCR amplification of samples from five selected published purification method ................................................................. 64
  4.3.4 Amplification of crude soil microbial DNA using other primers.................. 66

Chapter 5: Discussion

5.1 Overview..................................................................................................................... 68
5.2 DNA extraction methods.......................................................................................... 69
5.3 DNA purification methods....................................................................................... 73
5.4 PCR amplification of extracted and purified mangrove soil microbial DNA........ 78
5.5 Future experiment to be conducted........................................................................ 80
5.6 Conclusion.................................................................................................................. 80

References
ABBREVIATIONS

(a) Buffers / Media / Chemicals / Reagents / Enzymes

LB : Luria-Bertani media
Na$_2$HPO$_4$ : Sodium dihydrogen orthophosphate
PEG : Polyethylene glycol
Rnase A : Ribonuclease A
CsCl : Cesium chloride
SDS : Sodium dodecyl sulphate
Tris : Tris(hydroxymethyl)aminomethane
EDTA : Ethylenediamine tetraacetic acid
PVPP : Polyvinylpolypyrrolidone
CTAB : Cetyltrimethylammonium bromide
EtBr : Ethidium bromide
NaCl : Sodium chloride
RNA : Ribonucleic acid
DNA : Deoxyribonucleic acid
dNTP : Deoxyribonucleoside triphosphate
PCR : Polymerase Chain Reaction
TE : Tris-EDTA buffer
TAE : Tris-acetate-EDTA buffer
NH$_4$Ac : Ammonium acetate
KaAc : Potassium acetate
NaAc : Sodium acetate
(b) Units

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
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(c) Miscellaneous

OD\textsubscript{230} : Optical density at 230 nm
OD\textsubscript{260} : Optical density at 260 nm
OD\textsubscript{280} : Optical density at 280 nm
mw : molecular weight
uv : ultra violet
\% : percentage
(w/v) : weight : volume ratio
et al. : Et alia (and others)
(v/v) : volume : volume ratio
< : less than
& : and
temp. : temperature
wt. : weight