APPLICATION OF POLYMERASE CHAIN REACTION-RESTRICTION FRAGMENT LENGTH POLYMORPHISM TECHNIQUE IN DETERMINING THE IDENTITY OF SEVERAL MARINE SPECIES IN SEAFOOD PRODUCTS

By

LIM SOR SING

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirement for the Degree of Master of Science

October 2005
DEDICATED TO

My husband, daughter, parent and brothers
APPLICATION OF POLYMERASE CHAIN REACTION-RESTRICTION FRAGMENT LENGTH POLYMORPHISM TECHNIQUE IN DETERMINING THE IDENTITY OF SEVERAL MARINE SPECIES IN SEAFOOD PRODUCTS

By

LIM SOR SING

October 2005

Chairman : Associate Professor Foo Hooi Ling, PhD
Faculty : Biotechnology and Biomolecular Sciences

This study describes an investigation on the application of Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) nucleic acid based technique, as a routine analytical technique to generate DNA fingerprints for 24 fresh marine samples. Data obtained from the DNA fingerprints were used to identify 12 processed marine samples. Samples representative of various species and marine products submitted to different processing conditions were selected to verify the applicability of the techniques. A specific part of mitochondrion (mt) genome, cytochrome b (cytb) gene with 359 base pair (bp) fragment was successfully amplified from all the investigated samples by using ‘universal’ primer pairs. The obtained fragment of cytb gene (359 bp) was digested with different Restriction Endonuclease (RE) resulting in sample specific Restriction Fragment Length Polymorphism (RFLP). A total of 14 fishes, 7 prawns and 3 crabs with the exception of Doublelinded tonguesole (Paraplagusia bilineata), Rainbow sardine (Dussumieria acuta), Western king prawn (Metapenaeus latisulcatus), Sharp-rostrum prawn (Parapenaeopsis hardwickii), Giant freshwater prawn (Macrobrachium rosenbergii), Affluent prawn (Thenus orientalis), Giant tiger prawn (Penaeus
*semisulcatus*, Indo-pacific swamp crab (*Scylla serrata*), Red and Blue swimming crab (*Solenocera subnuda*) could be differentiated using RE *Hae*III, *Mbo*II, *Fok*I, and *Msp*I followed by agarose gel electrophoresis. For processed marine samples, only four out of twelve were successfully identified. The DNA of unidentifiable samples may have been degraded during the steps of processing. The RFLP patterns obtained are conclusive even in the mixture of Western king prawn (*Metapenaeus latisulcatus*) and African threadfin (*Alexis alexandrinus*) at a ratio of 1:100. Results of this study suggest that the PCR-RFLP based on *cytb* gene shows a reproducible, rapid and simple method for simultaneous identification of marine samples.
Abstrak ini dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

AMPLIFIKASI TEKNIK TINDAKBALAS BERANTAI POLIMERASE-POLIMERIFIK PERPISAHAN MENGIKUT KEPANJANGAN SERPIHAN UNTUK PENGENALPASTIAN IDENTITI BAGI BEBERAPA SPESIS HAIWAN LAUT DALAM PRODUK MAKANAN LAUT

Oleh

LIM SOR SING

Oktober 2005

Pengerusi : Profesor Madya Foo Hooi Ling, PhD
Fakulti : Bioteknologi dan Sains Biomolekul

Penyelidikan ini menghuraikan satu kajian tentang aplikasi teknik “Polymerase Chain Reaction-Restriction-Fragment Length Polymorphism (PCR-RFLP)”, teknik yang berdasarkan DNA sebagai teknik untuk menghasilkan maklumat DNA bagi 24 jenis sampel haiwan laut. Data yang didapati daripada maklumat DNA bagi sampel-sampel haiwan laut telah digunakan untuk mengesan sunber bagi 12 produk haiwan laut. Berbagai-bagai jenis haiwan laut dan produk haiwan laut yang diproses dengan pelbagai teknik pemprosesan yang berlainan dipilih untuk kajian ini supaya kebolehgunaaan teknik ini dapat dikenalpasti. Bahagian spesifik daripada genom mitokondrion (mt) iaitu gen cytokrome b (cytb) yang bersaiz 359 base pair (bp) telah berjaya diamplifikasi daripada semua sampel kajian dengan menggunakan sepasang primer gen berstruktur umum. Serpihan gen cytb yang bersaiz 359 bp dihuraikan dengan enzim pemotong untuk menghasilkan polimerifik perpisahan mengikut saiz serpihan secara spesifik untuk sampel-sampel yang berlainan. Semua sampel haiwan laut kajian kecuali Ikan Sisa Nabi (Paraplagusia bilineata), Ikan Sardin (Dussumieria acuta), Udang Susu (Metapenaeus latisulcatus), Udang Minyak
(Parapenaeopsis hardwickii), Udang Galah (Macrobrachium rosenbergii), Udang Lobok (Thenus orientalis), Udang Harimau (Penaeus semisulcatus), Ketam Batu (Scylla serrata), Ketam Laut atau Ketam Renjong (Solenocera subnuda) mampu dibezakan dengan menggunakan enzim pemotong HaeIII, MboII, FokI, dan MspI serta dianalisisi dengan kaedah analisa gel elektroforesis. Bagi produk-produk haiwan laut pula, hanya empat daripada dua belas sampel tersebut mampu dikenalpasti. DNA mungkin termusnah semasa melalui langkah-langkah pemprosesan. Keputusan Polimerifik Perpisahan mengikut saiz serpihan yang dicapai memang tidak dapat dinafikan walaupun digunakan untuk mengenalpasti adukan Udang Susu (Metapenaeus latisulcatus) dan Ikan Hebek (Alexis alexandrinus) dengan kadar 1:100. Keputusan yang dicapai melalui penyelidikan ini mencadangkan bahawa Amplifikasi Tindakbalas Berantai Polimerase-Polimerifik Perpisahan mengikut saiz serpihan menggunakan gen cytb adalah teknik yang dapat diulangi, cepat dan mudah untuk mengenalpasti sampel haiwan laut berdasarkan ujian DNA.
ACKNOWLEDGEMENT

This work was carried out with a hope to contribute towards the expansion of the knowledge on biotechnology. The completion of this thesis would have been impossible without the assistance and direct involvement of many kind-hearted individuals. I am very much indebted to all my mentors and have no way of repaying such a debt except to express my sincerest gratitude.

First and foremost, I would like to take this opportunity to express my deepest appreciation and gratitude to my project supervisor, Associate Prof. Dr. Foo Hooi Ling for her valuable comments, patience, guidance, and strong support for the very enriching and though-provoking discussions which helped to shape the thesis. Next, I would also like to acknowledge the kindness of my co-supervisor, Prof. Dr Son Radu and Associate Prof. Dr. Raha Abdul Rahim for their contribution of time, feedback, advice and encouragement.

Appreciation is also extended to my previous project supervisor Dr. Clemente Michael Wong Vui Ling for the initiation of this project. I am also indebted to Prof. Dr. Ho Yin Wan and Prof. Dr. Norhani of Biology Department, UPM for their courtesy allowing me to use their laboratory facilities.

For all the lecturers and staffs of the Faculty of Biotechnology and Biomolecular Science, Universiti Putra Malaysia, thanks for giving me fully commitment and cooperation during the process of doing my Masters degree project.
My heartfelt thanks also go to Madam Haw, Encik Khairul, Wai Ling, Kala, Kqueen, Chin Mei, Chiun Yee, Mosla, Hiao Ling, Lida, Yin Sze, Sia Yen and all my fellow friends for their sacrifices, encouragement, and generous co-operation throughout my project.

Lastly, I am forever indebted to my beloved family members Lim Kim Hue, Tan Lee Hua, Lim Chin Kee, Lim Chin Giap, Ng Eng Boon and Ng Siau Sian for their understanding and everlasting love and care during the course of my study.
I certify that an Examination Committee met on 14th October 2005 to conduct the final examination of Lim Sor Sing on her Master of Science thesis entitled “Utilization of PCR-RFLP to Determine the Identities of Seafood Sample” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Member of the Examination Committee are follows:

HO CHAI LING, PhD
Faculty of Biotechnology and Biomolecular Science
Universiti Putra Malaysia
(Chairman)

MARIANA NOR SHAMSUDIN, PhD
Associate Professor
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Internal Examiner)

SUHAIMI MUSTAFA, PhD
Faculty of Biotechnology and Biomolecular Science
Universiti Putra Malaysia
(Internal Examiner)

THONG KWAI LIN, PhD
Professor
Faculty of Science
Universiti Malaya
(External Examiner)

HASANAH MOHD. GHAZALI, PhD
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:
This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirements for the degree of Master of Science. The members of the Supervisory Committee are as follows:

**FOO HOOI LING, PhD**
Associate Professor
Faculty of Biotechnology and Biomolecular Science
Universiti Putra Malaysia
(Chairman)

**RAHA ABDUL RAHIM, PhD**
Associate Professor
Faculty of Biotechnology and Biomolecular Science
Universiti Putra Malaysia
(Member)

**SON RADU, PhD**
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

---

**AINI IDERIS, PhD**
Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:
DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

LIM SOR SING

Date:
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td></td>
<td>iii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td></td>
<td>v</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENT</td>
<td></td>
<td>vii</td>
</tr>
<tr>
<td>APPROVAL</td>
<td></td>
<td>ix</td>
</tr>
<tr>
<td>DECLARE</td>
<td></td>
<td>xi</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td></td>
<td>xiv</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td></td>
<td>xv</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td></td>
<td>xvii</td>
</tr>
</tbody>
</table>

## CHAPTER

### I INTRODUCTION

1.1 Objectives  

### II LITERATURE REVIEW

2.1 Method for Samples Identification  
2.1.1 Water-soluble Protein-based Method  
2.1.2 DNA-based Method  
2.2 Meat  
2.3 Marine Samples  
2.4 Processed Marine Samples  
2.5 Skeletal Muscle  
2.6 Mitochondrion  
2.7 Mitochondrion DNA (mtDNA)  
2.8 Cytochrome b (cytb) gene  
2.9 Polymerase Chain Reaction (PCR)  
2.10 PCR Sensitivity  
2.11 Restriction Enzyme (RE)  
2.12 Polymerase Chain Reaction (PCR)-Restriction Fragment Length Polymorphism (RFLP)

### III MATERIALS AND METHODS

3.1 Marine Samples  
3.2 Total DNA Extraction  
3.3 DNA Quality and Quantity Analysis  
3.4 Polymerase Chain Reaction (PCR)  
3.5 TOPO TA Cloning®  
3.5.1 Cloning and Transformation  
3.5.2 Selection for Positive Clones  
3.6 Plasmid DNA Extraction  
3.7 Automated DNA Sequencing  
3.8 PCR-RFLP Fingerprinting Analysis  
3.9 Sensitivity Test  

### IV RESULTS AND DISCUSSIONS

4.1 Total DNA Extraction
4.2 Polymerase Chain Reaction (PCR)  57
4.3 TOPO TA Cloning®  62
4.4 Plasmid DNA Extraction  65
4.5 Automated DNA Sequencing  67
4.6 PCR-RFLP Fingerprinting Analysis  72
    4.6.1 Marine Samples  72
    4.6.2 Processed Marine Samples  90
4.7 Sensitivity Test  100

V GENERAL DISCUSSION AND SUMMARY
5.1 Total DNA Extraction  104
5.2 Polymerase Chain Reaction (PCR)  105
5.3 PCR-RFLP Fingerprinting Analysis  108

REFERENCES  114
APPENDICES
BIODATA OF THE AUTHOR