# 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*

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for Degree of Master of Science

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**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 Doublelined 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 kebolehgunaan 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 *Hae*III, *Mbo*II, *Fok*I, dan *Msp*I serta dianalisasi 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.

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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:

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## 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.

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**LIM SOR SING**

Date:

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