

DEVELOPMENT OF PCR-RAPD TECHNIQUE TO DISTINGUISH
DIFFERENT POPULATIONS OF OYSTER (*Crassostrea iredalei*)

BY

NORZEHAN BINTI ZAKARIA

This project report is submitted in partial fulfillment of
the requirements for the Degree of
Bachelor of Science (Marine Science)

Faculty of Applied Science and Technology
UNIVERSITI PUTRA MALAYSIA TERENGGANU

1999

1100024160

ACKNOWLEDGEMENTS

First and foremost, I would like to express my utmost gratitude to my highly respected supervisor, Professor Dr. Lokman Shamsudin, for his invaluable advice, patience, guidance and support throughout the duration of my project.

My sincere appreciation also goes to my moderator, Associate Professor Dr. Siti Khatijah Yussof for access to her facilities in the laboratory and her support and advice in this work. Lots of thanks to Dr. Arif for his guidance and support.

A big thank you to Shima, Vijay, Wong and Subha and all member in Virology Lab, UPM Serdang for teaching and encouraging me to finish this project.

Mt heartfelt thanks to Mr. Ng Chong Sing of Bio-diagnostic for regular updates on advances in the molecular technique.

My warmest appreciation is also extended to Abang shah, Mr. Jaral, JD, Peah, Keong, Lilian, Izah, Podeh, Teddy, Ilee and all my friends and coursemate for their friendship and companionship.

Above all, I would like to express my love and gratitude to my father, mother, Kak Zam and Adik and unforgettable Mak Jang and her family for their constant love and support.

ABSTRAK

Tujuan penyelidikan ini ialah untuk menilai kegunaan berbagai jenis petanda genomik DNA dan mengoptimalkan DNA untuk mengidentifikasikan perbezaan populasi tiram di Malaysia, *Crassostrea iredalei*. Ia merupakan makanan yang penting dari segi ekonomi dan popular di kalangan pengguna di Malaysia. Oleh itu, kajian dilakukan ke atas dua populasi tiram, *Crassostrea iredalei*, dari Tumpat (Kelantan) dan Gong Batu (Terengganu). Kaedah konvensional digunakan semasa kajian ini untuk mengekstrak genomik DNA. Dua primer dikelaskan menggunakan teknik PCR-RAPD, iaitu OPA-02 dan OPA-19. Kedua-dua primer adalah stabil pada suhu 'annealing' 37°C. Penanda menunjukkan saiz tiram berada dalam kepekatan MgCl₂, Taq Polymerase, dNTP, primer-primer dan tempelat DNA. Perbezaan hanya dijumpai dalam kepekatan Taq Polymerase, iaitu 0.6 unit untuk OPA-02 dan 0.8 unit untuk OPA-19. Penghasilan semula ('reproducibility'), menunjukkan amplifikasi dan polymorfisme DNA yang lebih baik.

ABSTRACT

The main objectives of this study were to evaluate the ability of a variety of recently developed genomic DNA and to optimize DNA markers to identify different populations of Malaysian oysters, Crassostrea iredalei. It was an economically important food that has gained popularity among consumers in Malaysia. Therefore, a study was done on two population of Malaysian oyster, Crassostrea iredalei, from Tumpat (Kelantan and Gong Batu (Terengganu). The conventional method was used during this study to extract the genomic DNA. Two primers were typed using the PCR-RAPD technique, which were OPA-02 and OPA-19. Both of the primers were stable at annealing temperature 30°C. The marker showed that the size of this oysters ranged 200 bp – 1,650 bp. Optimization was achieved using known concentration of MgCl₂, Taq Polymerase, dNTP, primers and DNA template. The optimum concentration of the Taq Polymerase concentration comprised of 0.6 unit for OPA-02 and 0.8 unit for OPA-19. Reproducibility was obtained during DNA amplification and polymorphism.