

CHARACTERIZATION OF PCR-RFLP PROFILES ON PARTIAL CO1  
GENE OF HARPACTICOID COPEPOD, *Leptocoris conariensis*  
(Lang, 1965), UMT STRAIN

MOHD FAZHAN BIN MOHD HANAFIAH

MASTER OF SCIENCE (AQUACULTURE)  
UNIVERSITI MALAYSIA TERENGGANU  
MALAYSIA

2010



**CHARACTERIZATION OF PCR-RFLP PROFILES ON PARTIAL CO1  
GENE OF HARPACTICOID COPEPOD, *Leptocaris canariensis* (Lang, 1965), UMT  
STRAIN**

MOHD FAZHAN BIN MOHD HANAFIAH

October 2010

Institute of Tropical Aquaculture

**MOHD FAZHAN BIN MOHD HANAFIAH**

**Thesis submitted in Fulfillment of the Requirement for the Degree of Master of  
Science (Aquaculture) to Institute of Tropical Aquaculture  
Universiti Malaysia Terengganu**

**October 2010**

**CHARACTERIZATION OF PCR-RFLP PROFILES ON PARTIAL COI GENE  
OF HARPACTICOID COPEPOD, *Leptocaris canariensis* (Lang, 1965), UMT  
STRAIN**

**MOHD FAZHAN BIN MOHD HANAFIAH**

**October 2010**

**Institute of Tropical Aquaculture**

*Leptocaris canariensis* (Lang, 1965) is a benthic copepod that belongs to Order Harpacticoida. It is essential in aquaculture sector due to smaller size and good nutritional content suitable for the growth and survival of the fish larvae. It is also an important prey for other copepod and predators to mosquito larvae. The identification of the copepod species was done based on morphological characteristic only which is time consuming and need to be done on adult copepod only. There is no molecular study being done on *L. canariensis* itself. The aims of this study are to amplify and characterize the PCR-RFLP profile of partial COI gene of *L. canariensis*. The extractions of TGDNA of *L. canariensis* were done using Vivantis PCR Buffer A and the amplifications of partial COI gene were done using universal primer LCO-1490 and HCO-2198 and then the PCR product of partial COI gene were subjected to PCR-RFLP using eight restriction enzymes (REs) namely *KspAI*, *HindIII*, *TaqI*, *MboII*, *MnII*, *HpaII*, *MboI*, *AluI* and *RsaI*. The amplification of partial COI gene generated fragments size approximately 700 bp. Excluding *KspAI*, *RsaI* and *HindIII*, five REs successfully cleaved the partial COI gene of *L. canariensis* but only three restriction enzyme namely *MnII*, *HpaII* and *MboI* successfully cleaved and produced restriction profiles. The PCR-RFLP of partial COI gene successfully characterized based on the size of the fragment produced. The *MnII* cleaved the partial COI gene produced fragment size of 550 bp and 250 bp, *HpaII* cleaved produce fragment size of 600 bp and *MboI* cleaved the partial COI gene produced fragment size of 550 bp. This restriction profiles can be used to differentiate *L. canariensis* with other different copepods.

**PENCIRIAN PCR-RFLP KEATAS GEN SEPARA COI DARI KOPEPOD  
HARPAKTIKOID, *Leptocaris canariensis* (LANG, 1965), STRAIN UMT.**

**MOHD FAZHAN BIN MOHD HANAFIAH**

**October 2010**

**Institut Akuakultur Tropika**

*Leptocaris canariensis* (Lang, 1965) ialah copepod bentik kepunyaan Order Harpaktikoida. Ia memainkan peranan penting dalam sektor akuakultur sebagai makanan hidup yang sesuai untuk larva ikan dan udang kerana mempunyai saiz yang sangat kecil dan mempunyai zat yang lengkap. Kopepod ini juga penting dari segi ekologi sebagai makanan kepada kopepod lain dan pemangsa kepada larva nyamuk. Pengenalpastian spesis kopepod biasanya dijalankan dengan berdasarkan morfologi luaran copepod sahaja. Teknik ini sangat memakan masa dan hanya boleh dilakukan ke atas copepod dewasa sahaja. Setakat ini, tiada lagi kajian pengenalpastian *L. canariensis* dijalankan menggunakan teknik molekular. Tujuan kajian ini adalah untuk mengamplifikasikan gen separa COI mitokondria dan mencirikan PCR-RFLP profil dari gen separa COI untuk *L. canariensis*. Pemencilan seluruh DNA genom dilakukan dengan menggunakan buffer A PCR Vivantis dan amplifikasi dijalankan dengan menggunakan primer LCO-1490 dan HCO-2198. Seterusnya produk PCR gen separa COI digunakan di dalam PCR-RFLP yang menggunakan lapan enzim-enzim pembatas iaitu *KspAI*, *HindIII*, *MboII*, *MnII*, *HpaII*, *MboI*, *AluI* dan *RsaI*. Daripada lapan enzim-enzim pembatas hanya tiga enzim pembatas berjaya memotong dan menghasilkan profil pembatasan iaitu enzim *MnII*, *HpaII* and *MboI*. Enzim pembatas *MnII* memotong gen separa COI menghasilkan the produk serpihan bersaiz 550 bp dan 250 bp, manakala enzim pembatas *HpaII* memotong gen tersebut menghasilkan product serpihan bersaiz 600 bp dan enzim pembatas *MboI* memotong gen separa COI menghasilkan serpihan bersaiz 550 bp menunjukkan pencirian PCR-RFLP profil telah berjaya dilakukan dan boleh digunakan untuk membezakan *L. canariensis* dengan kopepod lain.