



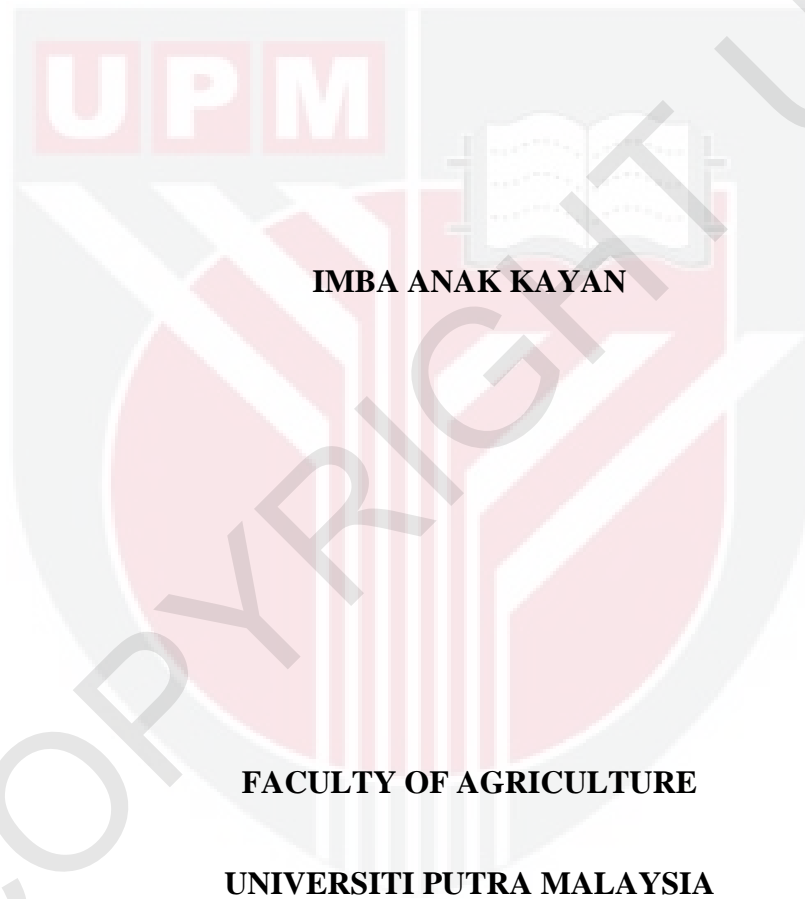
UNIVERSITI PUTRA MALAYSIA

***Molecular Identification of Cathepsin L Gene from Chrysomya
megacephala and Lucilia cuprina***

IMBA ANAK KAYAN

FP 2014 8

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*Lucilia cuprina***



FACULTY OF AGRICULTURE

UNIVERSITI PUTRA MALAYSIA

43400 SERDANG, SELANGOR DARUL EHSAN

2013/2014



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**Molecular Identification of *Cathepsin L* Gene from *Chrysomya megacephala* and
*Lucilia cuprina***

BY

IMBA ANAK KAYAN

A project report submitted to the Faculty of Agriculture, Universiti Putra Malaysia in partial fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science

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UNIVERSITI PUTRA MALAYSIA

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CERTIFICATION FORM

This project entitled “Molecular Identification of *Cathepsin L* Gene from *Chrysomya megacephala* and *Lucilia cuprina*” is prepared by Imba anak Kayan and submitted to the Faculty of Agriculture, Universiti Putra Malaysia in partial fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science.

Student's name

IMBA ANAK KAYAN

Student's signature

Certified by,

.....
(Dr. Lau Wei Hong)

Project Supervisor,

Department of Plant Protection,

Faculty of Agriculture,

Universiti Putra Malaysia,

43400 Serdang, Selangor.

Date:

ACKNOWLEDGEMENT

Firstly, I would like to express my sincere gratitude to my supervisor Dr. Lau Wei Hong for her guidance, supervision, support, attention, time, advice and as well as for providing me necessary information regarding my project. I really appreciate that she willing to help, teach and guide me until my project done.

Secondly, I would like to express my special gratitude to Mr. Fahad Nazir Khoso, Ms. Siti Mahsuri binti Talib, Mr. Chooi Yit Seng, Mr. Manan Tikon and Mr. Johari bin Mohd Sarikat for their kind co-operation, assisting and encouragement which help me in completion of this project.

Thirdly, special thanks to Ms. Nor Ezren binti Mahadi, Ms. Serena Ch'ng Chooi Ling, Ms. Fateen Noorasyiqin binto Sarkowi, Mr. Muhamad Ilham bin Omar, Mr. Abu Huzaifah bin Baharudin, Mr. Mohd Imran bin Suboh and my entire colleague who gave me a lot of support in developing my project. My project would not been possible without the kind support and help from many individuals. Last but certainly not least, a great thanks to my family for their constant support and understanding in everything I do in my life.

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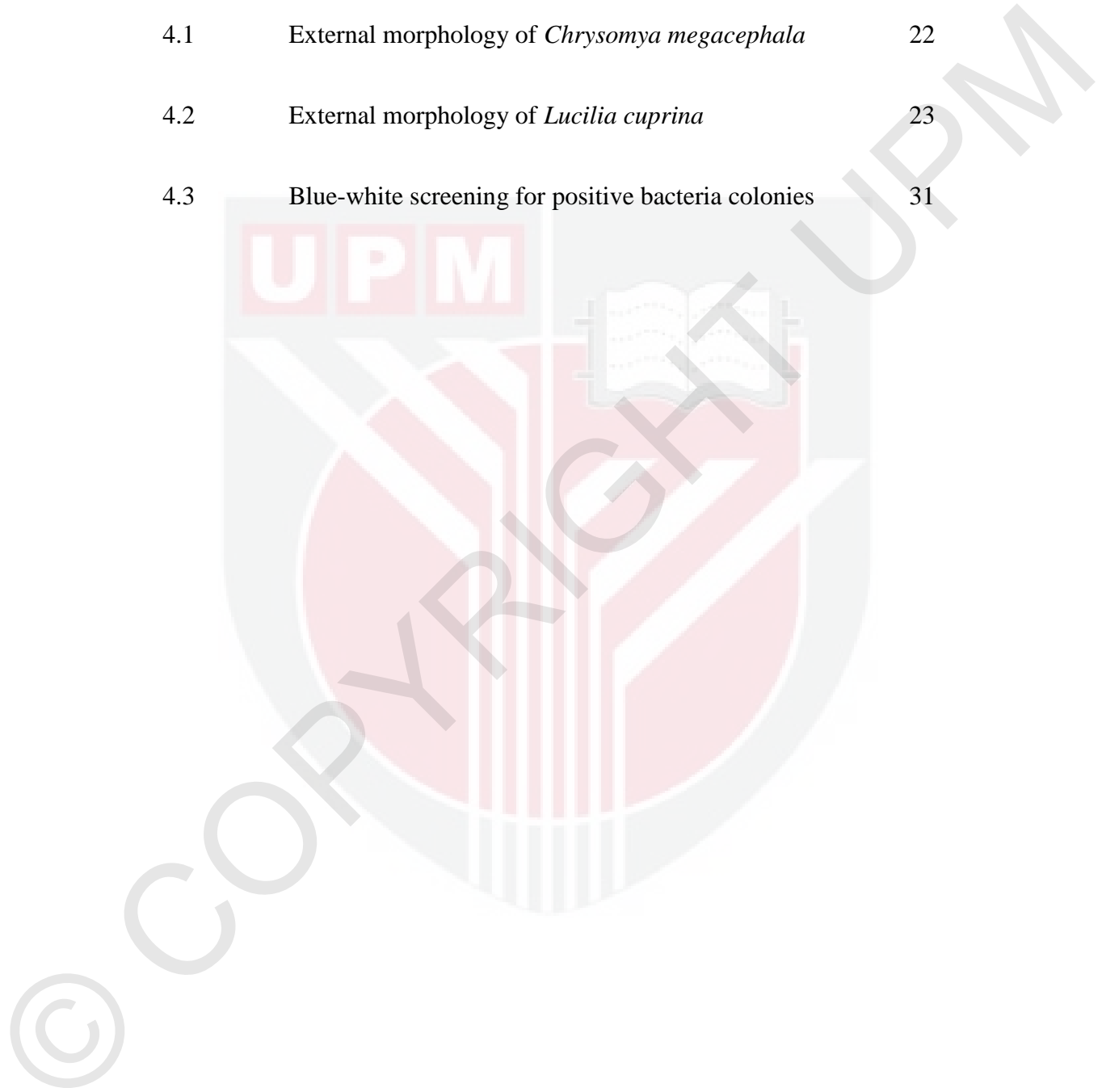
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LIST OF ABBREVIATION

g	Gram
μl	Microliter
L	Liter
°C	Degree Celsius
%	Percent
Min	Minutes
rpm	Rotation per minute
Kbp	Kilobase pairs
Bp	Base pairs
V	Volt
dNTP	Deoxyribonucleotide triphosphates
sp	Species
spp	Species (plural)
<i>CO1</i>	<i>Cytochrome c oxidase subunit 1</i>
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
PCR	Polymerase Chain Reaction
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
CaCl ₂	Calcium chloride
LB broth	Luria-Bertani broth

EtBr	Ethidium Bromide
SDS	Sodium dodecyl sulfate
EtOH	Ethanol
S.O.C medium	Super Optimal Broth
IPTG	Isopropyl β -D-1-thiogalactopyranoside
X-Gal	5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside
TAE Buffer	Tris Acetate-EDTA Buffer
NCBI	National Center for Biotechnology Information
MEGA	Molecular Evolutionary Genetics Analysis

ABSTRACT

Chrysomya megacephala and *Lucilia cuprina* are among the most common blow flies in Malaysia. These blow flies are easily captured and can be found anywhere in Malaysia. The objective of this study was to investigate the presence of *cathepsin L* gene from *C. megacephala* and *L. cuprina*. The *cathepsin L* has a potential to become a good candidates for insect pest control. A total of 97 *C. megacephala* and 45 *L. cuprina* were captured from Sri Serdang wet market, Putra Food Court and Pasar Borong Selangor. These flies were divided into groups based on their morphological characteristics and reared in the insectary. For the more accurate identification of species, *mitochondrial cytochrome c oxidase subunit 1 (CO1)* gene of *C. megacephala* and *L. cuprina* was amplified and sequenced. Analyses by neighbour-joining methods showed the potential of this region to provide the necessary species-level identifications. A cDNA encoding a cathepsin L-like cysteine protease was isolated from *C. megacephala* and *L. cuprina* purified RNA by a PCR approach. Plasmid PCR products of the expected size (~500 bp) were visualized by agarose gel electrophoresis. Phylogenetic tree analysis demonstrated that the *cathepsin L* gene presence in *C. megacephala* and *L. cuprina*. This is the first report of *cathepsin L* gene from *C. megacephala* and *L. cuprina* in the world.

ABSTRAK

Chrysomya megacephala dan *Lucilia cuprina* adalah antara lalat hijau yang banyak terdapat di Malaysia. Lalat hijau ini mudah ditangkap dan boleh didapati di mana sahaja di Malaysia. Objektif kajian ini dijalankan adalah untuk mengkaji kehadiran *cathepsin L* gen pada *C. megacephala* dan *L. cuprina*. *Cathepsin L* mempunyai potensi untuk menjadi calon yang baik untuk kawalan serangga perosak. Sebanyak 97 ekor *C. megacephala* dan 45 ekor *L. cuprina* telah ditangkap dari pasar basah Sri Serdang, medan selera Putra dan Pasar Borong Selangor. Lalat ini telah dibahagikan kepada kumpulan tertentu berdasarkan ciri-ciri morfologi dan dipelihara di rumah serangga. Untuk pengenalpastian spesies dengan lebih tepat, *cytochrome mitokondria c oxidase subunit 1 (COI)* gen daripada *C. megacephala* dan *L. cuprina* telah dipertingkatkan dandi susun menjadi jujukan. Analisis dengan kaedah jiran-menyeritai menunjukkan potensi rantau ini untuk memberikan keperluan pengenalan kepada setiap peringkat spesies. cDNA yang mengekod *cathepsin L* seperti cysteine protease telah diasingkan daripada *C. megacephala* dan *L. cuprina* dengan RNA tulen menggunakan pendekatan PCR. Saiz produk plasmid PCR yang dijangkakan (~ 500 bp) telah dilihat menggunakan agarose gel elektroforesis. Analisis pokok filogenetik menunjukkan kewujudan *cathepsin L* gen dalam *C. megacephala* dan *L. cuprina*. Ini adalah laporan pertama *cathepsin L* gen daripada *C. megacephala* dan *L. cuprina* di dunia.

CHAPTER 1

INTRODUCTION

Cathepsin L is a lysosomal endopeptidase. It is a member of the papain-like family of cysteine proteinases (Barrett and Kirschke, 1981; Ishidoh and Kominami, 1998). Its function is to degrade cellular and extracellular proteins particularly in acidic environment (Turk *et al.*, 2001). *Cathepsin L* has been proposed to be important in embryogenesis (Yamamoto and Takahashi, 1993; Uchida *et al.*, 2001). Various researchers have reported that *cathepsin L* is present in *Homarus americanus* (Laycock *et al.*, 1991), *Penaeus vannamei* (Le Boulay *et al.*, 1996) and many other organisms. The presence of *cathepsin L* in the digestive system of crustacean is unique but its function is yet to be identified (Le Boulay *et al.*, 1996). Besides, *cathepsin L* is an important digestive enzyme in Coleoptera, Diptera and Hemiptera. *Cathepsin L* has been considered as potential agent for pest control (Murdock *et al.*, 1987; Matsumoto *et al.*, 1995; Cristofolletti *et al.*, 2003).

Blow flies are dispersed worldwide including in Malaysia. From the studies, blowflies can cause losses to the animal industry (Zumpt, 1965; Greenberg, 1971; Ghandour, 1988). They can cause serious medical problems to human. *Chrysomya megacephala* is one of the most common blowflies in Malaysia. *Chrysomya megacephala* is the dominant vector of helminth parasite eggs (Sulaiman *et al.*, 1988 and Sulaiman *et al.*, 1989). On the other hand, *C. megacephala* is an important pollinator of mango and farmer has increased *C. megacephala*'s population to increase pollination especially in Australia and Taiwan (Anderson *et al.*, 1982; Hu *et al.*, 1995).

Moreover, blow flies are the most useful insects for the investigation of victims' death date. Investigators are able to estimate the date of death when they analyze the flies found on a corpse and the record of the ambient temperature (Early *et al.*, 1986).

Lucilia cuprina are another type of blow flies species which are facultative ectoparasites, particularly on sheep. Their larvae infest and feed on the living tissues of warm blooded vertebrates. Therefore, they are economically important pests in many countries (Zumpt, 1965; Hall and Wall, 1995). As a result of natural patterns of movement and artificial dispersal by humans and livestock in the last few hundred years, *L. cuprina* considered to have a cosmopolitan distribution in many parts of their range (Zumpt, 1965; Spradbery, 1991).

Blow flies are easily captured and can be found anywhere in Malaysia. The presence of *cathepsin L* gene in blow flies is yet to be confirmed. Thus, the objective of this study was to investigate the presence of *cathepsin L* gene from *C. megacephala* and *L. cuprina* and to characterize the *cathepsin L* gene from these blow flies.

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