UNIVERSITI PUTRA MALAYSIA

ENHANCING BACULOVIRAL INFECTIVITY BY EXPRESSING POLYHEDRIN PROTEIN FUSED WITH *Bacillus thuringiensis* Cry1D TOXIN

HAMZAH BIN ABDUL AZIZ

FP 2014 4

ENHANCING BACULOVIRAL INFECTIVITY BY EXPRESSING POLYHEDRIN PROTEIN FUSED WITH *Bacillus thuringiensis* Cry1D TOXIN

By

HAMZAH BIN ABDUL AZIZ

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

May 2014

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

ENHANCING BACULOVIRAL INFECTIVITY BY EXPRESSING POLYHEDRIN PROTEIN FUSED WITH *Bacillus thuringiensis*Cry1D TOXIN

By

HAMZAH BIN ABDUL AZIZ

May 2014

Chairperson: Lau Wei Hong, Ph.D.

Faculty: Agriculture

Escherichia coli and baculovirus are the common commercially available vectors used for protein expression. Both allow expression of huge amount of heterologous proteins which are functionally similar to the native proteins. The *cry1D* gene of *Bacillus thuringiensis* subsp. *aizawai* was successfully cloned into TA cloning vector and pBACgus-2cp EK/LIC baculovirus expression vector, and expressed in *E. coli* BL21 and *Trichoplusia ni* cells, respectively. Negative results were obtained when expressed in Sf9 (*Spodoptera frugiperda* cell line) and TUAT-Spli-221 (*Spodoptera litura* cell line). The sequence identity of the 3.5 kb DNA insert was compared with other holotype *cry1D* genes and scored 100% sequence similarity with *cry1Da1* and *cry1Da2* genes. A 140 kDa protein was produced by the recombinant baculovirus (AcMNPV) and cross-reacted with His-Tag monoclonal antibodies in Western blot analysis. The insecticidal activity of the recombinant AcMNPV and the Cry1D protoxin expressed in *E. coli* cells was compared against *S. litura* and *S. exigua*. The recombinant AcMNPV was injected into third instar *Spodoptera exigua* larvae (the dose is equivalent to2 x 10^5 pfu) and the median lethal time (LT_{50}) of infected *S. exigua* larvae was approximately 70% shorter than that of the wild-type AcMNPV. This recombinant AcMNPV was unable to induce mortality in the second instar larvae of *S. litura*. In contrast, the Cry1D protoxin expressed in *E. coli* cells appeared to have higher toxicity against *S.litura* (LT_{50} = 4.14 days) than *S. exigua* (LT_{50} = 10.65 days). This protoxin induced lower toxicity against susceptible insect host when compared to the commercial Bt Flobac. This may be due to the instability of the Cry1D protoxin

when expressed *in vitro* in *E. coli c*ells. The recombinant AcMNPV only expressed Cry1D protoxin when replicating in the susceptible insect cells, thus the protoxin was stable within the susceptible insect and able to induce higher toxicity in *S. exigua* when compared to the commercial Bt Flobac. The wild-type AcMNPV was unable to infect *S. litura* in this study, thus the efficacy of the recombinant AcMNPV against *S. litura* could not be compared with those Cry1D protoxin expressed in *E. coli* cells. Experiments were also conducted to study whether the truncated-core active part of *cry1D* gene expressed in both *E. coli* and BEVS could induce mortality in *S. litura* and *S. exigua*. The truncated-core active Cry1D toxin was successfully expressed in *E. coli* cells but not in *T. ni* cells. However, negative response was recorded in both *S. exigua* and *S. litura* larvae when challenged with the truncated-core active Cry1D toxin. Therefore, the fusion of Cry1D protoxin into baculovirus was proven able to improve its efficacy as a biopesticide.

Abstrak tesis yang dikemukan kepada senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

MENINGKATKAN KEBERKESANAN BAKULOVIRUS DENGAN PENGEKSPRESAN PROTEIN POLIHEDRIN DENGAN TOKSIN Cry1D *Bacillus thuringiensis*

Oleh

HAMZAH BIN ABDUL AZIZ

Mei 2014

Pengerusi: Lau Wei Hong, Ph.D.

Fakulti: Pertanian

Escherichia coli dan bakulovirus adalah vektor pengekspresan yang boleh didapati secara komersial dan digunakan untuk pengekspresan protein. Kedua-duanya membolehkan pengekspresan protein-protein asing, dihasilkan dalam kuantiti yang tinggi dan berfungsi serupa dengan protein asli. Gen *cry1D* daripada *Bacillus thuringiensis* subsp. *aizawai* telah berjaya diklon ke dalam vektor pengklonan TA dan vektor pengklonan bakulovirus pBACgus-2cp EK/LIC, dan diekspres masing-masing dalam sel-sel *E. coli* BL21 dan *Trichoplusia ni*. Keputusan negatif diperolehi apabila diekspres dalam sel-sel Sf9 (terbit dari *Spodoptera frugiperda*) dan TUAT-Spli-221 (terbit dari *Spodoptera litura*). Identiti jujukan gen selitan bersaiz 3.5 kb telah kenalpasti melalui dibandingkan dengan gen holotaip *cry1D* yang lain dan mempamerkan 100% kesamaan jujukan dengan gen *cry1Da1* dan *cry1Da2*. Protein bersaiz 140 kDa telah berjaya dihasilkan dan dikenalpasti dengan analisis pemblotan Western melalui interaksi-silang dengan antibodi monoklonal His-Tag. AcMNPV rekombinan telah disuntik ke dalam larva-larva serangga *Spodoptera exigua* pada penjelmaan ke-tiga (dos bersamaan 2 x 10^5 pfu) dan masa separuh maut (LT_{50}) yang terhasil adalah lebih kurang 70% lebih pendek berbanding dengan AcMNPV-jenis liar. AcMNPV rekombinan tidak menyebabkan kematian terhadap larva serangga *S. Litura* pada penjelmaan ke-dua. Sebaliknya, protoksin Cry1D yang diekspres dalam sel-sel *E. coli* boleh mengakibatkan ketoksikan yang lebih tinggi terhadap larva serangga

S.litura (LT_{50} = 4.14 hari) daripada larva serangga *S.exigua* (LT_{50} = 10.65 hari). Protoksin ini juga boleh menyebabkan kesan ketoksikan kepada larva-larva serangga yang sama menjadi serangga sasaran untuk produk komersial Bt Flobac tetapi pada tahap kesan ketoksikan yang lebih rendah. Kejadian ini mungkin disebabkan oleh sifat ketidakstabilan molekul protoksin Cry1D tersebut apabila diekspres secara *in vitro* dalam sel-sel *E. coli*. AcMNPV rekombinan hanya boleh mengekspres protoksin Cry1D selepas berupaya untuk berkembangbiak dalam sel-sel serangga yang rentan, dari itu molekul protoksin Cry1D yang telah diekspres bersifat stabil dan boleh menyebabkan kesan ketoksikan yang lebih tinggi berbanding dengan produk komersial Bt Flobac apabila diuji terhadap larva serangga *S. exigua*. Dalam kajian ini, disebabkan AcMNPV-jenis liar tidak dapat menjangkiti larva serangga *S. litura*, perbandingan keberkesanan AcMNPV rekombinan dengan protoksin Cry1D diekspres dalam sel-sel *E.coli* terhadap larva serangga *S. litura* tidak dapat dibandingkan. Eksperimen mengkaji sama ada bahagian aktif protoksin Cry1D yang telah diekspres menggunakan sistem pengekspresan *E. coli* dan bakulovirus, masing-masingnya dan diuji kesan ketoksikannya terhadap larva serangga *S. litura* dan *S. exigua* juga ada dijalankan dalam kajian ini. Bahagian aktif protoksin Cry1D telah berjaya diekspres di dalam sel-sel *E. coli* tetapi tidak dalam sel-sel serangga *T. ni*. Walau bagaimanapun, tiada kematian (keputusan negatif) dicatatkan untuk kedua-dua larva serangga *S. exigua* dan *S. litura* apabila bertindakbalas dengan bahagian aktif protoksin Cry1D rekombinan. Dari itu, pelakuran protoksin Cry1D denganbakulovirusbolehmeningkatkankeberkesanannyasebagaibiopestisid.

ACKNOWLEDGEMENTS

All praise and glory belongs to Allah s.w.t. and all forms of benedictions are for prophet Muhammad s.a.w.

My deepest gratitude to my supervisors, Dr. Lau Wei Hong, Prof. Datin Paduka Dr. Khatijah Mohd. Yusoff, Prof. Dr. Ahmad Said Sajap and Associate Prof. Dr. Tan Yee How for their patience and generous guidance throughout the study.

Appreciation is accorded to Mr. Hussan Abdul Kadir from Insectory Laboratory, MARDI, Serdang for providing the *Spodoptera exigua* and *Spodptera litura* larvae for this study. Special thanks to all staff members at Department of Biochemistry and Microbiology, Faculty of Biotechnology and Biomolecular Sciences, who helped and provided the appropriate equipments and chemicals during this study. Thanks are also conveyed to all staff members at the Unit of Electron Microscopy, Bioscience Institute for helping in taking E. M. monographs. Not to forget Mr. Mohamad Zawawi Idris, senior laboratory assistant in Molecular Biology Laboratory, Department of Plant Protection, Faculty of Agriculture, who also helped in technical assistance throughout this study.

I would like to express my sincere gratitude to all labmates in Virology Laboratory at Faculty of Biotechnology and Biomolecular Sciences; and in Molecular Biology Laboratory at Department of Plant Protection. I wish to extend my appreciation to everyone, although not individually named here, who had contributed directly or indirectly to my research project and thesis. This study was supported by Sciencefund project no. 05-01-04-SF0304 from the Ministry of Science, Technology and Innovation (MOSTI), Malaysia. Last but not least, I want to take this opportunity to thank my parents, brothers and sisters for their endless love, care and encouragement. Without all of you, it would not be possible for me to complete my research project and thesis. May Allah s.w.t. bless you all for your kindness.

I certify that a Thesis Examination Committee has met on 14 May 2014 to conduct the final examination of Hamzah bin Abdul Aziz on his thesis entitled "Enhancing Baculoviral Infectivity by Expressing Polyhedrin Protein Fused with *Bacillus thuringiensis* Cry1D Toxin" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Hafidzi bin Mohd Noor, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Ganesan a/l Vadamalai, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Halimi bin Mohd Saud, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Chandra Prakash Srivastava, PhD
Senior Lecturer
Banaras Hindu University
India
(External Examiner)

NORITAH OMAR, PhD
Associate Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 18 August 2014

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Lau Wei Hong, PhD
Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Datin Paduka Khatijah binti Mohd. Yusoff, PhD
Professor
Faculty of Biotechnology and Molecular Sciences
Universiti Putra Malaysia
(Member)

Ahmad Said bin Sajap, PhD
Professor
Faculty of Forestry
Universiti Putra Malaysia
(Member)

Tan Yee How, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

_____

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that.

- this thesis is my original work;
- quotations, illustration and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic from) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergoes plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No.: _____

Declaration by Members of Supervisory Committee

This is confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature:	_____	Signature:	_____
Name of Chairman of Supervisory Committee:	Dr. Lau Wei Hong	Name of Member of Supervisory Committee:	Prof. Datin Paduka Dr. Khatijah binti Mohd. Yusoff
Signature:	_____	Signature:	_____
Name of Member of Supervisory Committee:	Prof. Dr. Ahmad Said bin Sajap	Name of Member of Supervisory Committee:	Assoc. Prof. Dr. Tan Yee How

TABLE OF CONTENTS