



UNIVERSITI PUTRA MALAYSIA

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

HAMZAH BIN ABDUL AZIZ

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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

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

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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 to 2×10^5 pfu) and the median lethal time (LT₅₀) 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* (LT₅₀ = 4.14 days) than *S. exigua* (LT₅₀ = 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* cells. 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.



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

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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 dikenalpasti 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 2×10^5 pfu) dan masa separuh maut (LT₅₀) 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 dengan bakulovirus boleh meningkatkan keberkesanannya sebagai biopestisid.

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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.

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