

EXPRESSION, PURIFICATION AND STRUCTURAL ANALYSIS OF IMMUNOSUPPRESSANT PROTEIN, CRV1, OF *COTESIA RUBECULA* POLYDNAVIRUS (CRPDV).

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Introduction. Polydnviruses (PDVs) are symbionts of parasitoid wasps as a natural strategy to overcome the immune defense of the Lepidopteran host. PDVs are double-stranded, circular DNA viruses found in some Ichneumonoidea families, being classified as bracovirus (BV) or ichnovirus (IV) according to the host parasitoid and viral morphology. One gene product of the BV of the parasitic wasp *Cotesia rubecula*, *CrV1*, inhibits the immune system responses of its endoparasitized lepidopteran host through interference with the haematocyte cytoskeletal structure. The mechanism of inactivation is still not clear. In this study, *CrV1* is constructed into a baculovirus vector pAcGHLT-B and co-transfected into baculoviruses. To further understand the molecular mechanism of inactivation of host proteins by *CrV1*, we aim at expressing and purifying this protein in *Sf9* cells to analyze the structure of the protein and perform functional motif analysis by bioinformatics methods..

Methods. We design primers and amplify *CrV1* from a cDNA clone in pBluescript SK vector. Then clone *CrV1* gene into a transfer vector pAcGHLT-B in frame with the glutathione S-transferase tag (GST) open reading frame (ORF). After that, using the recombinant pAcGHLT-B plasmid and *AcMNPV* linearized genome co-transfect into the *Sf9* cells. Amplify the recombinant virus in *Sf9* insect cells and produce recombinant protein. Then purify the protein with protein expression and purification kit. Crystallize the protein. At last, analyze the structure of the protein. pVL1392 and pAcUW31 transfer vectors with *CrV1* gene are constructed using the same method.

Result and discussion. The coding sequences of *CrV1* were amplified from a cDNA clone in pBluescript SK vector using rTth DNA polymerase with specific primers. The fragments were subcloned into pAcGHLT-B, pAcUW31, pVL1392 vector. After that, sequence the recombinant vectors. The recombinant baculoviruses were generated by co-transfection with a BD BaculoGlot™ Transfection Kit (BD Biosciences Pharmingen). Recombinant baculoviruses were amplified as previously described. The target protein was detected by SDS-Polyacrylamide gel electrophoresis (SDS-PAGE) and purified with kit.

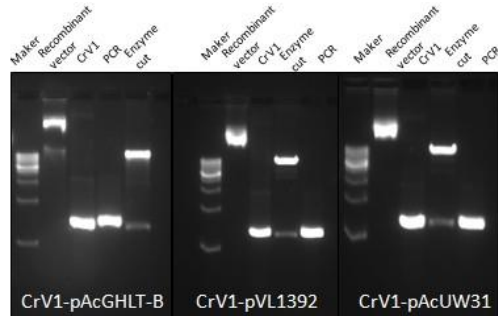


Figure 1. Construction of three transfer vectors

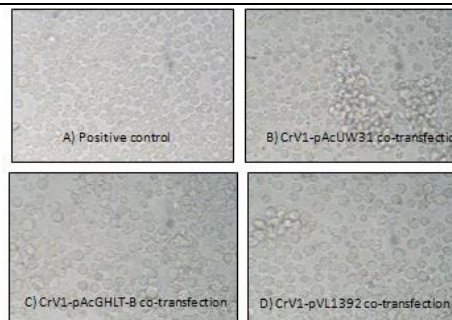


Figure 2. Co-transfection results of three recombinant vectors

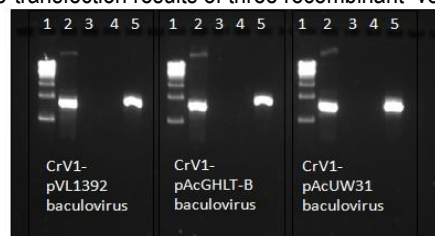


Figure 3. RT-PCR results of three recombinant baculoviruses

Lane 1: Marker lane 2: Positive control- *CrV1* gene; lane 3 : PCR results of RNA from each BV infected *Sf9* cells ; lane 4: PCR results of cDNA from healthy cells; lane 5: PCR results of cDNA from each BV infected *Sf9*

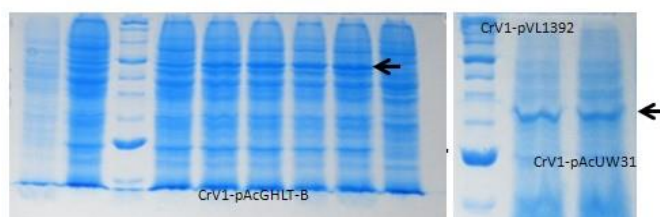


Figure 4 Expression of three recombinant baculoviruses

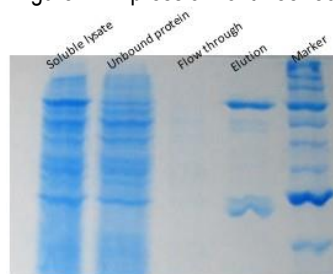


Figure 5 *CrV1* protein purification from pAcGHLT-B recombinant baculovirus

Conclusions and perspectives. Recombinant expression vectors with gene *CrV1* and *CcV1* were constructed. Recombinant viruses were obtained by co-transfection. The expression of *CrV1* protein was detected in three recombinant viruses. The GST-*CrV1* protein was also purified with kit. The GST tag should be cut with thrombin.

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