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Genomic Cloning and Promoter Analysis of *Cotesia plutellae* Polydnavirus

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Cotesia plutellae polydnavirus (CpBV) is obligate mutualistic insect virus found in parasitic wasp, *Cotesia plutellae*, which has been recommended as useful component to apply integrated pest management of the diamondback moth, *Plutella xylostella*, in southeastern Asia. The CpBV causes several obvious physiological changes such as immune suppression and developmental disturbance in parasitized hosts, *P. xylostella* larvae. Genomes of CpBV consist of several double-stranded, circular DNA molecules with variable size.

In this study, we cloned CpBV genomic segments using plasmid capture system (PCS), pPCS-S into *Escherichia coli* cell. The pPCS-S may transfer a pUC19 origin of replication and an ampicillin resistance marker inserted between Tn7 left (L) and right (R) end, and this pPCS-S donor was applied to clone segments of CpBV genome by *in vitro* transposition using TnsABC* transposase. In result, 53 genomic clones ranging from 0.3 to 26 kb were cloned and they were classified 29 different segments by their size and restriction endonuclease pattern. Among these clones, the complete nucleotide sequence of CpBV-S30 clone belonging to segment group 27 was determined and nine putative ORFs were predicted with FGENESV0. Comparison of the nucleotide sequences with EMBL databases or of the hypothetical proteins with SWISS-PROT databases using BLAST revealed that seven of nine ORFs showed homologies with known proteins, that is ORF301 with EP1 of *C. congregata* polydnavirus and CkV2.0 of *C. kariyai* polydnavirus, ORF302, ORF303, ORF305 and ORF309 with 94k protein of baculoviruses, ORF306 with catalytic domain of protein tyrosine phosphatase and ORF 307 with histone H4 proteins, respectively.

For the promoter analysis of these possible seven ORFs, EGFP gene as reporter was cloned into polyhedrin promoter deleted pBacPAK8 vector under the control of promoter of each seven CpBV-S30 ORFs, respectively. In addition, recombinant AcNPVs were made by co-transfection of these transfer vectors with bApGOZA DNA. In the transient expression using transfer vectors, ORF302 promoter showed highest activity among seven promoters in Sf9 and high-Five cells, but the activity was 40~50 % of that of AcNPV IE-1 promoter. In LdFB cells, ORF307

promoter showed highest activity that is comparable to that of IE-1 promoter. However, ORF301 and ORF306 promoters showed no activity in transient expression assay.

Meanwhile, in expression using recombinant AcNPVs harboring EGFP gene under the control of each CpBV-S30 promoters, ORF305 promoter showed highest activity in Sf9 and high-Five cells, which support replication of AcNPV, and the activity was about 24 % of that of AcNPV polyhedrin promoter in Sf9 cells. While the activity of polyhedrin promoter was observed from 14 hours post-infection (p. i.), the activity of ORF305 promoter was observed form 2 hours p. i. and reached to peak at 3 days p. i. The ORF305 promoter also showed high level of activity in LdFB, Bm5 and drosophila S2 cells which are non-susceptible host cells of AcNPV, in comparison to IE-1 and polyhedrin promoters. In addition, the ORF306 promoter, which did not show any activity in transient expression, showed moderate level of activity while the ORF301 promoter still was not active, and this was one of the evidences that CpBV-S30 ORFs promoter could be activated or enhanced by some factors attributed by AcNPV. Besides, ORF307 promoter also showed high level of activity in susceptible and non-susceptible insect cells although overall activity was lower than that of ORF305 promoter except in LdFB cells. In conclusion, CpBV-S30 ORF promoters investigated in this study could be useful promoters in variety of applications such as gene expression and insecticide development.