EXPRESSION OF DENGUE VIRAL ENVELOPE PROTEIN IN *Bombyx mori* CELLS USING A BACULOVIRUS EXPRESSION VECTOR SYSTEM

The *Bombyx mori* nucleopolyhedrovirus (BmNPV) expression system has been successfully used for high level expression of large amount of heterologous protein in *Bombyx mori* cells (BmN cell) or larvae. This system can be used as an economical alternative to large scale cell culture for production of biomolecules such as human growth hormone, parathyroid hormone and β-interferon, etc. This study was aimed to use BmNPV expression system to express dengue viral envelope gene (Den2E) in which the recombinant baculovirus was generated by homologous recombination. Construction of recombinant dengue baculovirus was archived using LacZ gene as a marker to facilitate the isolation of recombinant baculovirus. Lineared viral form created by the unique restriction site of Bsu36I in the LacZ gene was also used to improve recombination efficiency. Recombinant baculovirus harboring LacZ gene (BmLacZ) in which lacZ gene was inserted in lieu of polyhedrin gene in wild type BmNPV DNA was first prepared by co-transfection between wild-type BmNPV viral DNA and transfer vector containing lacZ gene (pBKblue). The resulting BmLacZ was then linearized by Bsu36I restriction endonuclease. A transfer vector, pBKDen2E, generated by cloning the coding region of Den2E gene into pBKblue and lineared BmLacZ DNA were then co-transfected into BmN cells. After homologous recombination, the Den2E gene was transferred from the pBKDen2E and replaced the LacZ gene in the BmLacZ DNA. The LacZ gene minus phenotype or colorless recombinant viral plaque was then obtained by three rounds of plaque purification. The purified recombinant BmDen2E was confirmed for insertion of Den2E gene by Southern blot analysis. SDS-PAGE and Western blot analysis of the extracted-BmN cells infected with recombinant BmDen2E using antibody specific to dengue viral envelope protein revealed that recombinant protein was synthesized in insect cells. This product has a molecular weight of approximately 58 kDa. Optimization of recombinant Den2E gene expression was investigated. It was found that maximum expression of recombinant protein can be obtained by using 2×10⁶ cells/ml of BmN cells infected with recombinant BmDen2E at MOI 5 for 72 h.