

Construction of An Improved Baculovirus Producing Recombinant Polyhedra That Contain *Bacillus thuringiensis* Insect Toxins

**Hee Jin Shim, Jong Yul Roh, Jae Young Choi, Ming Shun Li,
Joong Nam Kang and Yeon Ho Je**

School of Agricultural Biotechnology, Seoul National University

Baculovirus polyhedra have been recently engineered to incorporate foreign protein such as *Bacillus thuringiensis* Cry1Ac protein for improvement of insecticidal activity. In this study, we generated a novel baculovirus insecticide producing recombinant polyhedra that contain Cry1Ac, Cry1Ca and EGFP fusion protein. The *cry1Ac*, *cry1Ca* and *egfp* genes were amplified by PCR from pProAc, pBacPHcry1C and pEGFP as the templates, respectively. The amplified genes were fused in pGEM5Zf(-) vector in order as *cry1A-egfp-cry1Ca*. This full fusion gene was transferred at the back of *polyhedrin* gene of pOB I to construct transfer vector, pBacPAC-F. The recombinant *Autographa californica* nucleopolyhedrovirus (AcNPV), ApPAC-F, was generated by cotransfection with pBacPAC-F and bApGOZA in Sf9 cells and was confirmed by PCR, SDS-PAGE analysis and observation with fluorescent microscopy. Cry1Ac, EGFP and Cry1Ca fusion protein was successfully incorporated into polyhedra. ApPAC-F showed dual toxicity against *Plutella xylostella* and *Spodoptera exigua* larvae and also had increased pathogenicity in comparison with the wild type AcNPV.