4-5. Expression of a Fusion Protein between Cry1Ac and Enhanced Green Fluorescent Protein in an Acrystalliferous Bacillus thuringiensis

Jong Yul Roh^{*}, Ming Shun Li, Jin Hee Chang, Jae Young Choi, Hee Jin Shim, Kyung Saeng Boo and Yeon Ho Je

School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea

The expression of a fusion gene comprised of the B. thuringiensis crystal protein, Cry1Ac1, and enhanced green fluorescent protein (egfp) genes in B. thuringiensis Cry-B strain was examined. The cry1Ac1 gene was cloned in the B. thuringiensis-E. coli shuttle vector, pHT3101, under the control of the native cry1Ac1 gene promoter while the egfp gene was inserted between ribosomal binding site and start codon of the cry1Ac gene (pProMu-EGFP). The B. thuringiensis Cry-B strain carrying pProMu-EGFP (ProMu-EGFP/CB) produced mRNA transcripts encoding the cry1Ac1-egfp fusion gene and produced an small inclusion body, 200 nm in size. Although its expression level was relatively lower than that of ProAc/CB, this recombinant strain expressed the fusion protein, confirmed by immunoblot analysis using GFP and Cry1Ac1 antibodies. Furthermore, the spore-crystal mixtures of ProMu-EGFP/CB exhibited insecticidal activity against Plutella xylostella larvae. However, fluorescence of its parasporal inclusion body was not detected because faint light of EGFP in the gram-positive cell was not discriminated between parasporal inclusion and cell body. The current results suggest that the front region-fusion expression of foreign protein in the B. thuringiensis crystal protein can be functionally expressed and produced fusion parasporal inclusion in B. thuringiensis.