

## Cloning, characterization and expression of an insecticidal crystal protein gene from *Bacillus thuringiensis* isolates of Andaman and Nicobar Islands

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Biocontrol of pests via *Bacillus thuringiensis* (Bt)  $\beta$ -endotoxins represents the most successful use of a biological control agent to date. The most notable characteristic of *Bacillus thuringiensis* is its ability to produce insecticidal proteins. More than 300 different proteins have been described with specific activity against insect species. The six isolates of *Bacillus thuringiensis* from Andaman and Nicobar Islands which were previously characterized by PCR analysis for the presence of Coleopteran active cry genes were used for CryII full length gene amplification. A 2.16-kb DNA fragment of CryII gene was PCR amplified, cloned in expression vector pQE 80 L, and then used for transformation of E. coli M15 cells. The optimum expression was obtained with 1 mM IPTG at 37°C for 3 h. The sequence of the cloned crystal protein gene showed almost complete homology with a CryII toxin gene from *Bacillus thuringiensis* var. kurstaki, with scattered mutations in the toxic region. The deduced sequence of the protein has homologies of 91.0% with CryII and CryIIa, and 98.0% with CryIIb. Cloning of this gene may help to overcome the increasing resistance of pests to currently used insecticides. Based on the results obtained, the PCR method may be a valuable and reliable tool for specific detection and identification of cryII genes. The toxicity of Bt recombinant protein was determined against first instar larvae of *Mylokerus undecimpustulatus undatus* Marshall (Coleoptera: Curculionidae) and Adults; *Helicoverpa armigera* Hübner (Noctuidae: Lepidoptera) at 310  $\mu$ g/mL and 15.5  $\mu$ g/mL respectively. The novel cryII gene will be an important resource in constructing genetically engineered bacteria and transgenic plants for biocontrol of insect pests and Bt based biopesticidal formulations, aiming to reduce the use of chemical insecticides.

### Biography

Bryan J. Vogel is a partner in the New York office of the law firm of Robins, Kaplan, Miller, & Ciresi L.L.P. He is an Intellectual Property litigator with a practice emphasis in Life Sciences and Hatch-Waxman and biosimilars litigation and strategic counseling. He publishes frequently on general Intellectual Property topics, as well as matters specific to the Life Sciences community. He received his J.D. from George Washington University. In addition, he earned a B.S. in Chemical Engineering, with honors from Michigan State University, where he was also a member of Phi Beta Kappa and Tau Beta Pi (invited).