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Cloning and expression of novel coleopteran active cry genes from native *Bacillus thuringiensis* isolates

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Coleopterans are the most detrimental pests to field crops; forests, and stored products, research to develop effective biopesticides against beetle pest species is highly pertinent and urgent. Most coleopteran-specific Cry toxins have limited host range and efficacy that prevent their widespread use in agriculture. To date, low efficacy and lack of knowledge regarding the mode of action of Cry toxins in coleopterans have limited the commercialization of coleopteran specific Cry toxins. Characterization of the molecules directly involved in the mode of action of Cry toxins in Coleoptera will provide the tools necessary to increase the efficacy of Cry-based biopesticides against economically important beetles. The present study aimed to isolate, characterize novel Coleopteran active cry genes from local isolates of *B. thuringiensis*. cry1I, cry3A and cry26 genes were successfully isolated from local isolates of Bt. These full length gene sequences were deposited at NCBI and *Bacillus thuringiensis* Toxin Nomenclature Committee (BTTNC). Further these new/novel toxins were analyzed through protein homology modeling. Successfully these isolates were over expressed in pQE80L expression vector and then used for transformation of E. coli M15 cells. The recombinant proteins were purified through NiTED columns. The purified toxins were tested against different Coleopteran insects viz., Mango Asian gray weevil, *Myllocerus undecimpustulatus undatus* Marshal (Coleoptera:Curculionidae) larvae and adults, Brinjal ash weevil, *Myllocerus subfasciatus* Guerin (Coleoptera:Curculionidae) adults Myllocerus discolor (Coleoptera:Curculionidae) adults, Guava ash weevil, Myllocerus adults, Cashew stem and root borer *Plocaederus ferrugineus*, *Rhynchaenus mangiferae*, White root grubs (Coleoptera:Scarabaeidae), *Sagra femarota*, Aracanut stem borer *Leucopolis lepidopora*, Rice weevil *Sitophilus oryzae*, Maize weevil *Sitophilus zeamais*, Red flour beetle *Tribolium castaneum* (Coleoptera:Tenebrionidae), Pulse beetle *Callosobrchus sp*., American bollworm *Helicoverpa armigera* (Lepidoptera:Noctuidae), Diamond backmoth *Plutella xylostella* (Lepidoptera: Plutellidae). These new genes serve as candidate gene against different coleopteran insect pests and also new encounters due to climate change. The present research data is strengthened by an efficient expression, selection or screening system which will help us in exploiting the full potential of these environment friendly biological pesticides.

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