Molecular Characterization of Indigenous *Bacillus Thuringiensis Kurstaki* Isolates with Effective Pesticidal Activity against *Bactrocera cucurbitae* from Bangladesh

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Abstract: The indiscriminate use of agricultural pesticides has created serious health and environmental problems in many developing countries including Bangladesh. On the other hand, *Bacillus thuringiensis kurstaki* (*Btk*) has long been used commercially as pest control agents particularly against lepidopteran vegetable pests. In this study, new potential *Btk*-like isolates were collected from different sources (viz. soil, leaves, insects, stored product dust etc.) and regions of Bangladesh. Acetate selection-heat treatment-lecithinase production and haemolytic activity were initially performed to narrow down the spectrum for selection of *Btk*-like isolates. Specific biochemical tests (Es^+ , Sa^+ , Le^+ , Su^-) were performed to differentiate *Btk* from other subspecies. Based on these tests, of 103 Bt isolates, 38% (39 Bt isolates) were confirmed as *Bt kurstaki* isolates. The 39 isolates were investigated for the presence of *cryI*, *cryIA* and *cry2A* genes by PCR identification with gene specific primers and demonstrated some level of diversity with respect to major genes content in the strains. Nine of the isolates were found to carry all three genes while another nine contained both *cryI* and *cry2A* genes. 29, 22 and 16 of the isolates contained singly *cryI*, *cry2A* or *cryIA* gene respectively. Plasmid DNA was purified which ranged from 15-30 kb in most of the isolates which were very similar to the plasmid pattern of *Btk* HD-73. Bioassay was performed with the isolates harbouring Lepidoptera specific *cryIA* genes against vegetable pest *Bactrocera cucurbitae*. Five of the isolates having higher activity than *Btk* HD-73 were considered for determination of LC_{50} and LC_{90} . The results will be a useful basis for developing effective bio-pesticides to decrease the use of hazardous chemicals in Bangladesh agriculture.

Key words: Molecular characterization, *Bacillus thuringiensis kurstaki* (*Btk*), pesticidal activity, vegetable pest, Bangladesh.