

7. SUMMARY OF THE Ph.D. THESIS WORK

- Sampling of the host insects (*A. submarginata*, *A. bipunctata* and *O. postica*) was done from the tea plantations of Darjeeling foothill region and the adjoining plains (Terai and the Dooars).
- The entomopathogenic bacteria were isolated from the insect cadavers and moribund larvae collected from various tea gardens and tested following a standard procedure and Koch's postulate.
- Three bacterial strains of *Bacillus* Arc01, Arc02, Arc03 from *A. submarginata*; four strains Ab01, Ab02, Ab03 and Ab04 from *A. bipunctata* and two strains Org2A and Org6A from *O. postica* were selected for the study because they were more frequent in the natural population.
- All the morphological characteristics of bacterial isolates such as colony morphology, vegetative body structure, spore-shape presence of parasporal crystals were found to be similar to genus *Bacillus* and were comparable to commercially available *Btk* which was used as a reference in the study.
- Generation/ doubling time and cfu/ml of all the isolates were different thus making newly isolated bacteria different *Bacillus* strains.
- Biochemical tests indicated that all the newly isolated *Bacillus* strains differed with each other and with reference *Btk*, as biochemical test is done with the purpose of comparison of the unknown with the known. Similarity index based on biochemical tests showed highest similarity and dissimilarity among a pair of *Bacillus* isolates.

- In SDS-PAGE of crystal protein of reference strain *Btk*, Arc01, Arc03 and Ab04 showed 128, 128.4, 122.7 and 129.8 kDa bands, respectively, they were the size of ~130 kDa band corresponding to *cry1/cry9* toxin. Additionally, Arc01 had 64 kDa, Ab01 had 56.7 kDa, Ab03 had 56.2 kDa, Ab04 had 56.5 kDa and Org6A had 57.7 kDa band, all correspond to ~60 kDa of *cry2* toxin gene.
- The whole cell protein analysis of *Bacillus* isolates differed in protein content for different strains, highest for Arc03 (2.356 mg/ml) and lowest for Ab01 (1.094 mg/ml) and corresponding bands ranging from 10 to 126.6 kDa (Arc01, Arc02 and Arc03) 29 to 110 kDa (Ab01, Ab02, Ab03 and Ab04) and 30.8 to 78.5 kDa (Org2A and Org6A). The difference in the whole cell protein content and bands among different *Bacillus* strains and with reference *Btk* indicated that they are distinct strains.
- Apaydin et al. (2005) reported presence of a major plasmid band of 15kb in 33 *Bt* isolates with varying size between 15 kb and 22 kb in *Btk* from Korean soil. In present study too, the size of plasmids ranged from 16 kb (Ab01) to 20.7 kb (Arc01) which is in agreement with the above results. Yilmaz et al. (2013) found 19.3 kb plasmid band comparable to 19.4, 19.8 and 19 kb plasmid bands of Ab04, org 6A and reference *Btk*, respectively, in the present study.
- Low LC_{50} and LT_{50} values of Arc03, Ab04 and Org 6A indicated their high virulence than *Btk*.
- All the *Bt* strains of bacteria isolated from *A. submarginata*, *A. bipunctata* and *O. postica* were found to be non- pathogenic to the silk worm larvae. So it can be inferred that the newly isolated bacterial strains, Arc01, Arc02, Arc03, Ab01, Ab02, Ab03, Ab04, Org 2A and Org 6A with proper formulation are

apparently safe for spraying in the tea plantation for controlling the respective pest species.

- The most virulent *Bacillus* species i.e Arc01, Ab04 and Org6A when sequenced for 16S rDNA gene showed 99% similarity with *Bacillus thuringiensis* sequence.
- Sequencing of PCR products further confirmed that these newly isolated *Bacillus* strains that formed parasporal inclusions and exhibited toxicity against lepidopterans were novel isolates of *Bacillus thuringiensis*.