Summary and Conclusions

The present study was carried out to isolate native \textit{B. thuringiensis} isolates from soil samples predominantly collected from North East region along with some other states of India. These isolates were further characterized to identify their spectrum of insecticidal activity against lepidopteran insect pests and to estimate the (\textit{cry}) gene content by using different techniques like ELISA, PCR and SDS-PAGE. For selected efficient isolates, 16S rDNA analysis was also performed to characterize them at species level. At last, evaluation of different culture media based on agro byproducts was done for maximal growth, endotoxin production and insecticidal activity of \textit{B. thuringiensis} strain AUG-5.

- A total of 216 soil samples were primarily collected from North East region along with some states viz. Delhi, Haryana, Maharashtra, Punjab, Uttarakhand and West Bengal of India. Out of these isolates, 105 isolates were confirmed as \textit{B. thuringiensis} on the basis of the production of parasporal inclusions and being Gram positive, whereas 111 isolates were found as non-Bt isolates.
- Soil samples obtained from West Bengal and Punjab states showed 0\% presence of \textit{thuringiensis}, whereas samples collected from Govindghat, Haldwani (Uttarakhand), Jalgaon (Maharashtra) and Kohima, Zunheboto (Nagaland) showed 100\% presence of the \textit{B. thuringiensis} isolates.
- Colonies of all 105 isolates were rough in nature with wavy margin. Colonial appearance of \textit{B. thuringiensis} subsp. \textit{kurstaki} HD-1, HD-73, \textit{B. thuringiensis} subsp. \textit{israelensis} and \textit{B. thuringiensis} subsp. \textit{tolworthi} reference strains belonged to group A (colonies of pan cake, irregularly round, white to off white shade with bright translucent centre colonies, wavy margin), like the majority of \textit{B. thuringiensis} isolates (91.42\%). Best isolate AUG-5 showed the presence of bipyramidal and spherical crystal inclusions along with vegetative cells and spores.
In 105 native *B. thuringiensis* isolates, most of the isolates predominantly had bipyramidal type of crystals, whereas some showed the presence of spherical shaped crystals. A few isolates showed the presence of both type of crystals like bipyramidal and spherical, simultaneously.

Out of the 102 native *B. thuringiensis* isolates, tested for insecticidal activity against neonates of *H. armigera* only two isolates were found to be more toxic (AUG-5 with 95.93% mortality at 1 µg/g and GTG-7 with 83.33% mortality at 10 µg/g) in comparison of standard strain HD-1 which caused 58.33% mortality at 10 µg/g on the 7th day of treatment.

Out of the 102 native *B. thuringiensis* isolates, bioassayed for insecticidal activity against neonates of *S. litura*, only AUG-5 was found to be most toxic which caused 73.33% mortality at the concentration of 1 µg/g on 7th day of treatment, in comparison of standard toxins Cry2Ab2 and MVPII Cry 1Ac which caused 60% and 56.67% at the concentration of 10 µg/g and 1 µg/g on 7th day of treatment, respectively.

All 105 isolates were found to be as catalase positive. Biochemical tests based on pH change, substrate utilization and other biochemical reaction were performed along with antimicrobial susceptibility test for some selected native *B. thuringiensis* isolates, which showed that all isolates except GTG-4 were positive for trehalose and citrate utilization and all isolates except *B. thuringiensis* subsp. *israelensis* and GTG-4 showed positive reaction for esculin hydrolysis. For the results of antimicrobial susceptibility test, all isolates except GTG-4 showed resistance for the antibiotic ampicillin.

In case of quantitative estimation of Cry1Ac and Cry2Ab toxins, the higher content of Cry1Ac and Cry2Ab were produced by AUG-5 which was recorded as 104.8 ng/mg and 3792 ng/mg, respectively.

For selected 10 best native *B. thuringiensis* isolates, *cry1* genes and *cry1A* genes were amplified in seven isolates (AUG-5, GTG-7, GTG-9, GTG-70, GTG-3S, GTG-4S and GTG-6S), *cry2* gene was amplified in three isolates (AUG-5, GTG-7, GTG-4S and GTG-6S) and two isolates GTG-9 and GTG-70 were found positive for *cry9* gene.

- In selected 10 best native *B. thuringiensis* isolates, four isolates (AUG-5, GTG-7, GTG-4S and GTG-6S) produced a protein profile similar to that of lepidopteron specific *B. thuringiensis* subsp. *kurstaki* HD-1 with molecular weights of 130, 63-75, 48 and 35 kDa.

- Of the 82 native *B. thuringiensis* isolates, 73 isolates were identified as *B. thuringiensis*, 5 as *B. subtilis* (GTG-57, GTG-59, GTG-60, GTG-69, and GTG-92), 1 as *Bacillus cereus* (GTG-89), 2 as *B. pumilus* (GTG-11, GTG-31) and 1 as *B. amyloliquefaciens* and submitted to NCBI.

- Out of 11 different media based on agro byproducts, Medium VI produced the highest amount of biomass (5.44 g/L), as compared to other media including reference LB medium, whereas the lowest biomass of 1.58±0.17 g/L was produced by LB-1X(BOD).

- LB-1X medium produced the highest amount of Cry1Ac toxin (108.88 ng/mg), whereas the highest amount of Cry2Ab toxin (4235 ng/mg) was recovered in LB-2X medium.

- The highest CFU count and spore count were recorded in Medium III 84.67±0.88 × 10^8 CFU/mL and 87.33±0.17 × 10^8 spore/mL, respectively.

- In comparison of 11 different media, Medium II consisting of 2% wheat flour, 2% soybean meal and 1% Wesson salt could be as good alternative to LB medium. Medium II caused the highest mortality (63.3%) after one day of the treatment as compared to other treatments of other media against of *H. armigera*. In case of *S. litura*, Media II and III caused 100% highest mortality than the reference toxin Cry2Ab2 which, caused mortality of 60%, each at 10 µg/g concentration, after 7 days treatment.

- Hence, isolate AUG-5 could be further developed as biopesticide.