Conclusion
Sodium acetate selection and dry heat treatment methods were found inefficient for isolation of Bt which could be due to low density of Bt spores in soil. Bt enrichment method which allowed propagation of both spores and vegetative cells of Bt was successful for isolation from these samples. Growth kinetics and spiking study supported enrichment of Bt during isolation. Different strains of Bt can be isolated by the enrichment method. Bt isolation can be done from the samples with Bt spores as low as 100 spores per gram of soil. From the results of Bt isolation, spiking study and vegetative whole cell protein profile it can be concluded that Bt enrichment method is more efficient.

Bt was found to be present in all regions of India with different soil types and climatic conditions, and diverse environments of phylloplane and sewage. Bt isolation from river sedimentary soil samples suggested Bt spores to be present in soil since long period of time and ability to survive in soil. Native Bt isolates could have novel cry genes and toxicity as indicated by novel crystal inclusions and serotypes. A great of diversity of Bt isolates in India can be concluded from diverse protein profiles observed during SDS-PAGE analysis. Each region of the country has unique Bt strains with different combinations of Cry toxins. Isolates from phylloplane and soil sample from same area showed different protein profiles and thus soil could not be the source of origin for isolates in phylloplane environment. Detection of several novel serovars indicated diversity of Bt serovars remains to be explored. Isolation from more samples needs to be carried out to obtain isolates with higher toxicity to S. littoralis, at the same time bioassay with other insects should be done to explore toxicity potential of the native isolates.
Soil type can be concluded to affect distribution of cry genes to some extent. Presence of unique cry genes and cry gene profiles in different regions indicated geographical distribution of cry genes and Bt strains. Though presence of specific cry genes could be geographically related, diversity of cry genes may not be affected since rarefaction analysis represented similar degree of cry gene diversity. Since no major difference in the distribution and diversity of cry genes in isolates from agricultural and non agricultural samples was observed, it can be concluded that the insecticidal activity may not be an important factor for selection of Bt and some other factors might be involved for prevalence of Bt in soil.

Native Bt isolates harbored single to multiple cryIA genes. Isolates from different regions have unique combination of cryIA genes. Isolates from Indian regions have higher frequency of cryIAc genes as revealed by RFLP analysis and amplification with gene specific primers. In case of cry2 genes, RFLP analysis showed high frequency of an unknown gene which had 95% similarity to cry2Ab and cry2Ah genes. Thus it could be a variant of these genes or a novel cry2 gene. The cry2 genes are less divergent than cryI genes, since only five patterns were observed compared to 14 patterns by cryIA genes. Degenerate primers designed in present investigation can be useful for RFLP analysis as well as cloning of novel cryI genes. Moreover RFLP analysis with just one enzyme could be sufficient to determine cryI gene diversity due to full length amplification of genes.

Conserved blocks 2 and 3 were found to have sufficient homology to design primers for recombination of various cryI genes. The second approach with 233 bp of overlap sequence yielded higher amount of assembled products. Thus length of overlap region affects the recombination. As a result higher length of overlap sequence was more preferable in case of larger size for recombination. However the first approach with 18 bp
overlap sequence can also be employed successfully. The cry1 genes can be easily recombined by ssOE-PCR technique to construct various hybrid toxins. Compared to traditional OE-PCR, the ssOE-PCR technique was found more efficient for recombination of larger size of fragments and cloning of recombinant products.

Replacement of Domain III has diverse effects in different toxins. In case of Cry1Ac it has important role in toxicity since hybrid toxin Cry1AcAcEa exhibited lower toxicity compared to native toxin. Similarly hybrid toxin Cry1AcAbAb showed lower toxicity which could be due to important role of domain I of Cry1Ac toxin in pore formation. Similar results were obtained in other studies involving exchange of domain I. Thus it can be concluded that increase in activity could be difficult by exchange of domain I. Domain swapping with other toxins needs to be performed to achieve desired change in toxicity.

The in silico methods of structure prediction were found useful for structural characterization of Cry1 proteins. Structural alignment studies with Cry1 and Cry3 toxins supported the role of loop regions of domain II in specificity determination. Loop regions showing topological differences can be selected for mutagenesis studies to change specificity or improve toxicity.