SUMMARY

Bacillus thuringiensis is a ubiquitous, gram-positive and spore-forming bacterium. During sporulation, it produces intracellular crystal proteins (cry proteins), which are toxic to insects. Because of its insecticidal activity, it has been used for nearly fifty years to control certain insect species among the orders Lepidoptera, Coloeptera and Diptera. However, it is still necessary to search for more toxins to control other insect orders and to provide alternatives for coping with the problem of insect resistance. The genetic diversity of *B. thuringiensis* strains shows differences according to the regions where they were isolated. Thus each habitat may contain novel *B. thuringiensis* strains, which have some toxic effects on target spectra of insects.

The aim of this study was to isolate *B. thuringiensis* strains from soil environment and to identify the crystalline protein gene of the isolates. Twenty five samples were collected from different ecological environments of Lahore under sterile conditions.

Three approaches were applied for the isolation of *B. thuringiensis*: sodium acetate selection, heat treatment and end spore and crystal staining. Polymerase Chain Reaction (PCR) method was used for the characterization of *cry* gene of *B. thuringiensis* strains. The universal primer specific for *B. thuringiensis* were used. In addition, 16S rDNA gene based PCR-restriction fragment length polymorphism (RFLP) was carried out to confirm *B. thuringiensis* strains.

It was found that thirty isolates showed *B. thuringiensis* like colony morphology and subterminal endospore position. Twenty isolated bacterial cultures were gram positive bacilli. Three were cocci in shape. Two bacterial culture lost the colour of stain

after decolorization with alcohol. So, these two strains were gram negative. Fifteen isolates were screened on the basis of the test results, identify as *B. cereus* and *B. thuringiensis*. Those isolates which were actively motile and strongly hemolytic were selected as *B.t.* isolates.

Therefore, the final list of *B.t.* isolates was seven approx, which were identified on the basis of staining and biochemical tests and were further characterized for their toxicity against larvae of mosquito, *Adees aegypti*. Genomic DNA was isolated and a 650bp DNA fragment of *cry11* gene was amplified by PCR.

The toxicity bioassays with *B.t.* spores showed that six *B.t.* isolates harboring *cry11* genes (*viz.,* NF1*B.t.*,2,3,4,5,6,7) were most toxic to 3rd instar larvae of mosquito, *Adees aegypti*. Among seven *B.t.* isolates, NF5 *B.t.* 7.2, NF1*B.t.* 1.1 and NF2*B.t.* 4.2 were found the most toxic and were isolated from moist soil containing decaying cattle waste, dry waste animal dung and leaf liter soil.

The 16S rDNA study revealed that these isolates showed 99% homology with *B.t.* serovar *tolworthi*, *B.t.* str. *Al Hakam*, *B.t.* serover *thuringiensis*, *B.t.* serovar *konkukian*, and *B.t.* serovar *Chinensis*. *B.t.* serover *Indiana*, *B.t.* serover *kurstuki*. So, these isolates have a great potential to grow into a biopesticidal formulation for control of mosquitoes.