IDENTIFICATION OF cry2A GENE IN NATIVES STRAINS OF Bacillus thuringiensis ISOLATED SOILS OF MISIONES PROVINCE

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Vector and pest control in the regional agriculture has been characterized by the intensive use of chemical pesticides. The irrational and massive use of these products has given rise to major problems mainly in the environmental and phytosanitary areas. Therefore, there is a need for safe and effective pest control tools that are alternatives to chemical insecticides. Bacillus thuringiensis (Bt) is a cosmopolitan microorganism producing a parasporal inclusion containing the Cry and Cyt proteins. Until today 166 cry genes have been cloned and sequenced. Cry proteins encoded by the cry genes are the main virulence factor of Bt. In the presence of a reducing environment these proteins modify their structure becoming highly toxic against insects of various orders, particularly Cry2 proteins, encoded by the cry2 gene, which are toxic to Leptidoptera and Dipterans orders. The objective of this work was isolate and identify native Bt strains from soil samples from the province of Misiones for the subsequent identification of the cry2A gene. A total of 37 soil samples were processed from cultivated areas of the Guaraní town, province of Misiones. Morphological identification of bacterial colonies and microscopic observation were performed by selecting those strains that included the inclusion of a birefringent parasporal crystal. The molecular identification was carried out by amplification of the 16S rDNA gene. PCR amplification products were evaluated on 2% agarose gel; sequenced using the Macrogen Korea service and subsequently analyzed with the Geneius 8 program. The sequences were contrasted against the NCBI database using the BLASTn tool. Five strains were identified as Bacillus thuringiensis. The cry2A gene was investigated by conventional PCR technique. The presence of the cry2A gene was detected in 100% of the Bt strains studied. Our results indicate that local agricultural soils are an excellent source for the isolation of native Bt strains carrying the cry2A gene. This allows the advance in the development of a local bioinsecticide from native strains of Bt avoiding the incorporation to our ecosystem of exogenous microorganisms with influences less known for the region and as an alternative use of the chemical insecticides.

Keywords: Bacillus thuringiensis, cry2A gene, Cry proteins, Bioinsecticide.

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.