Characterization of a *Bacillus thuringiensis* subsp. *kurstaki* strain isolated from *Malacosoma neustria* L. (Lepidoptera: Lasiocampidae)

Hatice Kati, Kazım Sezen, Ali Osman Belduz & Zihni Demirbag*

Karadeniz Technical University, Faculty of Arts and Sciences, Department of Biology, TR-61080 Trabzon, Turkey; fax: ++ 90 462 325 3195, e-mail: zihni@ktu.edu.tr

Abstract: A *Bacillus thuringiensis* strain (MnD) was isolated from *Malacosoma neustria* L (Lasiocampidae, Lepidoptera) insect. This isolate was compared with the reference strains, by means of electron microscopy, SDS-PAGE analysis, plasmid pattern, *cry* gene content, and insecticidal activity. Isolate MnD produced bipyramidal and cubical-shaped parasporal inclusions. The presence of Cry1 and Cry2 proteins in this isolate was confirmed by observation of 130 and 65 kDa proteins by SDS-PAGE. Plasmid pattern of MnD showed similar bands to those of the reference strain, *B. thuringiensis* subsp. *kurstaki* HD-1. The only noticeable difference was the lack of two large plasmids (3.5 and 2.9 kbp). PCR analysis showed that the strain has two different *cry* genes, *cry1* and *cry2*. Toxicity tests were performed against seven insect species from Lepidoptera, Coleoptera and Diptera groups. Crystal-spore suspensions showed toxicity only against species of Lepidoptera (*Malacosoma neustria*, *Lymantria dispar* and *Hyphantria cunea*). This is the first study showing the significance future of a *B. thuringiensis* isolate from *M. neustria*.

Key words: *Bacillus thuringiensis*, *cry* gene, insecticidal activity, pest control, Cry protein.

Introduction

Bacillus thuringiensis strains are strongly recommended against agricultural pests in biological control. *B. thuringiensis* is a gram positive, aerobic, endospore-forming bacterium. The organism was first isolated from diseased silkworm (*Bombyx mori*) in 1901 (Ishiwata, 1901). It is naturally present in both live and dead insects (Damgaard et al., 1997). It has also been frequently isolated from many different natural environments, including soil (Martin & Travers, 1989), aquatic environments (Thanabalu et al., 1992), plants (Smith & Couche, 1991; Damgaard et al., 1997), and animal faeces (Bernhard et al., 1997).

B. thuringiensis is characterized by the production of parasporal crystals composed of protein molecules known as δ-endotoxins, insecticidal crystals proteins (ICPs) or crystal proteins (Cry proteins) that are toxic against the larvae of various insects (Höfte & Whiteley, 1989). ICPs produced during the sporulation of *B. thuringiensis* strains show specific insecticidal activity against insect species of different orders, such as Lepidoptera, Diptera, Coleoptera, Hymoneptera, Homoptera, Orthoptera and Mallophaga (Höfte & Whiteley, 1989; Feitelson et al., 1992; Becker & Margalit, 1993).

The search for new *cry* genes is on-going effort worldwide. So far 303 *cry* genes have been reported and classified into *cry1* to *cry46* according to the degree of amino acid sequence homology (for a full list, see the URL at: http://www.biols.susx.ac.uk/home/Neilcrickmore/Bt/). Cry proteins are synthesized as protoxins, which are then converted to toxin proteins by proteases in the insect midgut (Knowles, 1994). Crystal protein genes are located as satellite chromosome and/or chromosomal DNA (Kronstad et al., 1983). There is significant evidence indicating the involvement of specific plasmids in the production of crystals (Stahly et al., 1978; Gonzalez et al., 1981, Ozawa & Iwahana, 1986; Donovan et al., 1988). Crystal genes, in many cases, are found to be located on large plasmids, whereas small plasmids rarely harbor crystal genes (Honigman et al., 1986).

Isolation and characterization of *B. thuringiensis* from insects is important to evaluate relevant biological control agents against pest insects. *Malacosoma neustria* (Lepidoptera, Lasiocampidae) is one of the most important agricultural pests in Turkey. It forms various damages on many fruits and forest trees, especially apple, pear, cherry, hazelnut and oak. Chemical pesticides are currently utilized against *M. neustria*. It is crucial to develop an environmentally safer and more

* Corresponding author