Ozone and N-methyl-N'-nitro-N-nitrosoguanidine-induced sporless mutants in *Bacillus thuringiensis*

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Ahmed Farag[*] and Nariman, A. H. Aly[**]

[*] Département de Sciences Biologiques, Université de Montréal C.P. 6128, Sucursale (A), Montréal, Québec H3C 3J7, Canada.

[**] Microbial Genetics Department, National Research Centre, Cairo, Egypt.

ABSTRACT

Two sporless mutant strains (Spo$^-$ Cry$^+$) were derived from Bacillus thuringiensis var Kurstaki HD-1 after treatment with MNNG and ozone. The wild type strain of B. thuringiensis revealed dramatic decrease in the survival percentage after 1 hr exposure to MNNG at a dose of 100μg/ml. The MNNG-induced mutants produced three distinctly different crystal sizes. Similar results were obtained when B. thuringiensis was exposed to 50 ppm of ozone concentration for 15 min intervals until one hour. The ozone-induced mutants produced bipyramidal crystalline bodies similar to the parental strain with more toxic potentiality. Moreover, all the isolated mutant strains were characterized by lacking the ability to produce spores. Two proteins of molecular weights 130 and 66 kD were identified by SDS-PAGE, after the cellular lysis and crystals purification of B. thuringiensis wild type and its mutants Spo$^-$ Cry$^+$. The two proteins were observed in the two mutant sporless strains with no differences between the mutants obtained from MNNG and OZ treatments.

Key words: *Bacillus thuringiensis, Sporless mutants, MNNG, Ozone, Crystalline proteins, SDS-PAGE*

INTRODUCTION

Bacillus thuringiensis (B.t.) is a naturally occurring soil-borne organism that has recently gained popularity for its ability to control certain insect pests (Schnepf *et al.*, 1998). It is characterized by producing one or more crystalline proteins during the cycle of sporulation. This crystal protein is the toxic component of B.t., called the δ-endotoxin. The toxin binds to the larvae cells lining the midgut membrane and creates pores in it and then, insects stop feeding and starve to death (Gill *et al.*, 1992).

The increasingly rapid characterization of new crystal protein genes, triggered by an effort to discover proteins with new pesticidal properties, has resulted in a variety of sequences and activities that no longer fit the original nomenclature system (Crickmore *et al.*, 1998). Many proteins with different molecular weights were isolated from *B. thuringiensis* such as 130 kD which is a toxic protein for lepidopetra isolated from *B. thuringiensis*, krustaki and *B. thuringiensis* spotto. This protein degrades to sub-protein units of 66-68 kD that are toxic for lepidopetra and diptera (Donovan *et al.*, 1988). Moreover, two toxic proteins for diptera were isolated