ABSTRACT

A Study on the Isolation of *Bacillus thuringiensis* from the Environment: A Critical Look at Library Construction

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Different delta endotoxins produced by the bacterium *Bacillus thuringiensis* are known for both their high specificity and toxicity for a wide range of pests and are therefore of great economic importance as pesticidal agents. For this reason a global effort has been made to isolate new or improved strains of *B. thuringiensis*. However, current methodologies use techniques which are either selective or potentially restrictive on the type of *B. thuringiensis* which can be isolated. Because of the large cost (both in time and money) associated with the construction of *B. thuringiensis* libraries and their evaluation, a restrictive methodology at the critically early stage of *B. thuringiensis* isolation could result in a defective library incapable of yielding useful or novel strains. The work described herein sought to explore this subject by performing a screen that addressed these potentially flawed practices. A novel, high throughput method that minimized selective culture and which used a novel staining technique instead of Phase Contrast microscopy was used. The resulting library was characterized by spore and crystal morphology, SDS-PAGE protein analysis, PCR and probe hybridization. Although the study utilized a relatively small screen, a

novel, filamentous isolate, a isolate containing a rare nematocidal gene and an isolate possessing a very small parasporal-like body similar to a rarely reported and recently described spiked spore-structure of a non-*B. thuringiensis* strain were found. Besides isolating novel or rare bacteria, the screen as a whole was atypical due to the extremely low number of *cry1* isolates identified, again suggesting a departure from normal screen results. These results suggest that the new screening methodology might deliver a vastly richer and potentially more useful library of *B. thuringiensis* isolates as compared to that obtained with current techniques and that by extension, methodologies fundamentally different from current methods should generally be explored.

Keywords: *Bacillus thuringiensis*; parasporal body, novel, PCR, Hybridization, Bt, crystal.