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RESEARCH ARTICLE

# Characterization of Cry toxins from autochthonous *Bacillus thuringiensis* isolates from Mexico

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Toxin;
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*Diatraea considerata*;
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**Abstract**
*Background:* Chemical pesticides, widely used in agriculture and vector-borne disease control, have shown toxic effects on the environment and the people in contact with them. *Bacillus thuringiensis* is a widely used bacterium for alternative and safer control of insect pests. Its toxins are specific for insects but innocuous for mammals and may be used as powerful adjuvants when applied with vaccines. The objective of this work was to characterize some autochthonous *B. thuringiensis* strains, which could be used for the control of a local pest (*Diatraea considerata* Heinrich) that affects sugar cane crops in Sinaloa, Mexico. Also, to evaluate these strains as a source of Cry toxins, which may be used in the future as adjuvants for some vaccines.
*Methods:* Eight strains from field-collected dead insects were isolated. These were microbiologically identified as *B. thuringiensis* and confirmed by amplification and sequencing of 16S rDNA. Bioassays were performed to evaluate their pathogenicity against *D. considerata*, and Cry toxins were identified by proteomic analyses.
*Results:* An increased mortality among larvae infected with strain Bt-D was observed, and its toxin was identified as Cry1Ac.
*Conclusions:* The observed data showed that the selected strain was pathogenic to *D. considerata* and seemed to produce Cry1Ac protein, which has been reported as an adjuvant in different types of immunization.


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**PALABRAS CLAVE**
Toxina;
Proteómica;
*Diatraea considerata*;
*Bacillus thuringiensis*

**Caracterización de toxinas Cry de aislados de *Bacillus thuringiensis* autóctonos de México**

**Resumen**
*Introducción:* Los pesticidas químicos, ampliamente usados en agricultura y en el control de vectores transmisores de enfermedades, han mostrado efectos tóxicos sobre el medio ambiente y las personas expuestas a ellos. *Bacillus thuringiensis* es una bacteria ampliamente utilizada como una alternativa segura y eficaz en el control biológico de plagas agrícolas. Sus toxinas son específicas de insectos, pero inocuas para mamíferos, e incluso poseen gran potencial para ser usadas como adyuvantes en vacunas. El objetivo de este trabajo fue caracterizar cepas autóctonas de *B. thuringiensis* con efectividad contra el gusano barrenador (*Diatraea considerata* Heinrich) de la caña de azúcar en cultivos del estado de Sinaloa, México, y como fuente de proteínas Cry, con potencial de utilizarse como adyuvantes en vacunas.
*Métodos:* Se lograron aislar ocho cepas a partir de insectos muertos en campos agrícolas, las cuales fueron identificadas microbiológicamente como *B. thuringiensis*, lo que se confirmó por amplificación y secuenciación del 16S rDNA. La efectividad de los aislados para el control del gusano barrenador fue evaluada mediante bioensayos y las toxinas Cry fueron identificadas por análisis proteómico.
*Resultados:* Se observó una mortalidad elevada en las larvas infectadas con las cepas de estudio. Particularmente, la cepa Bt-D, de la cual el análisis molecular mostró que posee una toxina tipo Cry1Ac.
*Conclusiones:* Los resultados mostraron que la cepa Bt-D posee un elevado potencial patogénico hacia *D. considerata* y produce la proteína Cry1Ac, de la cual existen reportes de su aplicación como adyuvante en diferentes formas de inmunización.


## 1. Introduction

Chemical pesticides are commonly used in large-scale commercial crops and for vector-borne disease control worldwide, including Mexico, particularly in agricultural zones such as Sinaloa. Unfortunately, the use of these chemicals has been associated with several diseases, such as leukemia, diabetes and other degenerative disorders.[1] To achieve an equilibrium between social, environmental and economic interests, Integrated Pest Management indicates that one reliable option is the use biological control agents,[2,3] such as *Bacillus thuringiensis* (Bt).

Bt is a Gram-positive bacterium that develops very resistant spores during the stationary phase and simultaneously produces crystalline inclusions during sporulation. These inclusions are composed of proteins with highly specific insecticidal activity, which is why Bt has been widely used in products for the control of pests, mainly Lepidoptera, Diptera, and Coleoptera.[4] These proteins are commonly known as Cry proteins or δ-endotoxins and are classified into different families depending on their amino acid sequence.[5] There are reports that indicate that Lepidopteran insects are usually susceptible to the toxins of the Cry1 family. For example, *Diatraea grandiosella*, which affects sugar cane crops in Sinaloa, Mexico, is known to be susceptible to Cry1Ab, Cry1Ba and Cry9Ca.[6] Surprisingly, there are no reports on the use of Bt for the control of *D. considerata*.

Bt is not only a safe pest control agent, but also a source of other useful proteins and toxins (such as parasporins) that have shown cytotoxic effects on human cancer cells.[7,8] Moreover, some studies have reported that Cry toxins could be used as adjuvants in vaccines, since they enhance the immune response.[9–11] Therefore, in this work, eight regional Bt strains were isolated and characterized to evaluate their entomopathogenic effect on *D. considerata* and to identify Cry toxin subtypes.

## 2. Methods

### 2.1. Source of the strains

Reference strains (Bt kurstaki HD73 and HD1) used for this study were kindly provided by the Sciences Center of Sinaloa. Native strains were isolated from dead insects (*Spodoptera frugiperda*) collected from the fields and labeled as Bt-A, B, C, D, 5, 15, 22 and 64. Insects were macerated in 0.85% sterile saline solution and subjected to thermic shock (85 °C for 10 min and 4 °C for 2 min). Subsequently, a loopful of the solution was streaked in nutrient agar and incubated at 30 °C for 24 h. The colonies that showed typical *Bacillus* morphology (Gram-positive, opaque, whitish and irregular) were re-cultured in nutrient agar at 30 °C for 48 h. Then, bacteria were stained with Scheaffer-Fulton endospore stain and visualized in a light microscope at 100X. Cultures that showed spore-forming bacilli and parasporal crystals production were selected.