

A novel *Bacillus toyonensis* biovar Thuringiensis strain showing strong nematocidal activity

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Introduction

Bacillus thuringiensis (Bt) is a gram-positive bacterium that produces parasporal crystal proteins showing biocidal activity against different invertebrate species. In addition, other gram-positive bacteria such as *Lysinibacillus sphaericus*, *Paenibacillus* spp. and *Brevibacillus laterosporus* have been described for showing insecticidal activity. In 2022, a *Bacillus* sp. strain has been described for the first time as producing parasporal crystals with insecticidal activity and after species identification, the bacterium was designated *Bacillus toyonensis* biovar Thuringiensis.

Objective

The objective of this work was to carry out the isolation of autochthonous *Bacillus* spp. strains from the province of Córdoba (Argentina) and the characterization of their pesticidal activity.

Methods

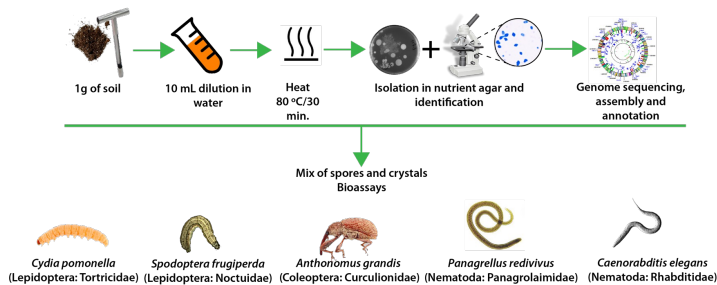


Figure 1. Schematic representation of methods for the isolation of the bacterium from soil samples from the province of Córdoba, Argentina.

Results

A strain from Río Tercero city designated Bto_100 was isolated, which produced both the typical Bt colony phenotype (flat, matte white color, dry, and uneven borders) and the crystalline inclusions under the phase contrast microscope (Figure 2).

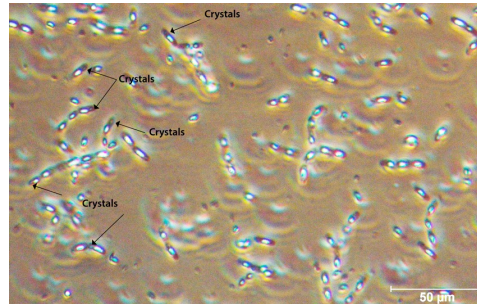


Table 2. Phase contrast micrography (1000x) of the strain showing parasporal crystals.

The draft genomic sequence of strain Bto_100 showed a total size of 6,072,356 bp in 439 contig sequences with a G+C percentage of 34.9%. The genomic sequence encoded 6362 putative gene sequences and 46 RNAs (RAST server).

Analysis of the genome sequence (Table 1) showed the presence of 5 coding sequences showing significant similarity with known pesticidal proteins from Bt. The TYGS taxonomic server (<https://tygs.dsmz.de>) identified the strain as belonging to *Bacillus toyonensis* species (Figure 3).

Bioassays performed with crystal-spore mixtures showed no activity against the insects tested but a 100% of mortality against *P. redivivus* and *C. elegans* (Figure 4).

Table 1. General features and putative pesticidal proteins found in the genome.

Genome features	Bto_UNVM-104
Genome Size (bp)	6,072,356
Contigs	439
CDs	6362
G+C%	34.9
Pesticidal proteins	5
Cry7G	1
Cry7B	1
Cry73A	1
Mpp2A	1
Xpp22A	1

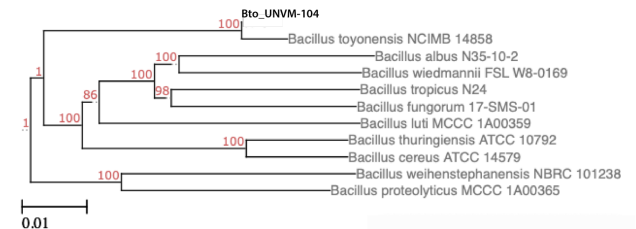


Fig. 3. Tree inferred from TYGS (Type Strain Genome Server) tool for the identification of bacterial species (data type: genome sequence).

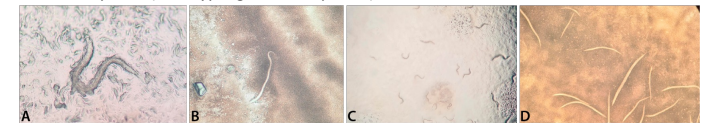


Fig. 4. Bacterial-feeding bioassays with nematodes. A) *P. redivivus* control and B) dead worm, C) *C. elegans* control and D) dead worms.

Conclusions

A novel *B. toyonensis* biovar Thuringiensis strain was isolated from Argentinian soil samples which showed the production of parasporal crystals and putative pesticidal genes into its genome. The strain was nontoxic for the insects tested but highly toxic to both nematode species tested. More research is needed in order to unveil the strain potential for the control of phytopathogenic nematodes.