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MICROBIOLOGICAL IMAGE

Argentinean *Bacillus thuringiensis* strains exhibiting distinct morphology of their parasporal crystals

Cepas argentinas de *Bacillus thuringiensis* con distinta morfología en sus cristales paraesporales

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Bacillus thuringiensis is a Gram-positive and sporulated bacterium exhibiting insecticidal activity against a wide range of insects.[3] During sporulation, this bacterium produces a number of different proteins forming crystalline inclusions adjacent to the spores (parasporal crystals). Among these insecticidal proteins, the most abundant are those commonly known as Cry (Crystal) proteins, which are responsible for exerting a toxic activity (upon ingestion) against insects of different species.[5] For this reason, *B. thuringiensis* has proved to be the most efficient and used bioinsecticide to date.[2] However, *Spodoptera cosmioides*, *Spodoptera eridania* and *Agrotis* sp. (Lepidoptera) are species that are not yet controlled by some transgenic crops (e.g. Intacta RR2Pro soybean). Thus, in an attempt to enlarge the host spectrum of this bacterium it is necessary to search for novel strains. In this work we show a sporulated *B. thuringiensis* Bt-UNVM_84 strain exhibiting a number of rare amorphous to spherical crystal combinations, whereas sporulated *B. thuringiensis* strain Bt-UNVM-94 showed quasi symmetric bipyramidal parasporal crystals, by using scanning electron microscopy (SEM) (Fig. 1). Strains Bt-UNVM_84 and Bt-UNVM_94 were isolated from Oncativo (Córdoba, Argentina) and Cululú (Santa Fe, Argentina), respectively. The insecticidal activity of these different *B. thuringiensis* strains is currently under investigation. Each strain was grown in liquid CCY sporulation medium[6] for ~48 h (150 rpm) until no vegetative cells were observed under a light microscope. The presence of parasporal crystals was first determined using Coomassie blue stained slides[1] (1000×) under a Nikon E100 light microscope and confirmed later by a Nikon *Ti*-Eclipse phase

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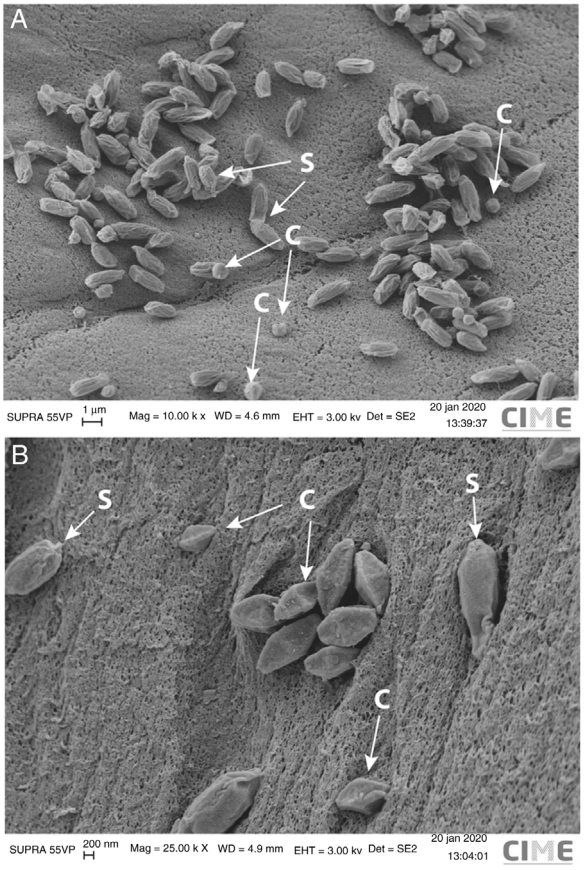

Figure 1 Scanning electron microscopy of parasporal crystals from *Bacillus thuringiensis* strains Bt-UNVM_84 and Bt-UNVM_94 (C = crystal, S = spore). (A) *B. thuringiensis* strain Bt-UNVM_84 showed combinations of amorphous to spherical parasporal crystals of ∼0.7–0.9 μm from two points along the diametral axis. (B) *B. thuringiensis* strain Bt-UNVM_94 exhibited quasi symmetric bipyramidal parasporal crystals of ∼1.0–1.2 μm from two points along the longitudinal axis. Crystal size was measured using ImageJ.[4] Parameters used for image acquisition are shown: Mag = magnification (K× = 1000×), WD = work distance, Eht = energy high tension, Det = detector type and SE2 = secondary electron.

contrast microscope (1000×) (data not shown). For the SEM analysis, aliquots of 1 ml were centrifuged for 5 minutes (16,000 *g*) at room temperature. Each pellet was washed three times with sterile distilled water and fixed with 100 μl 4% formaldehyde. Each fixed preparation was then sent to Centro Integral de Microscopía Electrónica (CIME – CONICET – UNT) for SEM examination.

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