RESEARCH NOTE

Bacillus thuringiensis subsp. *oswaldocruzi* and *Bacillus thuringiensis* subsp. *brasiliensis*, two Novel Brazilian Strains which Determine New Serotype H38 and H39, Respectively

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It is now a fact that the soil bacterium *Bacillus thuringiensis* may be present in a number of various habitats or ecosystems including phyllophane, stored products, dusts, diseased insects and tobacco residues. The objective of the present study was initially to detect and to count *B. cereus* in powdered spices *Cuminum cyminum* L. and *Piper nigrum* L. currently used as commercial preparations in Rio de Janeiro. A high level of *B. cereus* colony forming units was encountered, particularly from commercial pepper. Isolation of presumed *B. cereus* colonies was performed according to the APHA (ML Speck 1984, *Technic Compendium of Methods for Microbiological Examination of Foods*. Washington D.C., p. 701). After resuspending spice powders (1g/10 ml) in sterile phosphate buffer pH 7-2 and plating dilutions on selective medium, *B. cereus* cells were selected as egg-yolk lecithinase positive colonies, that were further reisolated and kept on soil-extract agar or as lyophilized powders.

In this study, at least 224 strains of *Bacillus* were isolated from both kind of spices, among them 192 *B. cereus* were found; 28 of these strains were identified as *B. thuringiensis* on the basis of the presence of parasporal protein inclusions, and further characterized. Most of them were shown to belong to known serotypes with the exception of isolates LFB-855, LFB-856 and LFB-869. A flagellar suspension of these strains was not agglutinated by antisera specific for the 37 subspecies known at that time. Reciprocally, antisera prepared against each of the three new isolates did not agglutinate any H-suspension from the previous subspecies, whereas they reacted with homologous suspensions to give high titers (25 600). On this basis (H de Barjac 1981. In HD Burges p. 35-43 *Microbial Control of Pests and Plant Diseases*, 1970-1980), LFB-855 and LFB-856 were found to share a common novel antigen we designated as H38, with the subspecies name *oswaldocruzi* whereas LFB-869 was shown to display another new antigen determining H39 serotype to which was assigned the subspecies name *brasiliensis*.

Both subspecies were analyzed for morphological and biochemical charaters. Cultures of the new isolates displayed rod-shaped cylindrical cells with the usual dimensions for *B. thuringiensis* species (PHA Sneath 1986. In *Bergey's Manual of Systematic Bacteriology*, vol 2, p. 1108) and they produced ovoid, non deforming and subterminal spores. For strains LFB-855 and LFB- 856, parasporal inclusions appeared ovoid or rhomboidal-shaped, heterogeneous in size with diameters ranging 0.5 to 1 µm, some of them looking attached to the exosporium. For strain LFB-869, crystals appeared diamond-shaped, with size ranging 0.6-0.8 µm, a few of them that appeared larger (1 to 3 µm) were bipyramidal-shaped.

Like the other *B. thuringiensis* subspecies the new strains fall into a class of bacteria considered as aerobe or facultative anaerobe (AFA), as shown using selective anaerobic media without nitrate. The biochemical characters according to Sneath (*loc. cit.* p. 1102-1139) are summarized in Table. LFB-855 and LFB-856 (H38) appeared quite similar, while LFB-869 (H39) displayed same differences regarding VP reaction, sucrose utilization or salicin degradation.

SDS-polyacrilamide gel electrophoresis of parasporal crystals is shown in Fig. The new subspecies differ in their protein pattern: for strain LFB-855, subsp. *oswaldocruzi* a doublet band at

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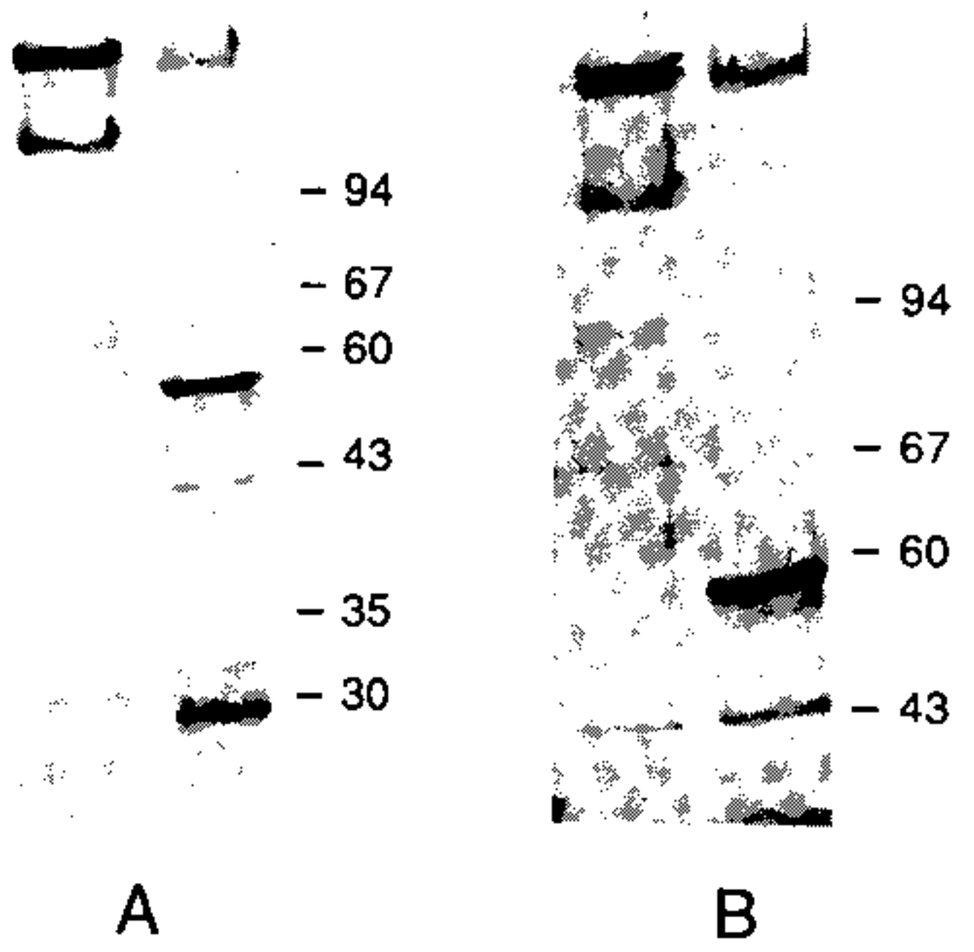
TABLE

Biochemical characters of the new serotypes H38 (LFB-855 and LFB-856) and H39 (LFB-869)

	LFB FIOCRUZ 855	LFB FIOCRUZ 856	LFB FIOCRUZ 869
Nitrate reduction	+	+	+
VP reaction	+	+	-
Anaerobic growth	AFA[a]	AFA	AFA
Raffinose	-	-	+
Mannitol	-	-	-
Salicin	-	-	+/-[b]
Sucrose	-	-	+
Mobility	+	+	+
Starch hydrolysis	+	+	+
Lecithinase	+	+	+

[a]: aerobe, facultative anaerobe
[b]: depending on the medium for reaction, or on the techniques in use, salicin may appear as + in most cases



SDS-PAGE analysis of parasporal crystals from strains LFB-855 (H38) and LFB-869 (H39). A and B: gels 12.5% and 10.5% respectively with regard to acrylamide concentration. Lane 1: *Bacillus thuringiensis* subsp. *oswaldocruzi* (H38). Lane 2 : *Bacillus thuringiensis* subsp. *brasiliensis* (H39)

140-145 kDa is seen, with a minor component around 100 kDa. For strain LFB-869, subsp. *brasiliensis,* two major components at 57 and 28 kDa are seen; in addition, two minor bands at 135 and 46 kDa are also present. To our knowledge such a pattern has not yet been reported. Relationships between the major components and the minor ones were not determined.

The strains were tested against three mosquito species, using the technique of H de Barjac and I Thiéry (1979 WHO/VBC/79, 744). No significant activity was found against the three dipteran species: *Aedes aegypti*, *Culex pipiens* and *Anopheles stephensi.* The strains appeared also inactive towards the lepidoptera: *Spodoptera littoralis.* It would be necessary to test against a wide variety of insects before concluding that the new subspecies have no insecticidal activity at all.

The results presented in this note as well as the origin of the strains confirm the world-wide occurrence of *B. thuringiensis* and the diversity of the protein components in parasporal crystals for which specific target insects remain to be found.

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