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Traceability of potential enterotoxigenic *Bacillus cereus* from bee-pollen samples from Argentina at different sampling throughout the production process

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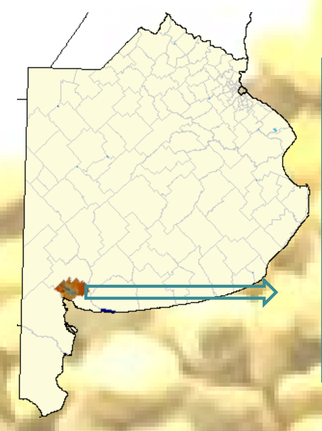
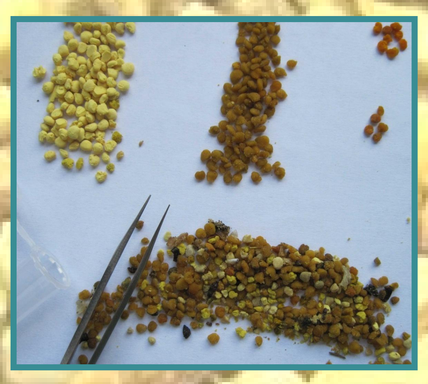
Materials and Methods



Beekeepers from South West of Buenos Aires Province, Argentina

1. Bee-pollen from hives collected by using pollen traps
2. Freezing (-10 °C for two months)
3. Dehydration (40°C for 24 h)
4. Cleaning, and packaging

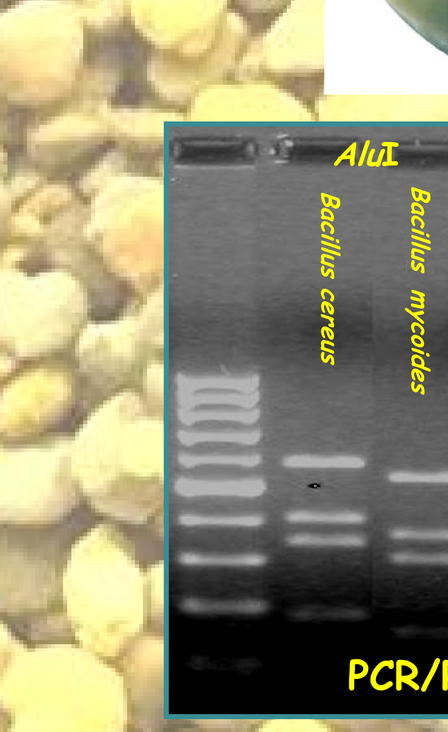


Thirty-six bee-pollen samples from three beekeepers of SW area were analyzed by testing three samples from each beekeeper at each sampling point (1), (2), (3), and (4).

Results and conclusions

Colony counts on PEMBA revealed that *B. cereus* incidence increased significantly from collection to dehydration and slightly decreased at the final step of cleaning.
A total of 47 isolates of *B. cereus* yielded 24 different rep-fingerprint patterns by using BOX and ERIC primers.
A positive correlation was observed between rep-fingerprint patterns and the enterotoxigenic profiles obtained.
Also, cross-contamination occurred as shown by differences in fingerprint patterns after freezing, dehydration and cleaning steps compared to the initial collection step.

Presence of sequences associated with virulence genes by PCR. Genes encoding for hemolysin BL (*hblA*, *hblB*, *hblC*, *hblD*), enterotoxin-T (*bce*T), cytotoxin K (*cyt*K), non-hemolytic complex (*nheA*, *nheB*, *nheC*), sphingomyelinase (*sph*) and cereulide (*ces*).



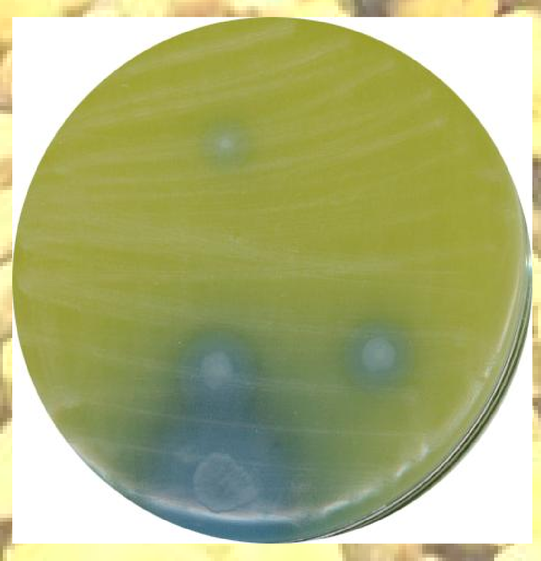
Introduction

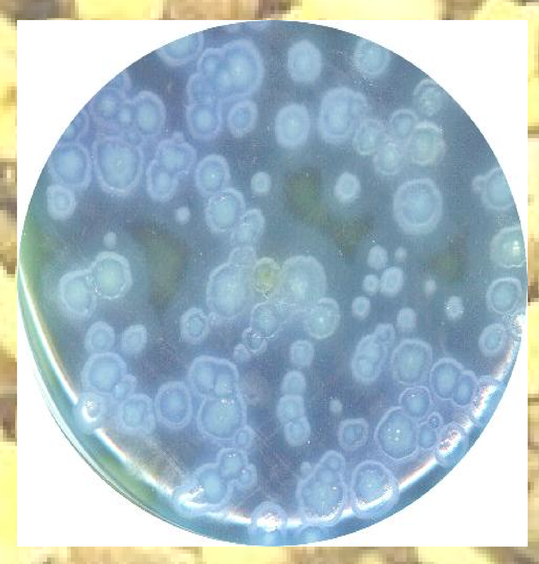
Bee-pollen is the result of the agglutination of pollen grains collected from flowers and mixed with nectar and salivary secretions by honeybees. Bee-pollen is a functional food sold for human and animal consumption but also is a favorable microhabitat for many spore-forming bacteria. Among them, *Bacillus cereus* is a ubiquitous Gram-positive spore-forming bacterium found in soil, plants, and enteric tracts of insects; these niches include honey and pollen. *B. cereus* can produce several toxins and other virulence factors causing an emetic or diarrheal syndrome after ingestion.

Aims

This work aimed to study the traceability of potential enterotoxic *Bacillus cereus* based on colony counts, rep-fingerprinting and toxigenic profiles at four sampling points of the production process.

Isolations in PEMBA





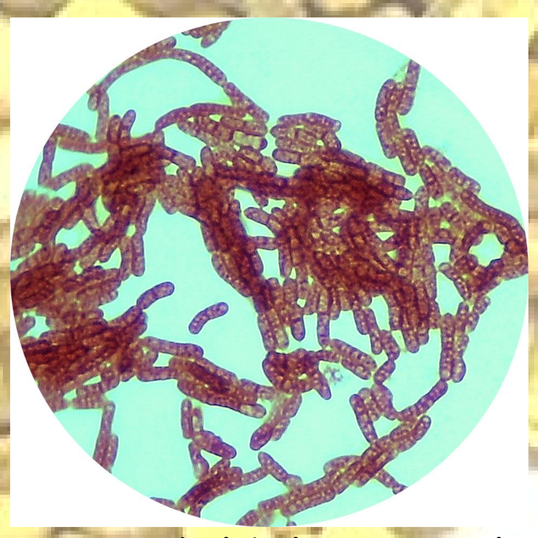
Bacillus cereus sensu stricto:

(1) N = 8 isolates

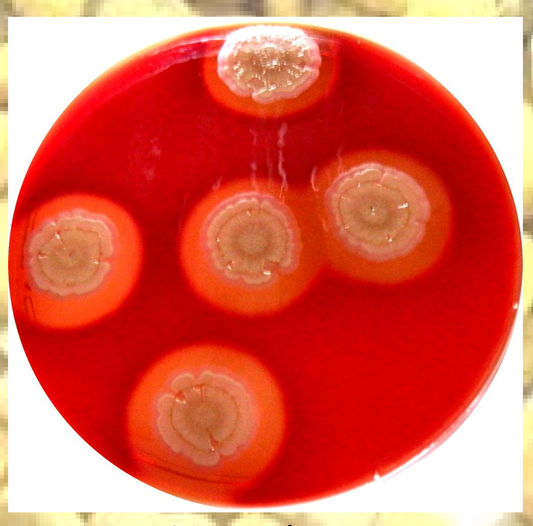
(2) N = 12 isolates

(3) N = 14 isolates

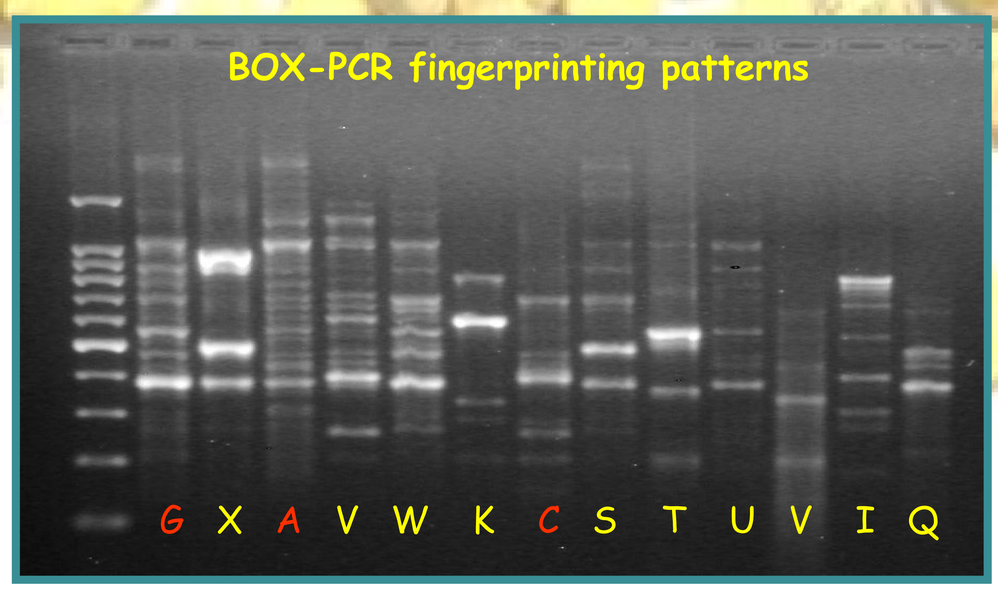
(4) N = 13 isolates



Unstained globules in cytoplam



Haemolysis



Thirteen rep-PCR patterns were obtained at the last sampling point (4) (Cleaning and packaging). Only 3 patterns (A, C, and G) were maintained throughout the production process.