



Update on Diagnostic Methods for Differentiating *Bacillus thuringiensis* from *B. cereus sensu lato* in Food

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Creative Hybrid Chemistry

Background – *B. thuringiensis* and *B. cereus* Look the Same in Standard Food Safety Tests

B. thuringiensis
type strain HD-1

B. cereus type
strain 14579



Example of presumptive Bc test results

 **MetropoliLab**

TESTAUSSELOSTE 2012-18336
Elintarvike

1(1)
31.10.2012

Ottopiste Caternet Finland Oy
Näytteen ottaja Tilaaajan toimesta

Analyysi	Menetelmä	18336-1 Miniluumutomaatti	Yksikkö
Bacillus cereus -ryhmä	* NMKL 67:2010	53 000	pmy/g

*=näyte tutkittu akkreditoidulla menetelmällä



Exceeds local threshold of 10,000 CFU/g

The Overlap of Biopesticides with the Food Safety Industry in a Different than Traditional Chemistries

EFSA Risk Assessment, SCOPAFF
EC No 1107/2009, EFSA residue limits
Country level pesticide regulations/programs
Usage decision made by growers

EU General Food Law, EC No 178/2002
Academic and Government Food Safety Research
Food Testing Laboratories (E.g. Eurofins and Biomerieux)
Safety decisions made by processors and food companies

In Food Safety the Primary Concerns are Consumer Safety and Mitigating Litigation

German Court: Compensate Cucumber Grower Wrongly Accused in E. Coli Outbreak

By **News Desk** on October 27, 2015



Research Agencies Methods Under Development for Public Health Determination

- **One Health European Joint Program**
 - 41 research institutes and 19 MS
- **ANSES ToxDetect**
 - Development and Harmonization of Innovative Methods for the Comprehensive Analysis of Food-Borne Toxigenic Bacteria, ie Staphylococci, *Bacillus cereus* and *Clostridium perfringens*
- **Proposed systematic method for Bc differentiation and identification:**
 - Strain isolation
 - PCR detection of toxin genes
 - panC-typing
 - Molecular typing of strain diversity in food remnants
 - ISO7932 differentiation method for identifying Bt



One Health illustrates the interconnectedness and interdependence of human, animal, and ecosystem health.

COST Action 16110 – Control of Human Pathogenic Micro-organisms in Plant Production Systems

- **HUPLANT Control** – “to promote open communication on science, agricultural practice and behaviour of food borne pathogens in plant microbiomes”
- **HUPLANT (COST ACTION 16110) TRAINING SCHOOL – Bacillus: identification, phylogeny and potential pathogenicity**
 - January 28 to February 1, 2019, Aarhus University, Roskilde, Denmark
 - Identification and quantification of *B. cereus sensu lato* bacteria in environmental samples.
 - Identification and phylogeny of *B. cereus sensu lato* at species level and at level of phylogenetic groups.
 - The pathogenic potential of *B. cereus sensu lato* bacteria from environmental samples.
- **IBMA to support to research project with Wageningen Plant Research:**
 - Discrimination between *Bacillus thuringiensis* biocontrol strains and *Bacillus cereus sensu lato* strains in food products derived from plants.

New Method Under Development Based on a Technology Accessible to Food Industry

- **BtID Project: Identify, trace & mitigate Bt based bioinsecticide contamination from farm to fork**
 - ADRIA Food Technology Institute and ITAB French Research Institute of Organic Farming
- **MALDI Platform** – Common in clinical labs (thousands of units across EU), but quickly expanding into food safety industry. Reasonable cost, fast results.
- **Bacillus differentiation platform**
 - Strains characterized based on their MALDI-ToF spectrum (fingerprint)
 - Strains characterized based on broad range of cytotoxicity tests, molecular markers and physiological attributes to categorize likely risk
 - Platform would not be strain specific, but attribute likely risk level

 **UMT**
SPORE-RISK
RISQUES ASSOCIÉS AUX
BACTÉRIES SPORULÉES



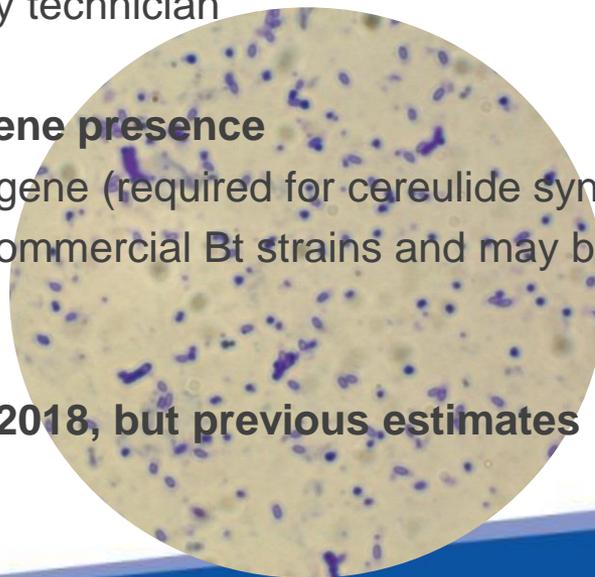
Bruker MALDI-ToF Biotyper

ISO 18465:2017 – Quantitative determination of emetic toxin (cereulide) using LC-MS/MS

- ISO 18465:2017 describes the quantitative analysis of the emetic toxin cereulide using high performance liquid chromatography (HPLC) or ultra performance liquid chromatography (UHPLC) connected to a tandem mass spectrometer (LC-MS/MS).
- **Cereulide** – heat stable toxin which can survive in food after its produced; causes vomiting (emetic)
 - Food poisoning strains are more likely to be emetic than environmental Bc strains
 - Methods to measure cereulide have been used in the industry to evaluate Bc contamination in food or factories
- **Not an alternative to Bt identification**
 - High cost (\$200 or more per sample), but high throughout
 - No tolerance set for Cereulide in food, therefore positive result triggers recall
 - Availability may be limited

ISO7932:2004 Amendments Still Pending Publication

- **Positive visual identification of Bt crystals**
 - 5 colonies isolated from presumptive Bc test plated on a sporulation medium and incubated up to 120 hrs. Crystals are identified with a blue dye and the relative number of Bt-positive colonies is used as semi-quantitative measure (e.g. 5 of 5 will be considered 100% Bt)
 - Issues
 - Long incubation time is not in line with shelf-life of perishable vegetables
 - Interpretation can vary by technician
- **Further analysis of toxin gene presence**
 - PCR for toxin genes - *ces* gene (required for cereulide synthesis) and CytK1 gene
 - Neither target is found in commercial Bt strains and may be a faster alternative to the crystal method
- **Publication is expected in 2018, but previous estimates have already passed.**



Trends in Food Safety that May Effect Biocontrol

- The food safety industry may not have a clear understanding of crop production, especially strategies for crop protection and trends towards the introduction of biologicals.
- Modern detection methods are moving towards better resolution which will invariably uncover more microbes which will need to be addressed.
 - WGS as a food safety tool is still in its infancy, but will uncover more microbes than ever and possibly open more species to greater scrutiny.
- Host-microbe interaction in the rhizosphere is valuable for agriculture, but recent research has focused on the potential for internalization of food borne pathogens which may lead to unexpected overlap.

Successful Formulation and Application of Plant Growth-Promoting *Kosakonia radicincitans* in Maize Cultivation

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