

# UMT ACTIA 19.03 ALTER'IX

## A JOINT TECHNOLOGICAL UNIT ON FOOD SPOILAGE DUE TO SPOREFORMERS & MOLDS



*The University Lab on Sporeformers & Moulds*



*The Food Technology Institute on Food Safety & Quality*



## The Bt/Bc Challenge in the Food Lab :

- how to identify, distinguish & trace these strains -

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# Outline

01

Introduction to the industrial issue

02

Diversity of *B. cereus* Group

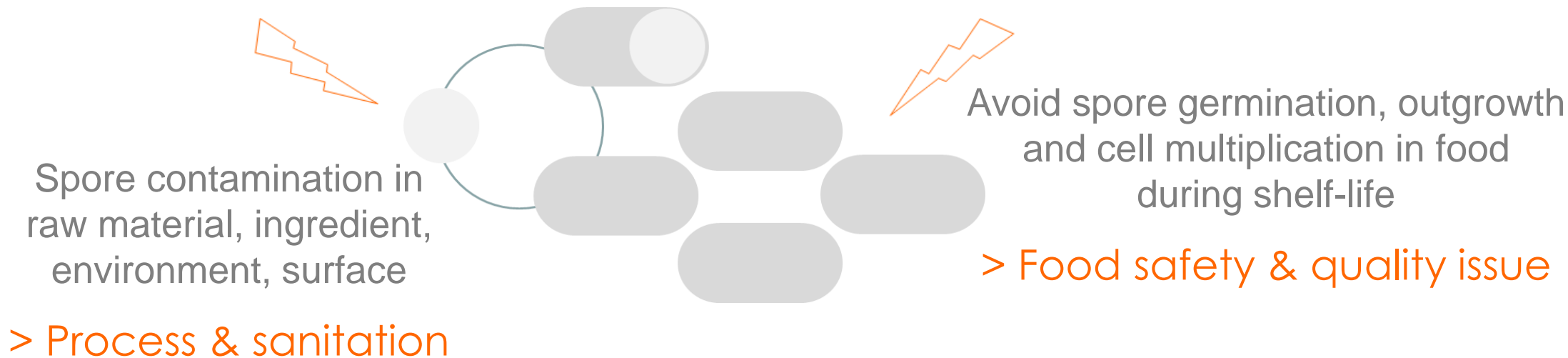
03

How to differentiate & trace isolates

# 01

## *Bacillus species*

- ❑ Sporeforming bacteria are ubiquitous, exhibit a wide range of diversity and are characterised by a complex life cycle

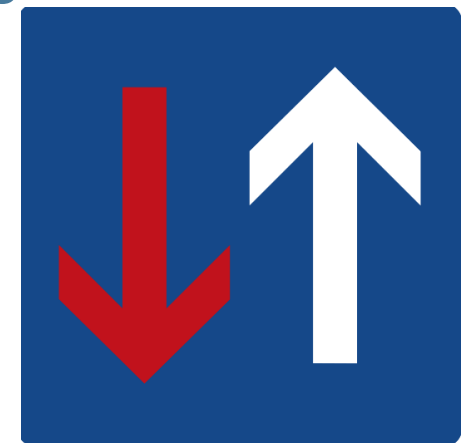


➡ Persistence of sporeformers in food industrial sites & huge economical losses

## Context (food safety)

- ❑ In Europe, a **process hygiene criteria** is established for *B. cereus* in dried infant formula ( $n=5$ ,  $c=1$ ,  $m=50\text{cfu/g}$ ,  $M=500\text{cfu/g}$ )
- ❑ Applied failing level varies between 2-5 log cfu/g *B. cereus* in food worldwide and **food industrials have lower specifications**
- ❑ **Even though under-estimated**, reported **outbreaks** are **increasing** with a difficulty to retrieve clinical isolates (strong evidence/weak evidence)

➔ Food poisoning is related to contamination level, strain virulence & encountered scenario



## Context (biocontrol)

- ❑ *Bacillus thuringiensis* (Bt)-based product is used worldwide as biopesticide in agriculture, forestry and mosquito control
- ❑ Pest control is ensured by the ingestion of Bt insecticidal crystalline protein inclusions that are activated in larvae midgut
- ❑ Efficient, easy-to-use, low cost commercial biopesticide which represents an alternative to chemicals
- ➔ Persistence of Bt spores on vegetables yielding high presumptive *B. cereus* counts



## 02

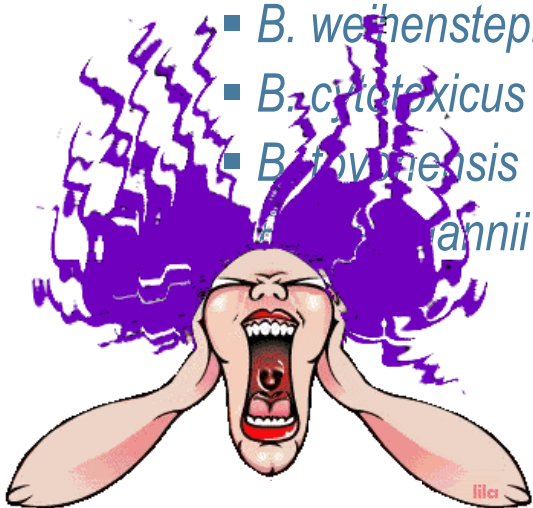
# *Bacillus cereus*

- ❑ ISO7932:2004 introduces the term presumptive *B. cereus* (= *B. cereus sensu lato* = *B. cereus* Group) for typical colonies to be enumerated on MYP agar at 30° C
  - ❑ Genetically closely related strains with enormous variation in pathogenic potential, often linked to plasmid encoded virulence factors
  - ❑ Classification relies mainly on phenotypic features, *i.e.* pathogenic potential, colony morphology, motility, growth and enzymatic abilities
- ➔ Group of closely related strains showing high phenotypic diversity

# Bacillus cereus

## □ Different species have been described, *i.e.*

- *B. mycoides* (Flügge 1886)
- *B. anthracis* (Smith 1952)
- *B. cereus sensu stricto* (Smith 1952)
- *B. thuringiensis* (Smith 1952)
- *B. pseudomycoides* (Nakamura 1998)
- *B. weihenstephanensis* (Lechner 1998)
- *B. cytotoxicus* (Guinebretière 2013)
- *B. toyonensis* (Jimenez 2013)
- *B. pinnipinnis* (Miller 2016)
- *B. maniponensis* (Jung 2011)
- *B. gaemokensis* (Jung 2010)
- *B. bingmayongensis* (Liu 2014)
- *B. paranthracis* (Liu 2017)
- *B. pacificus* (Liu 2017)
- *B. tropicus* (Liu 2017)
- *B. albus* (Liu 2017)
- *B. mobilis* (Liu 2017)
- *B. luti* (Liu 2017)
- *B. proteolyticus* (Liu 2017)
- *B. nitratireducens* (Liu 2017)
- *B. paramycoides* (Liu 2017)





## Industrial issue

- ❑ Strains involved in industrial biotechnology settings, *i.e.* crop protection, probiotic, feed additive, potential plant growth promoter...
- ❑ Toxinogenic strains involved in food safety, *i.e.* emetic and/or diarrheic toxins causing food poisoning outbreaks
- ❑ Strains involved in food spoilage, *i.e.* deterioration of food texture and sensory attributes that renders foodstuffs no longer suitable for human consumption



How to distinguish hazard & assess risk for presumptive *B. cereus* contaminations ?

03

## Available tools



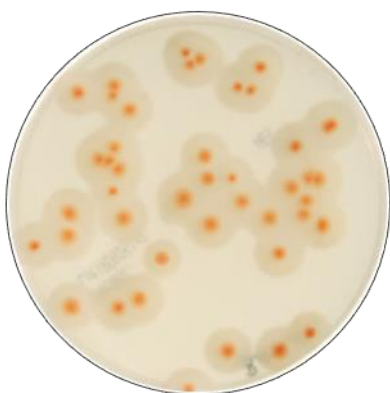
## Available tools

### ☑ Identify presumptive *B. cereus* in food

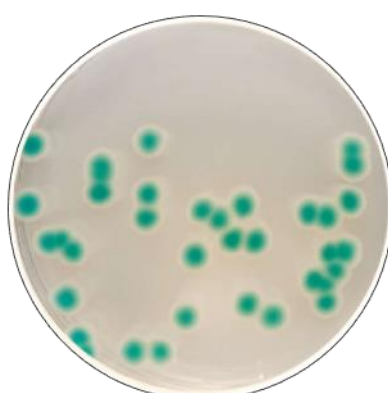
MYP



Bacara



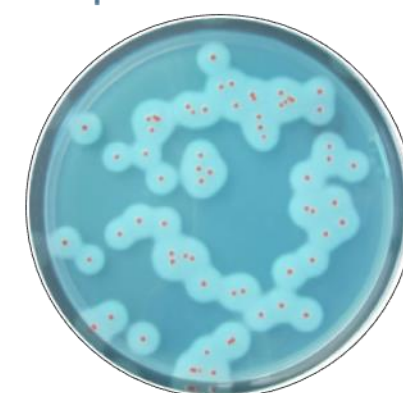
Brilliance



COMPASS



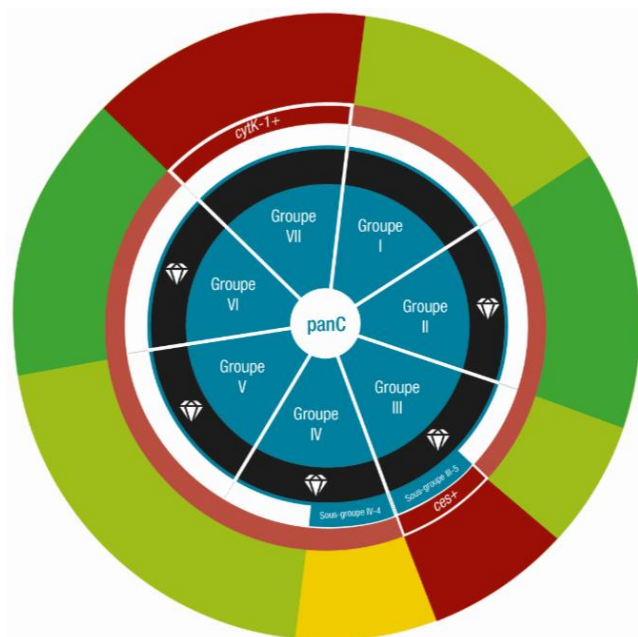
Rapid'Bcereus



➔ Chromogenic media validated according to ISO16140  
for the enumeration of presumptive *B. cereus*  
(ISO7932:2004)\*

## Available tools

### ☑ Differentiate presumptive *B. cereus* in food



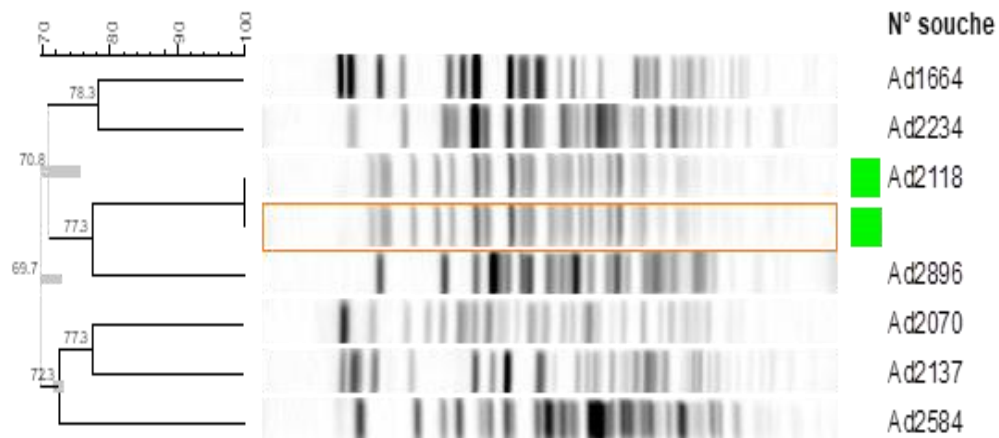
☐ Affiliation to phylogenetic Group based on panC specific sequence & identification of potential hazard (Guinebretière *et al.* 2008)

- ■ Potential pathogenicity
- ■ Potential spoilage activity
- Potential commercial biopesticide

➡ 7 major phylogenetic *B. cereus* Group, associated to specific hazard

## Available tools

### Trace strain of presumptive *B. cereus*



trace biopesticide strains from field to fork

trace back the sources of contamination along production lines (surface, ingredient, raw materials, end-product ...)

➔ Clustering of molecular fingerprint enables identification of contamination source & ensure hygiene of production lines

## Conclusions & significance

- ✓ Commercial biocontrol agents represent a few clonal related strains which belong to phylogenetic group IV of presumptive *B. cereus* (IV-4 and IV-7)
- ✓ High ecological niche adaptation of presumptive *B. cereus* is well characterized by the affiliation to phylogenetic groups which enable hazard identification and risk assessment
- ✓ Tools are available to identify, differentiate and trace strain for better knowledge on prevalence



# Acknowledgements

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Thank you for your attention

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