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SPORE-RISK
RISKS LINKED TO
SPORE-FORMING BACTERIA

Bacillus cereus: from a food industrial perspective

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ACTIA

UMT SPORE-RISK
RISKS LINKED TO SPORE-FORMING BACTERIA

SPORE-RISK

A JOINT TECHNOLOGICAL UNIT ON RISKS LINKED TO SPORE-FORMING BACTERIA

Laboratoire Universitaire LUBEM de Biodiversité et d'Écologie Microbienne

UBO université de bretagne occidentale

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The University Lab on Sporeformers & Moulds

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The Food Technology Institute on Food Safety & Quality

Bacillus species

☑ sporeforming bacteria are ubiquitous and exhibit a wide range of diversity in phenotypic behavior

☑ high prevalence of spores in raw materials and ingredients, representing dormant but highly resistant contamination

☑ in food, when conditions are favourable, germination, growth and cell multiplication of specific strains may lead to food poisoning or food spoilage

➲ Persistance of sporeformers in industrial plants & huge economical losses

Bacillus cereus

☑ food spoilage, *i.e.* deterioration of food texture and sensory attributes that renders foodstuffs no longer suitable for human consumption

☑ food safety, *i.e.* emetic and/or diarrheic toxins yielding to food poisoning outbreaks, with a few fatal cases

☑ food poisoning mainly occurs with takeaway and catering restaurant due to temperature abuse and improper handling/storage

➲ World food consumption patterns may yield increasing food quality & safety issues associated to *B. cereus*

Industrial issues

☑ *B. cereus* are ubiquitous, resist physical and chemical treatments and may grow at refrigeration temperatures

☑ no food safety criteria in regulation EC No 2073/2005, but only a process hygiene criteria for dried infant formula (50-500 CFU/g)

☑ level of 1 000 CFU/g to screen raw material or end product

How to assess risk regarding *B. cereus* hazards ?

Content

01 Complex life cycle

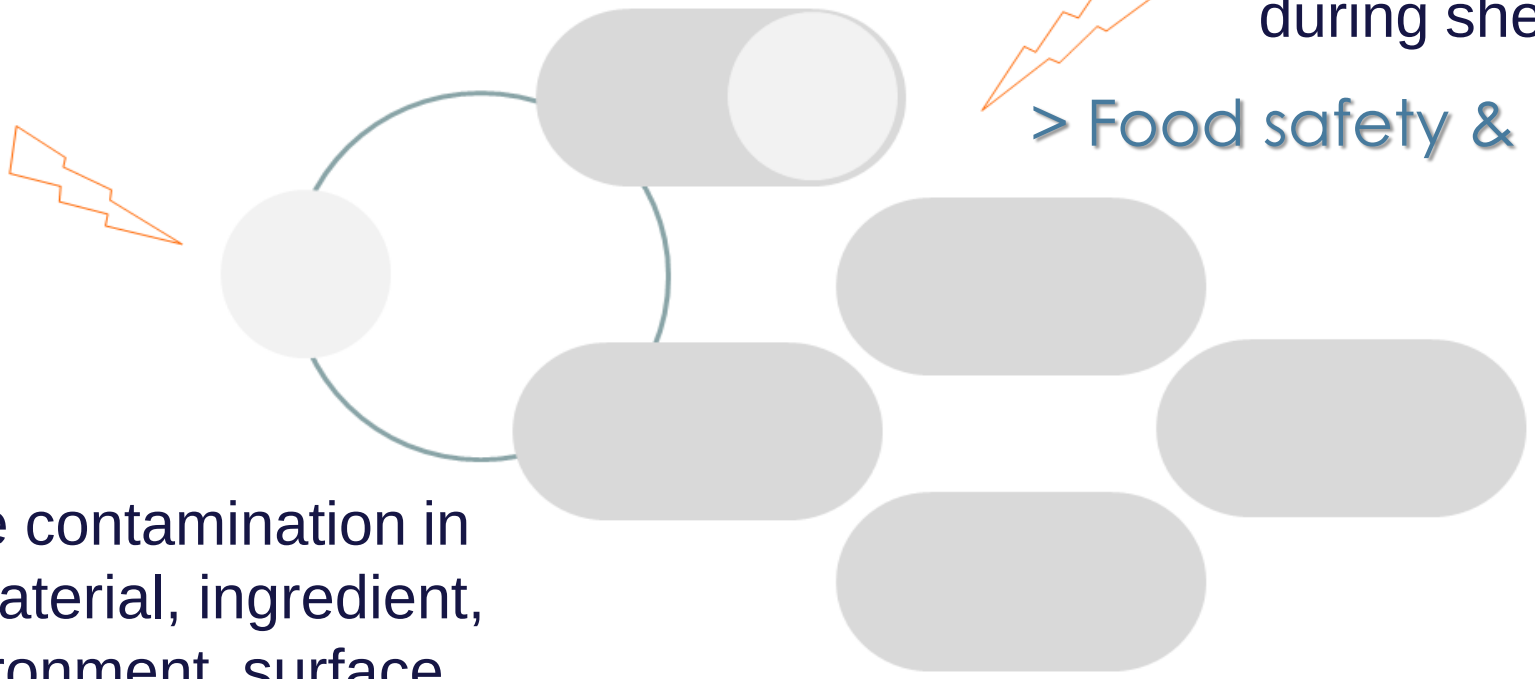
02 B. cereus biodiversity

03 Methods to screen & control contaminations

Complex life cycle

Avoid spore germination, outgrowth and cell multiplication in food during shelf-life

> Food safety & quality issue

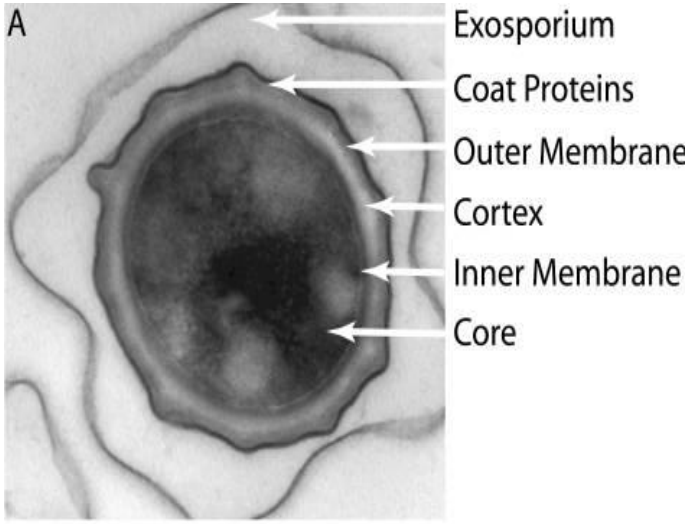


Spore contamination in raw material, ingredient, environment, surface

> Process & sanitation

01 Complex life cycle

Sporulation

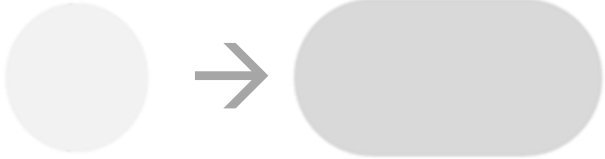


- ☑ synthesis of a resistant, dormant spore, usually due to unfavorable nutrient conditions
- ☑ well described cycle, progressive resistance to UV, chemical, radiation and heat treatments
- ☑ spore may remain dormant for extended time period … until favorable growth conditions

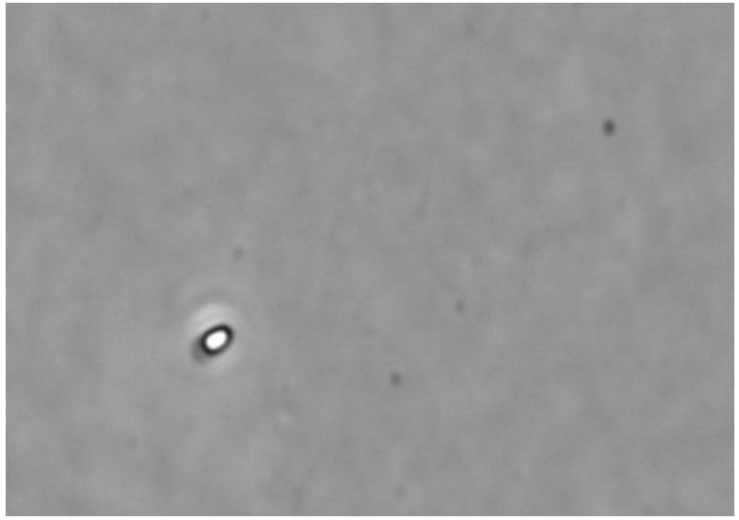
Transmission electron micrograph of *Bacillus anthracis* endospore
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC303457

01

Complex life cycle



Germination & outgrowth

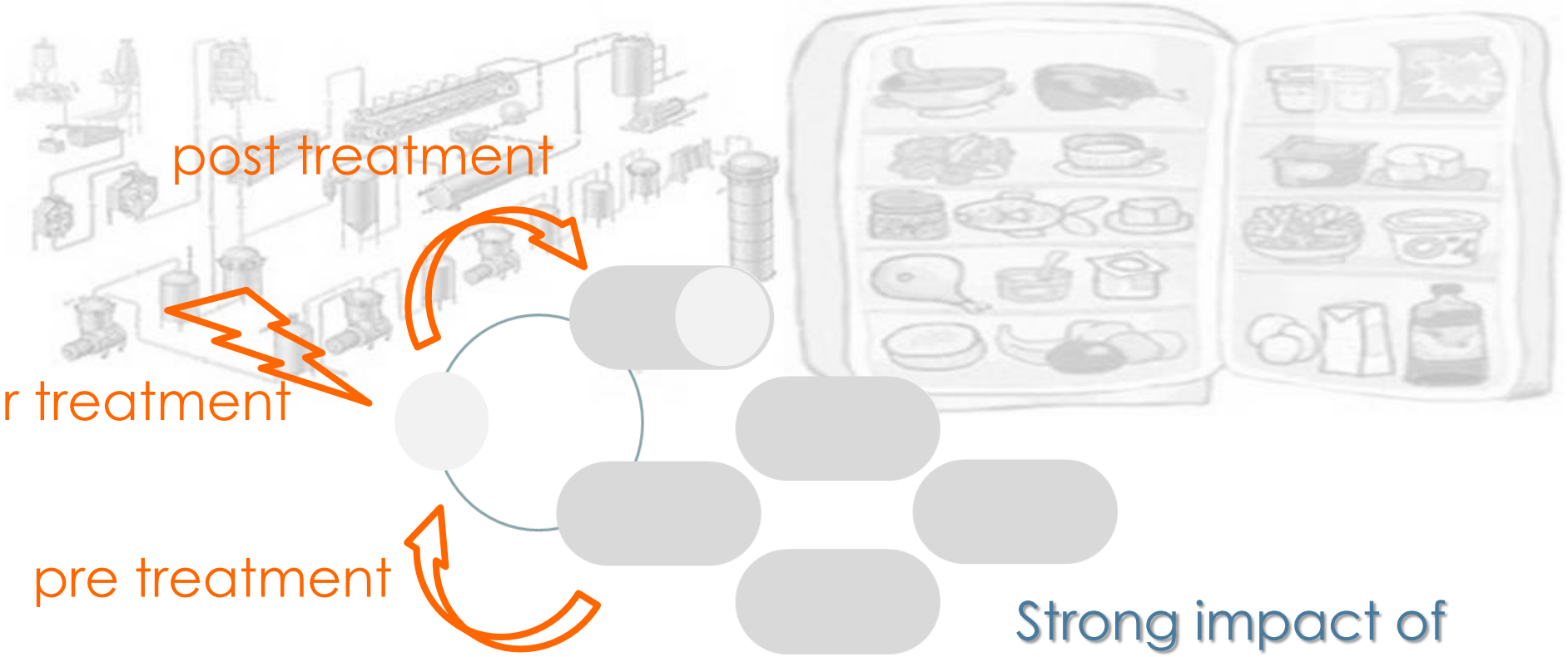


Stringer *et al.* 2005

- ☑ loss of resistance and initiation of vegetative growth when presence of germinant (=nutrient)
- ☑ rapid process but combination of factor is needed to activate super dormant spores
- ☑ germinosome, temporal gene expression …

01

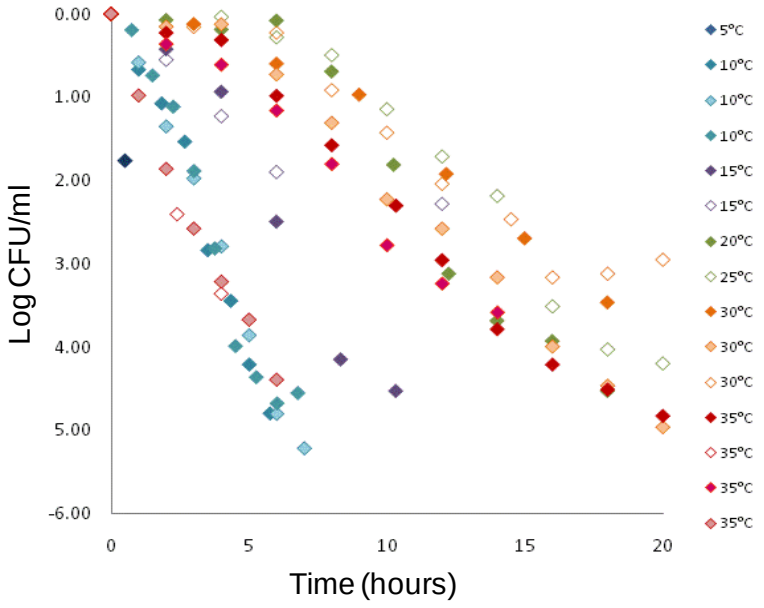
Complex life cycle



Strong impact of environmental conditions on spore load, resistance and germination ability

Complex life cycle

- Impact of sporulation conditions on spore resistance
  - Comfort classical lab practices & robustness of challenge test assays

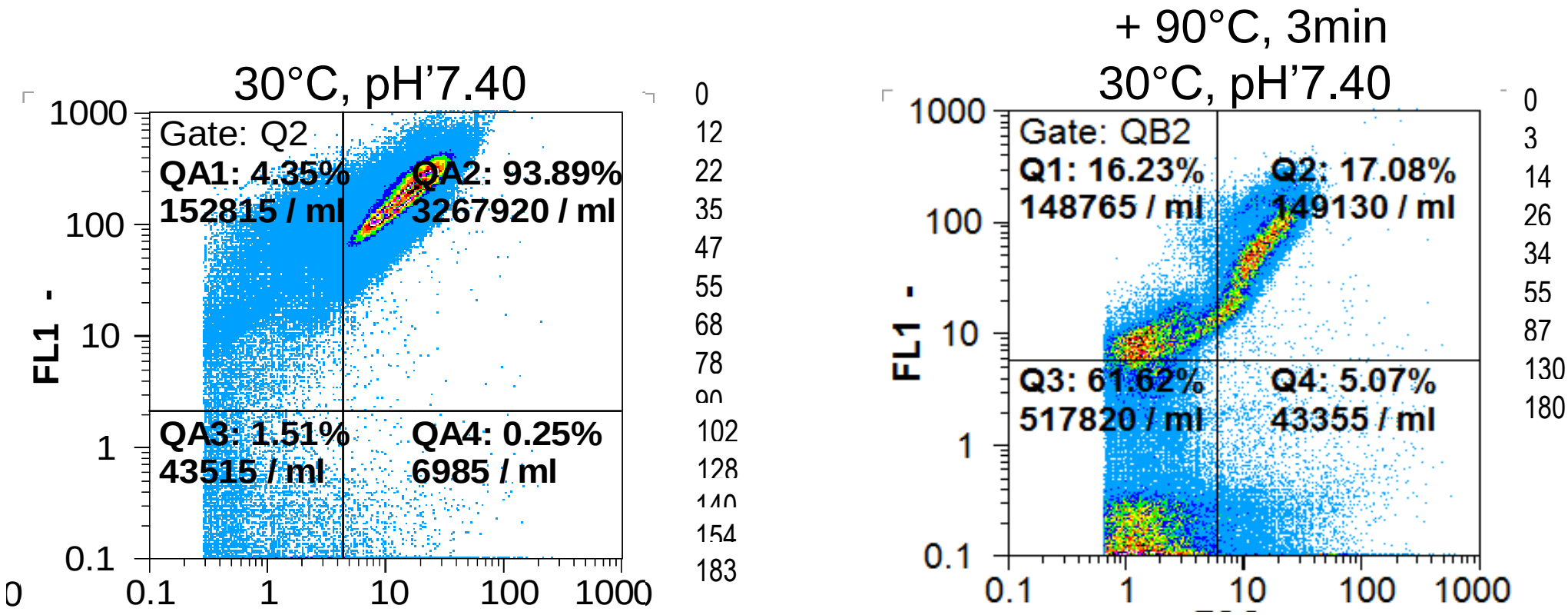


☑ *B. weihenstephanensis* KBAB4 spore heat resistance ($D_{90°C}$) is lower when spores are produced at 10°C than at 30°C

Baril *et al.*, IAFP Milwaukee, 31 july-1 august 2011

Complex life cycle

Impact of process on germination abilities



Trunet *et al.* , session IAFP à Cardiff, 20-22 avril 2015

Complex life cycle

Mathematical modelling to account for complex life cycle of *Bacillus*

T_{min}, T_{opt}, T_{max}
pH_{min}, pH_{opt}, pH_{max}

☑ Growth cardinal values may be used to account for all complex life cycle of *Bacillus*

☑ Generic concept validated for several strains of *Bacillus*, *i.e.* psychrotolerant, mesophilic, thermophilic species

02

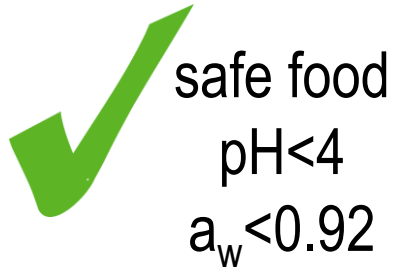
B. cereus biodiversity

02

B. cereus biodiversity

☑ data sheet on *B. cereus* foodborne biological hazards

-Growth limit : 4 °C (Tmin), 55°C (Tmax)
4.3-5 (pH min), 6-9.3 (pHmax)
0.92-0.93 (a_w min)



-Toxin production : 10-40°C
-Heat resistance : $D_{120°C}$:0.03-2.3min

➲ Rapid cooling of cooked food & proper temperature storage (4°C< >63°C)

02

B. cereus biodiversity

B. cereus group = *B. cereus sensu lato* (DNA/DNA homology >98%)

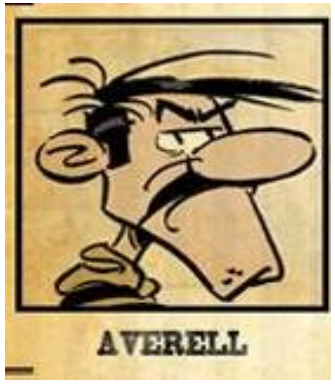
Morris & Goscinny ®

- *B. anthracis* (Smith 1952) — Anthrax
- *B. cereus sensu stricto* (Smith 1952) — Food poisoning
- *B. mycoides* (Nakamura 1995) — Growth @refrigeration
- *B. pseudomycoides* (Nakamura 1998)
- *B. thuringiensis* (Smith 1952) — Microbial pest control
- *B. weihenstephanensis* (Lechner 1998) — Growth @refrigeration
- *B. cytotoxicus* (Guinebretière 2013) — Food poisoning
- *B. toyonensis* (Jimenez 2013) — Probiotic

➲ Wide biodiversity of *B. cereus* strains which could be involved in both food quality & food safety issue

02

B. cereus biodiversity



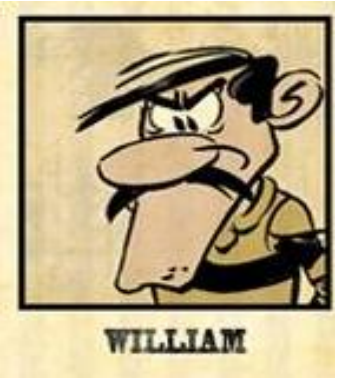
☑ ***Bacillus weihenstephanensis***

- Psychrotolerant bacteria, growth at 4-7°C.

  PCR distinction targeting specific biomarker (16SrDNA, CspA cold shock protein)
- Some strains may produce emetic toxin at temperatures >20°C (Thorsen *et al.* 2006)
- Food spoilage of dairy, RTE, egg-based products…

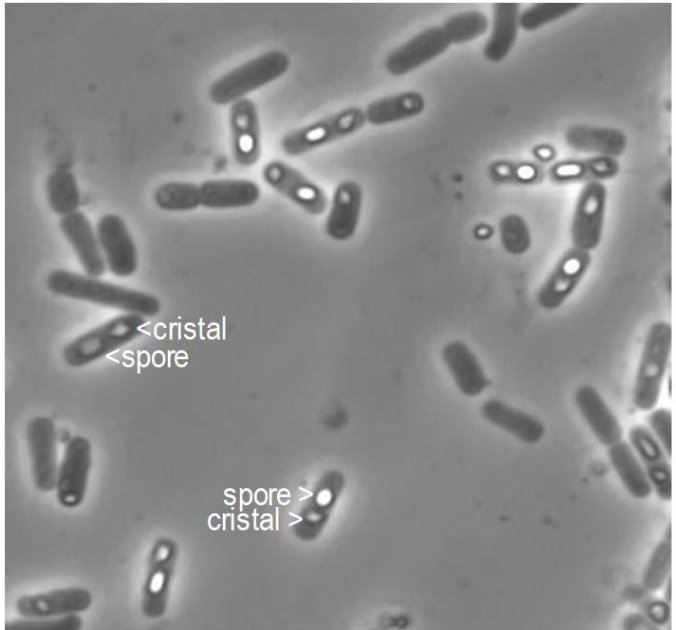
02

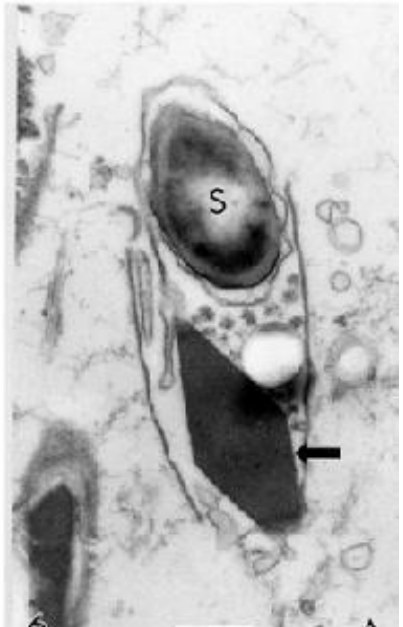
B. cereus biodiversity



☑ *Bacillus thuringiensis*

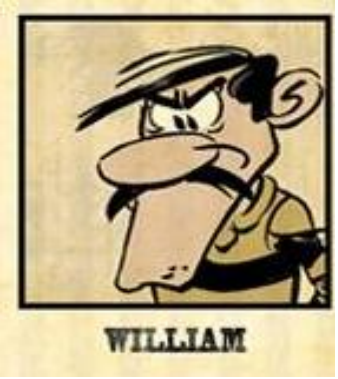
- Large diversity of virulence factors, *i.e.* Vip protein, thuringiensine, and parasporal body=crystal
- Crystal = protein inclusion (*cry* et *cyt* encoding genes) produced during sporulation and showing insecticidal properties





02

B. cereus biodiversity

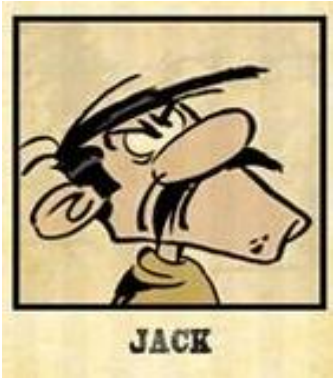


☑ *Bacillus thuringiensis*

- Large diversity of virulence factors, *i.e.* Vip protein, thuringiensine, and parasporal body=crystal
- Crystal = protein inclusion (*cry* et *cyt* encoding genes) produced during sporulation and showing insecticidal properties
- Different Bt serovars comply with EC regulation (*kurstaki, aizawa, israelensis etc*…) , +300 commercial product formulations
- Worldwide crop protection against caterpillars, beetles, flies and mosquitoes

02

B. cereus biodiversity



☑ **Bacillus cereus sensu stricto**

- Hemolytic and motile strains
- Food poisoning due to temperature abuse and improper handling/storage of cooked products

➲ Diarrheic strain

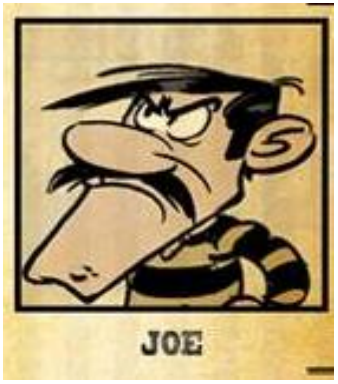
- Heat-labile enterotoxins (NHE, HBL, CytK), during growth in small intestine after consumption of contaminated food.
- Optimal production of enterotoxins at 32°C [10-43°C]

➲ Emetic strain

- Often associated to consumption of contaminated cooked rice
- Cereulide, heat stable toxin, pre-formed in food.
- Optimal production at 12-15°C [12-37°C]

02

B. cereus biodiversity



☑ ***Bacillus cytotoxicus***

- More distant cluster
- Heat tolerant strains, growth at 20-50°C, highly virulent strains. PCR distinction using *cytK1* marker (Guinebretiere *et al.* 2013)
- 5-10 clinical isolates but high prevalence in dehydrated ingredient (Contzen *et al.* 2014)

☑ ***Bacillus anthracis***

- Higly monomorphic and clonal lineage
- Ethiological agent of anthrax, mammals and human zoonotic pathogen, may be lethal
- Virulence factors harboured by 2 mega-plasmids encoding for anthrax toxins (pXO1) and capsule (pXO2)

02

B. cereus biodiversity

- *B. cereus* strains show great genome plasticity leading great adaptation ability

- While certain virulence factors are located on large plasmids, genes encoding for diarrheic toxins are chromosomally
… which makes it difficult to distinguish pathogenic strains !

03

Methods

03

Methods

☑ *B. cereus* diarrheal toxin detection using commercial kits
TECRA BDE-VIA (NheA du NHE), OXOID BCET-RPLA (L2 du HBL)

☑ *B. cereus* emetic toxin detection, no commercial kit

+ EN ISO 18465, Mai 2017- Quantitative determination of emetic toxin (cereulide) using LC-MS/MS

☑ PCR gene quantification (*ces, hbl, cytK* …)
Ehling-Schulz *et al.* 2005
Guinebretiere *et al.* 2006
Fricker *et al.* 2007

03

Methods

☑ *B. cereus* enumeration

ISO 7932:2004 – Horizontal method for the enumeration of presumptive *Bacillus cereus* – colony count at 30°C

MYP, reference agar medium

- Colonie : pink (mannitol^{-}),
  +/- precipitate (lecithinase$^{+/-}$)
- Confirmation : hemolysis at 30°C

http://nf-validation.afnor.org/en/food-industry/bacillus-cereus/

NF Validation

Certify the analytical performances of test kits

HOME NF VALIDATION **FOOD INDUSTRY** WATER ANALYSIS

Bacillus cereus

Reference method(s): NF EN ISO 7932

You will find below listed the certified analytical methods and holder companies. The methods are classified by principle. Download (pdf format) the two public documents relating to each certified method: the **validation certificate** and the summary validation **study report**.

Section updated and published on December 21st, 2016

Culture media

BACARA
Aim of method: Enumeration of presumptive *Bacillus cereus*
Holder: bioMérieux
Certificate / Summarized study report

COMPASS Bacillus cereus Agar
Aim of method: Enumeration of presumptive *Bacillus cereus*
Holder: SOLABIA S.A.S.
Certificate / Summarized study report

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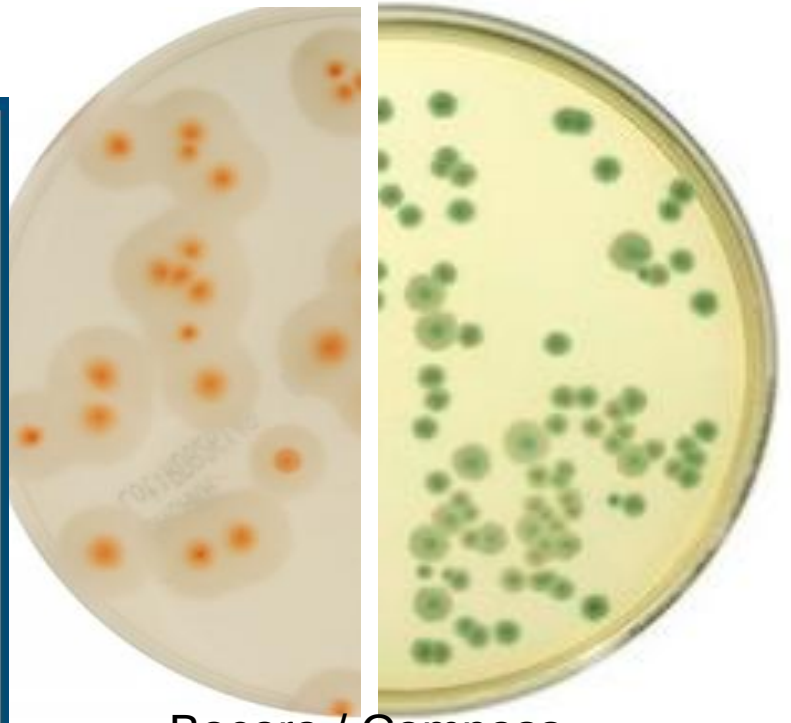
DOMAIN OF FOOD INDUSTRY



Bacara / Compass

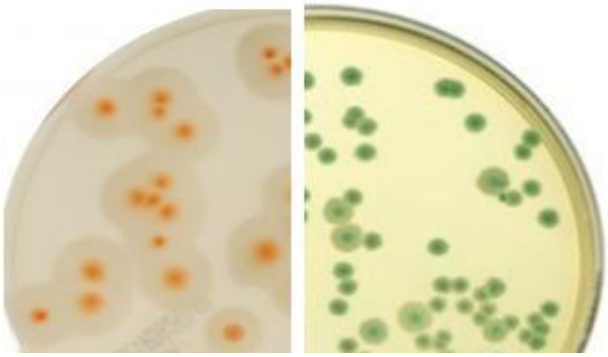
03

Methods

☑ *B. cereus* enumeration

ISO 7932:2004 – Horizontal method for the enumeration of presumptive *Bacillus cereus* – colony count at 30°C

MYP, chromogenic agar medium



- Colored colonies
- No confirmation needed

03

Methods

☑ affiliation to phylogenetic *B. cereus* group Guinebretiere *et al.* 2008, 2010 based on *panC* sequences

https://www.tools.symprevius.org/bcereus/english.php

How does this tool work :
panC gene sequence is used for identification to phylogenetic groups (I to VII), according classification proposed by Guinebretière *et al.* (2008).
This sequence is compared to known sequences in the database of UMR408, INRA-Université d'Avignon, France.

Phenotypic features attributed to the 7 phylogenetic groups can be consulted by this link.

Requirements:
The short sequence AACAAAC or AACAGAC is required to initiate alignment. For more information refer to link below.
Proposal and requirement for preparing *panC* sequence: please click here

The homology search algorithm can be consulted by this link
Contact : M.-H. Guinebretière

How to input sequences?

```
>Ad832
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```

START!

Souche	Groupe	% d'homologie	résultats complets
Ad832	III	99.72	résultats complets
Ad797	II	99.43	résultats complets
Ad796	IV	99.72	résultats complets
Ad790	VI	100	résultats complets
Ad788	II	100	résultats complets

03

Methods

☑ affiliation to phylogenetic *B. cereus* group
Guinebretiere *et al.* 2008, 2010 based on *panC* sequences

groupe	croissance à 7°C	croissance à 10°C	croissance à 43°C	Resistance thermique	TIAC	Présence de *nhe*	Présence de *cytk1*	Présence de *cytk2*	Présence de *ces*
I	-	+	-	/	-	+	-	-	-
II	+	+	-	++	+	+	-	+	-
III	-	-	+	+++	+++	+	-	+	+/-
IV	-	+	+	++	++	+	-	+	-
V	-	+	-	++	+	+	-	+	-
VI	+	+	-	+/-	-	+	-	-	-
VII	-	-	+	+++	+++	+	+	-	-

Guinebretière *et al.* 2008-2010, Bulletin épidémiologique de l'ANSES TIAC *B. cereus* de 2006-2010

03

Methods

☑ affiliation to phylogenetic *B. cereus* group
Guinebretiere *et al.* 2008, 2010 based on *panC* sequences

groupe	croissance à 7°C	croissance à 10°C	croissance à 43°C	Resistance thermique	TIAC	espèces
I	-	+	-	/	-	*B. pseudomycoides*
II	+	+	-	++	+	*B. cereus*, *B. thuringiensis*
III	-	-	+	+++	+++	*B. cereus*, *B. anthracis* *B. thuringiensis*,
IV	-	+	+	++	++	*B. cereus*, *B. thuringiensis*
V	-	+	-	++	+	*B. cereus*, *B. toyonensis* *B. thuringiensis*
VI	+	+	-	+/-	-	*B. weihenstephanensis*, *B. mycoides*, *B. thuringiensis*
VII	-	-	+	+++	+++	*B. cytotoxicus*

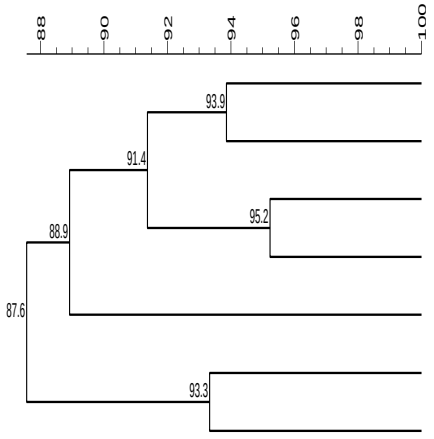
Guinebretière *et al.* 2008-2010, Bulletin épidémiologique de l'ANSES TIAC *B. cereus* de 2006-2010

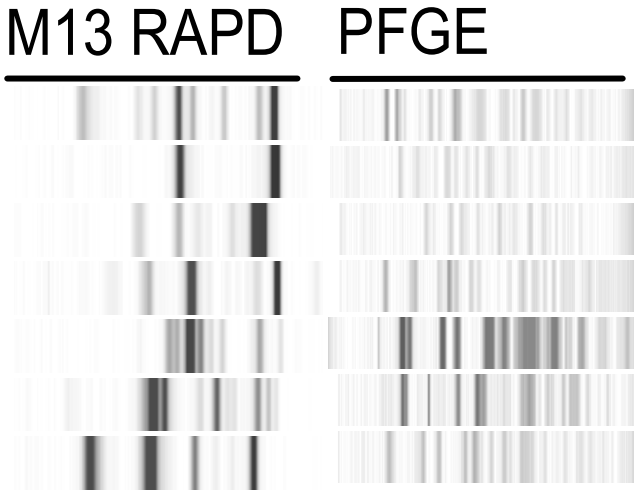
03

Methods

☑ determination of molecular fingerprint

- normalisation and biostatistic analysis
- cluster strain (M13 RAPD, Rep-PCR)
- Trace strain (PFGE)





03

Methods

☑ for detection and count or presumptive B. cereus

☑ for characterization and typing of *B. cereus* isolates

☑ for growth/destruction of population as a function of food formulation/process/shelf-life in food

For a given food :

-Is food formulation supporting growth of *B. cereus*?
-Is pasteurisation process ensuring destruction of *B. cereus*?
-Is food safe during shelf-life?

03

Methods

Case study

Raw material — 2°C / 4 days

↓

Mixing — 8°C / 1 hour

↓

Packaging

↓

Heat treatment — 90°C / 45 min

↓

Fast cooling — up to 2°C

↓

Storage / Distribution — 4°C / 20 days

End product — pH =6, a_w=0.98

03

Methods

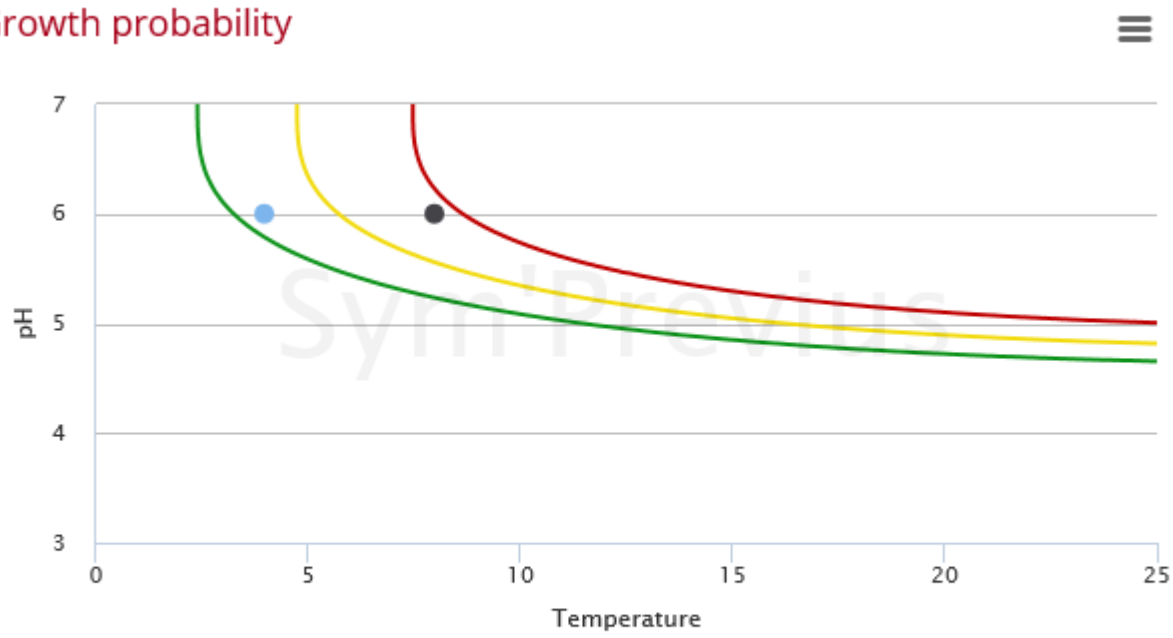
1. Does food formulation allow *B. cereus* growth ?

Raw material	2°C / 4 days
↓	
Mixing	8°C / 1 hour
↓	
Packaging	
↓	
Heat treatment	90°C / 45 min
↓	
Fast cooling	up to 2°C
↓	
Storage / Distribution	4°C / 20 days
End product	pH =6, a_w=0.98

03

Methods

☑ determination of growth/no growth boundaries for *B. cereus*



9 strains, a_w = 0.98

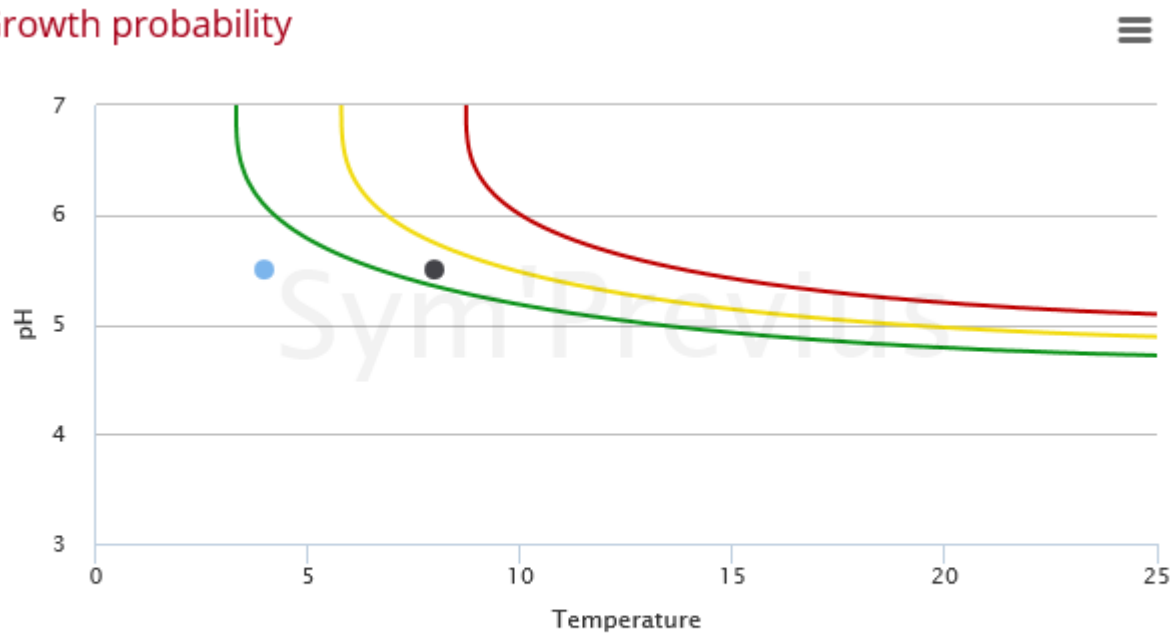
#1: 4°C, pH6
#2: 8°C, pH6

➲ Food formulation supports growth of *B. cereus,* but what would be the impact of a light change

03

Methods

☑ determination of growth/no growth boundaries for *B. cereus*



9 strains, a_w = 0.97

#1: 4°C, pH5.5
#2: 8°C, pH5.5

➲ Unless strictly stored at 4°C, food formulation supports growth of *B. cereus*

03

Methods

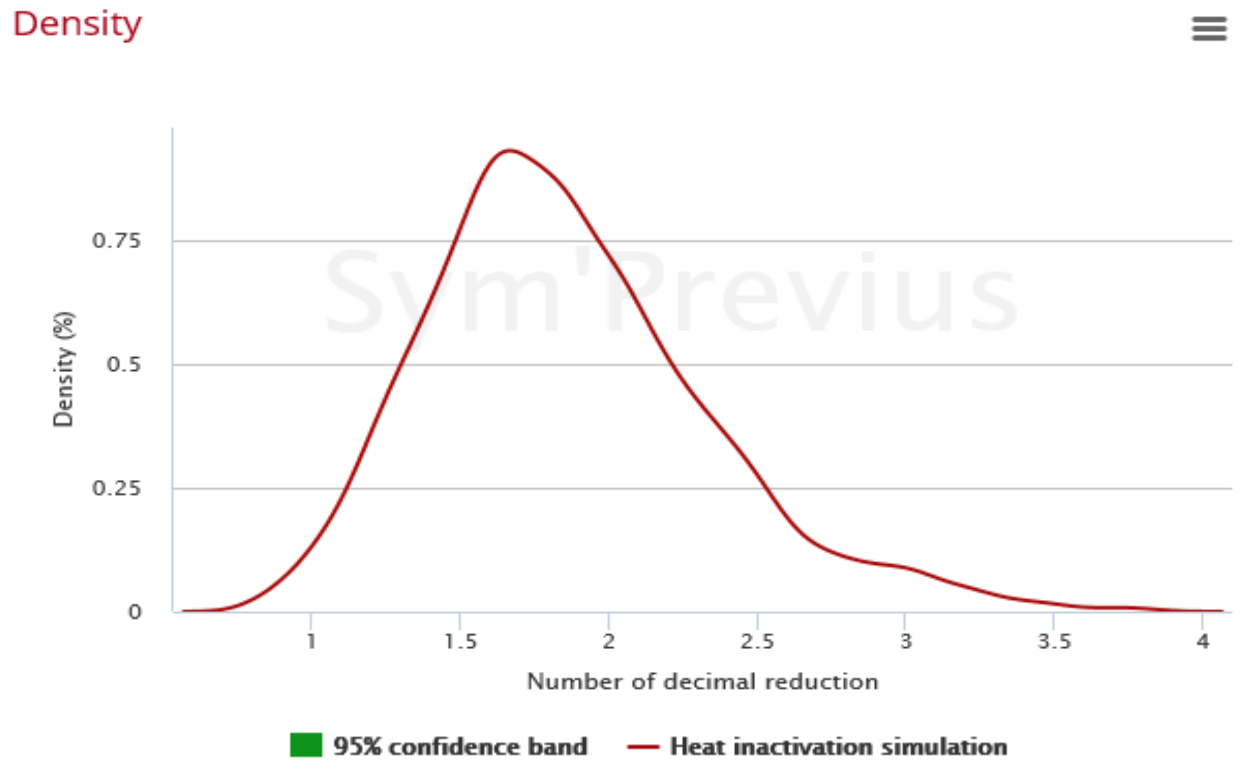
2. Does heat treatment ensure destruction of *B. cereus* ?

Raw material	2°C / 4 days
↓	
Mixing	8°C / 1 hour
↓	
Packaging	
↓	
Heat treatment	90°C / 45 min
↓	
Fast cooling	up to 2°C
↓	
Storage / Distribution	4°C / 20 days
End product	pH =6, a_w=0.98

03

Methods

☑ simulation of population destruction by this given heat treatment



90°C-45min

Targeted reduction:5

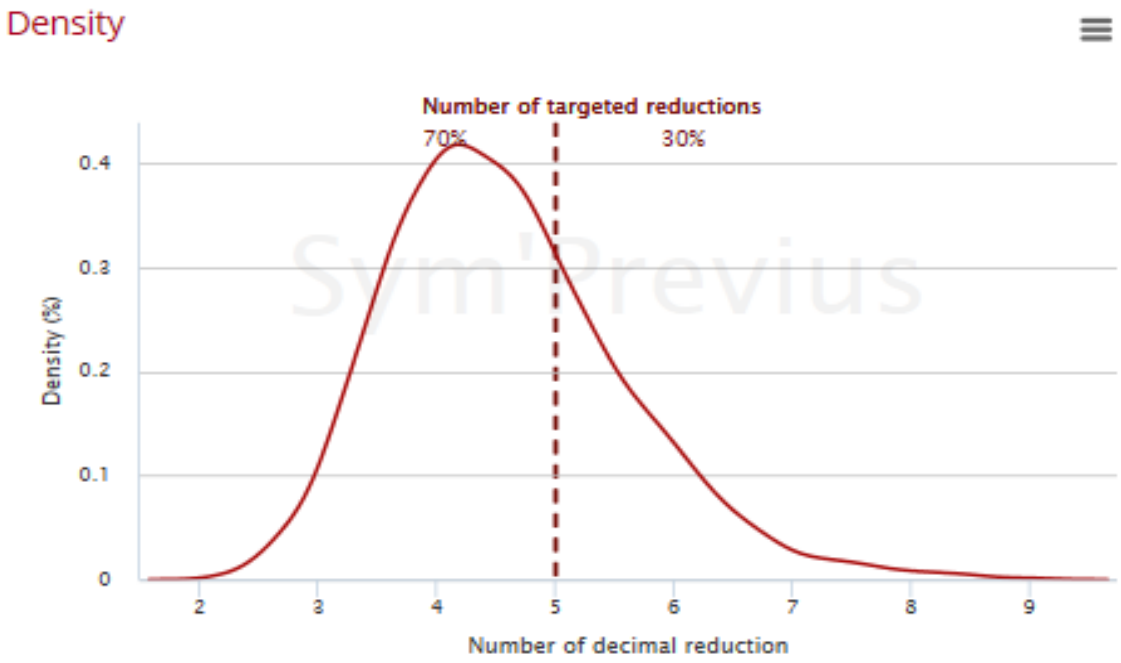
B. cereus strain A

→ decimal reduction ~2

03

Methods

☑ simulation of population destruction by this given heat treatment



90°C-45min
Targeted reduction:5
B. cereus strain A
→ decimal reduction ~2

B. cereus strain C
→ decimal reduction <5

➲ Depending of contaminating strain, targeted population reductions are not achieved by treatment

03

Methods

3.

What about *B. cereus* population during shelf-life ?

Raw material — 2°C / 4 days

↓

Mixing — 8°C / 1 hour

↓

Packaging

↓

Heat treatment — 90°C / 45 min

↓

Fast cooling — up to 2°C

↓

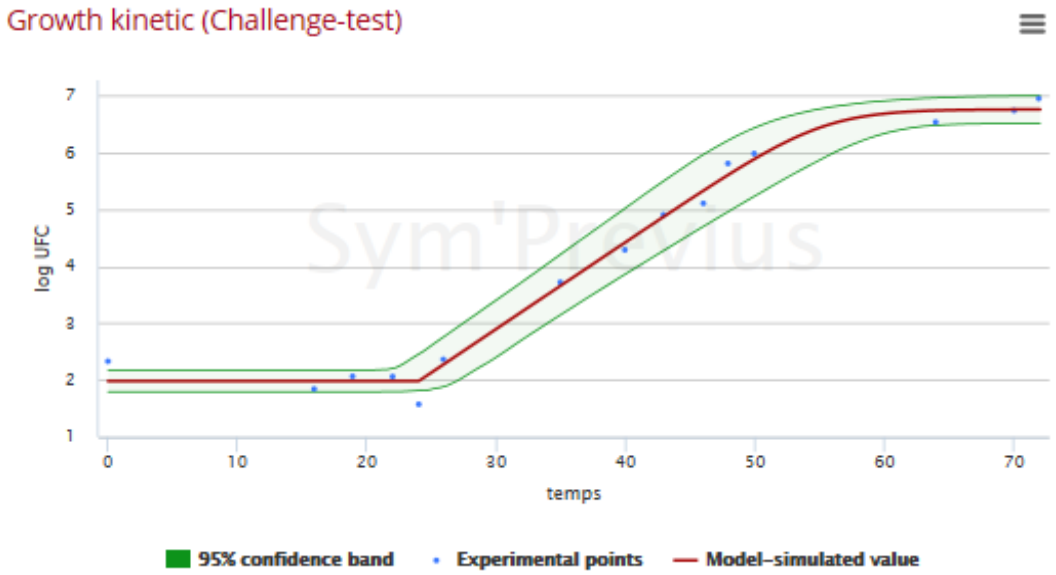
Storage / Distribution — 4°C / 20 days

End product — pH =6, a_w=0.98

03

Methods

☑ simulation of *B. cereus* growth during shelf-life



Estimated primary parameters

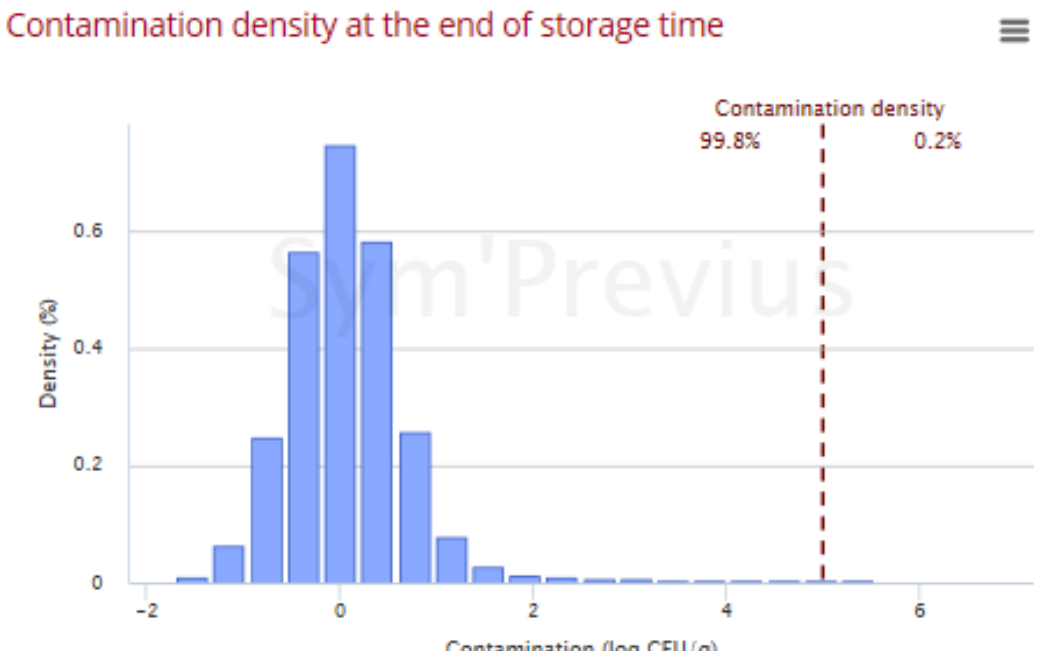
	Estimated values	Standard Deviation
No (Log UFC/g)	1.98	0.1
Nmax (Log UFC/g)	6.75	0.13
μmax (/h)	0.352	0.025
lag (h)	24	1.47
tg (h)	1.981	0.143

Based on 1 challenge test, growth rate is determined for a given combination contaminant/foodstuff

03

Methods

☑ simulation of *B. cereus* growth during shelf-life



temperature storage scenario:
1/3 @4°C, 2/3 @8°C
during 20 days

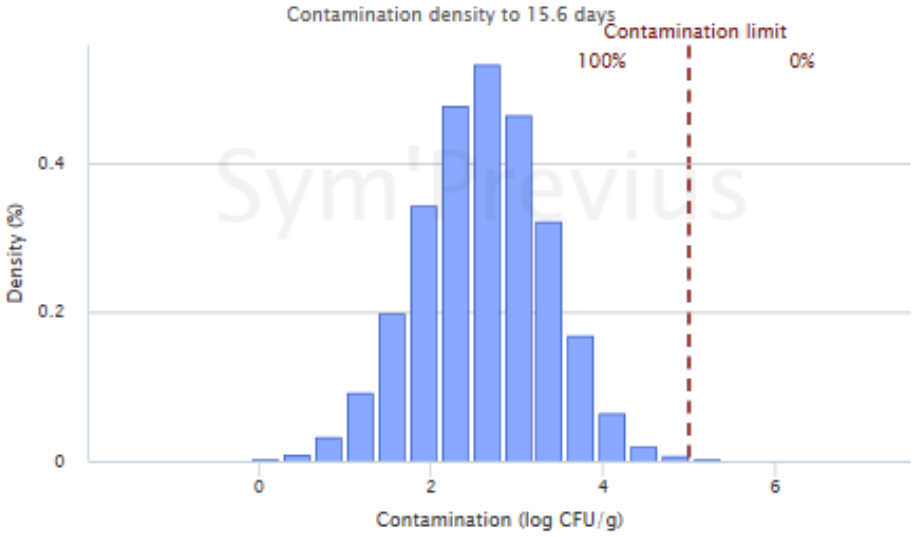
➲ If refrigerated storage condition is ensured, food shelf-life of 20days is validated

03

Methods

☑ simulation of *B. cereus* growth during shelf-life

Contamination density

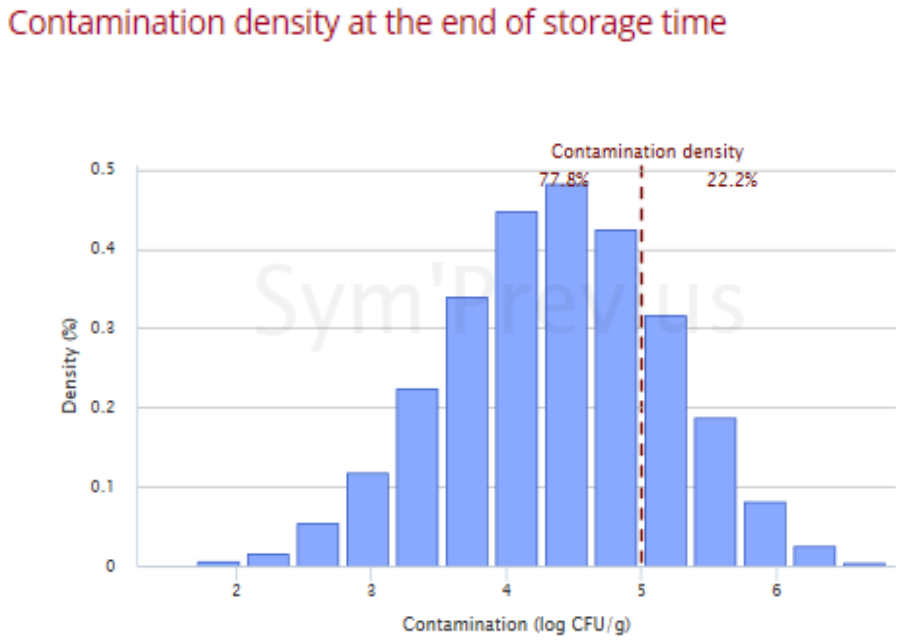


For less stringent refrigeration conditions or the contamination of psychrotolerant strain, *B. cereus* population will develop during shelf-life

03

Methods

☑ simulation of *B. cereus* growth during shelf-life



For less stringent refrigeration conditions or the contamination of psychrotolerant strain, *B. cereus* population will develop during shelf-life

➲ In a few clic, evolution of targeted population may be predicted for relevant industrial scenarii

Take home message

01 *B. cereus* group shows a wide range of behaviour & adaptation abilities

02 Food poisoning outbreaks are mainly due to temperature abuse and improper handling / storage of cooked food

03 Already available tools allow to account for biodiversity in order to ensure food safety & quality

Acknowledgements

ITAI

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LABOCEA
Laboratoire public
Conseil, Expertise et Analyse en Bretagne

Bt ID
http://itab-asso.net/btid/wakka.php?wiki=PagePrincipale

iBB Le réseau de l'Initiative Bio en Bretagne

International Organization for Standardization
ISO

afnor
NORMALISATION

ADRIA DÉVELOPPEMENT
UNIVERSITÉ DE BRETAGNE OCCIDENTALE
(LUBEM) À QUIMPER
ACTIA

UMT SPORE-RISK
RISQUES ASSOCIÉS AUX BACTÉRIES SPORULÉES

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Microbial Spoilers in Food symposium is back - Get ready !

SPOILERS IN FOOD

The scientific programme is now online!

contact@spoilers2017.com | www.spoilers2017.com

SPOILERS IN FOOD

Microbial spoilers in food 2017
28th-29th-30th June - Quimper

Microbial spoilers in food - June 28th - 29th & 30th 2017

Welcome to Microbial Spoilers in Food 2017

Super spoilers, wonder spores and diehard micro-organisms: New insights to integrate these super foes in food spoilage risk management.

There is always ONE, ONLY ONE, BUT ONE which is able to resist or resuscitate after inactivation processes, which is able to adapt or is naturally adapted to acid and/or low moisture formulations, as well as low temperature and/or heat treatment,... and this ONLY ONE is finally able to grow causing food spoilage and economical losses! This only one is always of major concern for food quality and production managers. How does one deal with them? One way to proceed is to learn more about these diehard microorganisms.

The challenge of this new edition of "Microbial Spoilers In Food" will be to gather scientists, food quality and production managers and project leaders in food innovation to exchange knowledge and experience on this specific topic.

This symposium will focus on

- Biodiversity of microbial food spoilers
- Physiology and metabolism of Spoiler microorganisms
- Characterization methodologies, enumeration methods for quality indicators
- How to fight super spoilers?

What they thought about Spoilers2013...

"It is the only event dedicated to spoilage. This meeting fills a gap and has everything to be part of the food science"

"Number of participants was perfect to get in contact with each other and even pay attention to posters"

"The program was well equilibrated and the level of the contribution was high. Do not change the concept, never change a winning concept, well done!"

"I thought the organisation was excellent before and during the congress, excellent reception, good coffee breaks and a very enjoyable gala dinner"

"Thank you so much for a wonderful conference, I thoroughly enjoyed it and learnt a lot!"