SPICED – Symposium

Survival, detection and toxinogenic potential of *B. cereus* group species in spices and herbs

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Federal Institute for Risk Assessment, Berlin

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B. cereus group (= *B. cereus* sensu lato)

https://commons.wikimedia.org/wiki/File:Bucatini_%28amatriciana_rossa%29.jpg

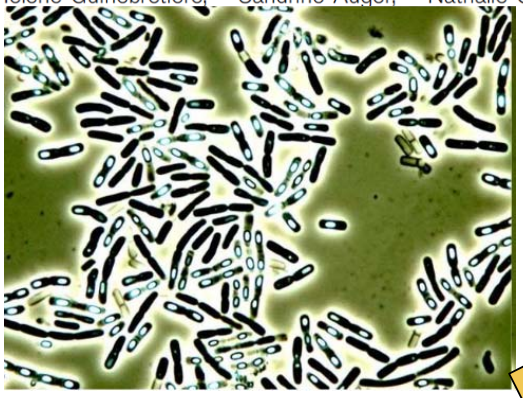
B. cereus

ic and Evolutionary Microbiology (2013), 63, 31–40 DOI 10.1099/ijs.0.030627-0

B. cytotoxicus

Bacillus cytotoxicus sp. nov. is a novel thermotolerant species of the *Bacillus cereus* Group occasionally associated with food poisoning

Marie-Hélène Guinebretière,[1,2] Sandrine Auger,[3,4] Nathalie Galleron,[3,4]



micbio.wzw.tum.de/cms/docs/Stammsammlung/Foto Bibliothek Bacillus.pdf

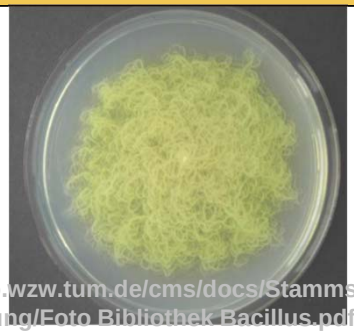
B. anthracis

http://phil.cdc.gov/phil/details.asp

B. thuringiensis

Wagner, A. (2015)

B. mycoides and pseudomycoides



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B. weihenstephanensis

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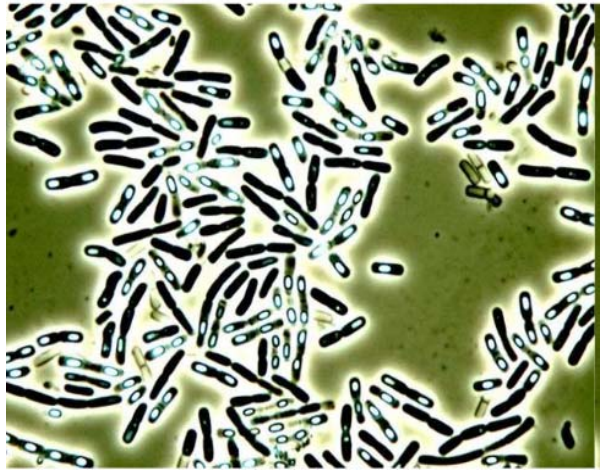
Bacillus (B.) cereus

- Gram-positive, motile, endospore forming rod
- facultative anaerobic
- optimal growth at 30 - 37 °C
- growth limitations: 4 °C, minimum pH 4.1, lowest aw 0.92
- heat resistant spores (survive pasteurization and cooking for several minutes)
- prevention of growth after cooking at T < 8 °C (optimal < 4 °C) or > 63 °C
- forms biofilms
- toxin producer
- ubiquitous in soil
- detectable in many kinds of food



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EFSA, 2005

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Outbreaks

Spice	Year	Country	Cases	Reference
Spicemix	2007	France	146	EFSA (2009, 2013)
Paprika	2009	Denmark	48	FAO und WHO (2014)
Curry	2009	Belgium	7	EFSA (2011)
White pepper	2010	Denmark	112	EFSA (2011, 2013)
Cinnamon	2011	Denmark	30	FAO und WHO (2014)
Turmeric	2011	Finland	19	EFSA (2013a)
Turmeric	2011	Finland	4	EFSA (2013a)
Cumin	2011	Finland	3	EFSA (2013a)
Black pepper	2011	Denmark	52	EFSA (2013a)

Food borne disease outbreaks from 1973 to 2012 in Europe associated to spices contaminated with *B. cereus*; modified according to Mader & Schaarschmidt, 2016

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Toxins

Enterotoxins

- nonhemolytic enterotoxin (Nhe)
- hemolysin BL (HBl)
- cytotoxin K (CytK)

- infective dose: >10^5-10^7 cells/spores consumed
- production in the gut
- symptoms: abdominal pain and diarrhoea
- incubation time: 8 - 16 h
- duration: 12 - 24 h
- associated to meat, vegetables, milk products

Emetic toxin (heat stable)

- cereulide

- ‚effective' dose: ~8 µg/kg body weight (also 0.02 - 2 µg/kg)
- production in food (10^5 - 10^8 cells)
- symptoms: nausea and vomiting
- incubation time: 1 - 5 h
- duration 6 - 24 h
- associated to rice and pasta

Blackburn, C. de W., 2009
Ceuppens et al., 2011

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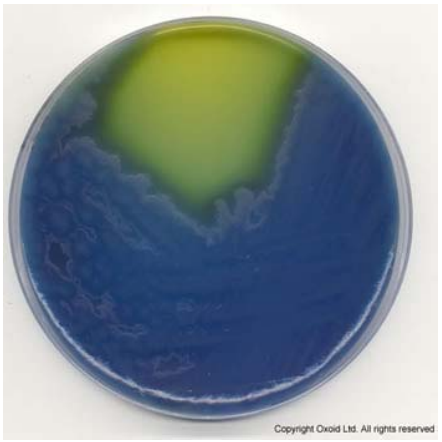
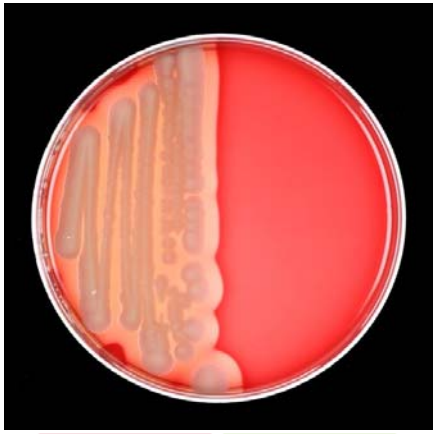
Detection – Cultural

ISO

- **7932:2005-03** Horizontal method for the enumeration of presumptive *Bacillus cereus*
- **21871:2006-04** Horizontal method for the determination of low numbers of presumptive *Bacillus cereus* (MPN)





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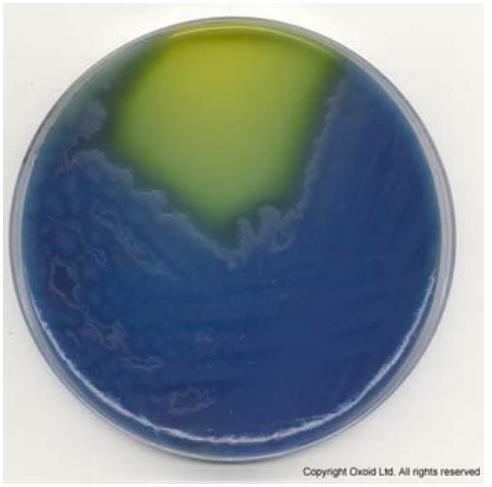
Detection – Cultural

Brilliance *B. cereus* Agar

PEMBA



Bacara

http://www.rapidmicrobiology.com/news/new-b-cereus-selective-agar-bacara-in-fda-bam/

MYP

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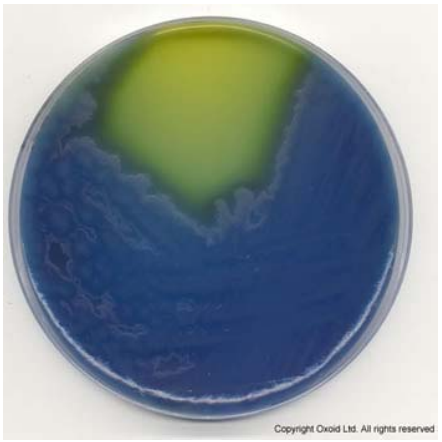
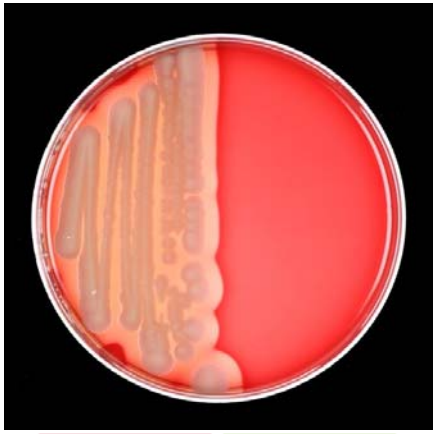
Detection – Cultural

ISO





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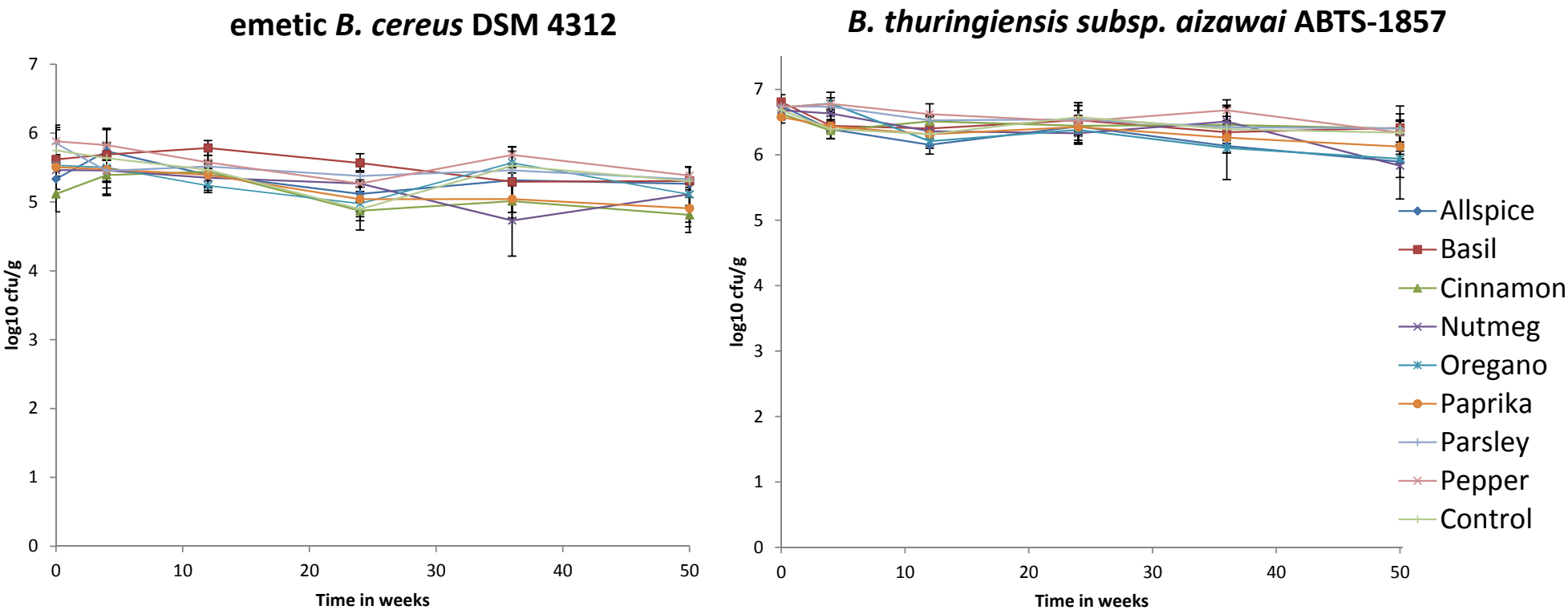
Survival

- spore suspension air dried on 0.5 g sand + 4.5 g spice (3 parallels)
- final concentration 10^5 to 10^6 cfu/g, stored in the dark at 23 ± 1 °C.
- mean reduction rates (log_{10}) of 0.39 ± 0.15 (*B.c.*) and 0.51 ± 0.23 (*B.t.*) after 50 weeks



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Characterization of *B. cereus* group isolates

- presumptive *B. cereus* in allspice (10^3 cfu/g), oregano, parsley (10^2 cfu/g), basil (~ 20 cfu/g), paprika and pepper (< 20 cfu/g)
- 59 isolates confirmed as *B. cereus* s.l., thereof 4 *B. weihenstephanensis*, 2 *B. mycoides* and 1 *B. thuringiensis* strain(s)
- several species in one sample
- all isolates carried toxin genes, except *B. mycoides*
- toxin gene combinations: *nheA*, *hblD* (27x); *nheA*, *hblD*, *cytK* (29x); *nheA*, *cytK*, *ces* (1x) → one emetic strain
- toxin production reflects genetic configuration (confirmed for Nhe, Hbl, cereulide)

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Multiplex real-time-PCR for *B. cereus* group species - Testing of isolates

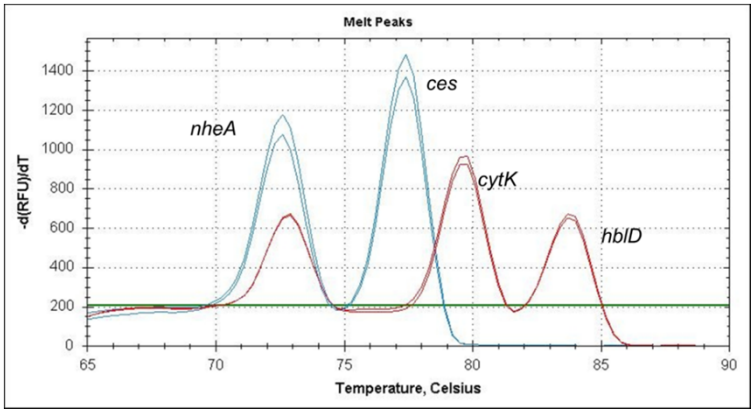
Species (Oliwa-Stasiak, K., et al., 2011; Wielinga, P.R., et al., 2011)

- *B. cereus / B. anthracis / B. thuringiensis*: *motB* gene (MotB_1)*
- *B. weihenstaphanensis*: *motB* gene (MotB_2)*
- *B. pseudomycoides*: *bpm* gene (Bpm_1)
- *cry1* positive *B. thuringiensis* strains: *cry1* plasmid gene (Tqpro_Bt)
- *B. anthracis* : lambda pro-phage type 3 sequence (Tqpro_PL3)

* *B. mycoides* with rhizoid growth

Toxin genes (Wehrle, E., et al., 2010)

HRM multiplex-PCR; for *cytK, nheA, hblD* und *ces*



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Multiplex real-time-PCR for *B. cereus* group species - Testing of spices and herbs

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- *cry1* positive *B. thuringiensis* strains: *cry1* plasmid gene (Tqpro_Bt)
- Internal amplification control: pUC18 fragment (Tm_pUC18)

* *B. mycoides* with rhizoid growth

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DNA extraction from spores in PBS buffer

	LOD[a] (cfu/ml)	Average C_q at LOD (*motB*)
DNeasy Blood & Tissue Kit	1.4×10^2	34.57 ± 0.74
MasterPure Gram Positive DNA Kit	1.4×10^3	35.00 ± 1.36
Invisorb Spin Plant Mini Kit	1.4×10^3	35.09 ± 1.20
Ultraclean DNA Isolation Kit	1.4×10^4	36.51 ± 1.11
PowerSoil DNA Isolation Kit	1.4×10^4	35.44 ± 1.25

[a] Limit of detection: the lowest concentration of spores/ml (*B. cereus* DSM 4312) for which six out of six reactions (three DNA extractions and duplicate PCR reactions) gave a positive result for the *motB* target

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DNA extraction from spores in spices and herbs

		Inoculum (cfu/g) at LOD[a]	Average C_q at LOD (*motB*)
Blood and tissue	Allspice	3.3×10^6	-
	Oregano	3.3×10^6	34.14 ± 1.95
	Paprika	3.3×10^5	34.43 ± 0.88
	Pepper	3.3×10^5	35.97 ± 1.24
CTAB method	Allspice	6.2×10^6	35.87 ± 0.89
	Oregano	6.2×10^2	36.25 ± 1.29
	Paprika	6.2×10^3	34.02 ± 1.38
	Pepper	6.2×10^2	38.22 ± 1.18

[a] Limit of detection: the lowest concentration of spores/g (*B. cereus* DSM 4312) for which twelve out of twelve reactions (three repetitions of duplicate DNA extraction and duplicate PCR) gave a positive result for the *motB* target.
CTAB (cetyltrimethylammonium bromide) method is based on ISO 21571:2013-08 and modifications according to Minarovicova et al., in press.

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Summary

- Low numbers of *B. cereus* in spices and herbs are normal → temperature regimes of dishes are crucial
- Different *B. cereus* group species with varying toxinogenic potential occur in spices and herbs
- Spores persist in spices and herbs
- Detection of *B. cereus* group species is possible at LODs of 10^2 to 10^3 spores/g with CTAB method and real-time PCR

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Thanks for your attention.
Questions and comments?

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