

Detection of spore-forming bacteria in dairy products

Paul Cotter/Kieran Jordan

Teagasc Food Research Centre, Moorepark, Fermoy, Co.Cork

Harvesting and ensiling



Silage consumption



Silage $<10^2 - 10^7$

Grass $<10^2 - 10^4$

Cow



Defecation

Soil $<10^2 - 10^5$

Faeces $<10^2 - 10^8$

Udder contamination

Manure fertilization



Manure storage $<10^2 - 10^7$



Milk $<10^1 - 10^2$

Eliminating thermotolerant bacteria to improve the quality of powdered dairy ingredients

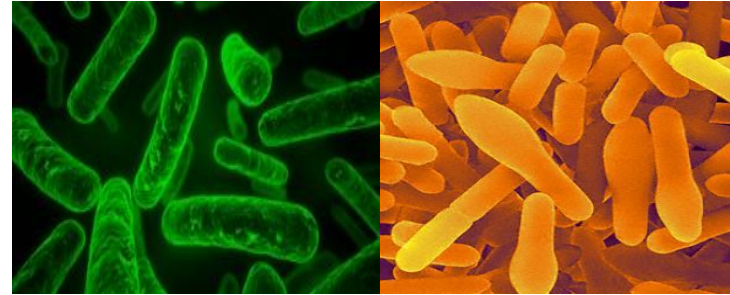
- (A) develop methods to facilitate the rapid identification of these bacteria,
- (B) identify the industrial cleaning-in-place agents that work most effectively against these microbes and
- (C) reveal food-grade antimicrobials which can (i) control the renewed build-up of these bacteria during processing and (ii) prevent their outgrowth when used as ingredients.

Thermotolerant bacteria

- ❑ *Bacillus cereus* spp. – Gram positive, spore forming, toxin producing food borne pathogen
- ❑ Sulfite reducing *Clostridia* spp. – Gram positive anaerobic spore forming food borne pathogens (SRCs)

Problem: Ubiquitous in nature – FOUND EVERYWHERE!

What are SRCs



- ❖ Group of phenotypically distinctive sporeformers belonging to the order *Clostridiales*
- ❖ Distinguished by their ability to reduce sulphite to sulphide under anaerobic conditions
- ❖ Multiple phenotype specific agar assays designed to detect SRCs
- ❖ All rely on the reduction of ferric sulphite to iron sulphide, and the accompanying colour change

Why are SRCs important

- ❖ *Clostridium* sp. are found widespread throughout the dairy farm environment
- ❖ Sporeformers can survive commercial pasteurisation
Germination later
- ❖ Include pathogenic and spoilage associated species
Clostridium botulinum , *Clostridium perfringens*, *Clostridium tetani*,
Clostridium butyricum & *Clostridium beijerinckii*
- ❖ Used as indicator organisms in the food industry

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A case of infant botulism with a possible link to infant formula milk powder: evidence for the presence of more than one strain of *Clostridium botulinum* in clinical specimens and food

M M Brett¹, J McLauchlin¹, A Harris², S O'Brien^{1†}, N Black⁴, R J Forsyth⁵, D Roberts¹ and F J Bolton^{1†}

Author Affiliations

Correspondence J. McLauchlin jim.mclauchlin@hpa.org.uk
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Abstract

Infant botulism was confirmed in a 5-month-old female by both isolation of *Clostridium botulinum* type B and by detection of type B botulinum neurotoxin in rectal washout and faeces. DNA fingerprinting of nine isolates from faeces yielded two different amplified-fragment length polymorphism (AFLP) patterns.

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SRCs: Use of molecular approaches to assess the factors which impact on the biota of milk

Focus on farm level and milk at-farm

SRC are poorly defined – initial task to isolate SRC from culture and sequence them to identify them!!

90% are clostridia, but some others not recognised as SRC – *B. licheniformis*

- To analyze the composition of the sulphate-reducing *Clostridium* (SRCs) and sulphate-reducing bacteria (SRBs) in milk**
- Progress: Identify common gene clusters between isolates; design primers**
- Use of culture-independent approaches to assess/evaluate the impact of a variety of factors (including seasonality and storage temperature) on the microbiota of milk**
- Progress: Currently being done**

Surveillance of powders

- ❖ Working with dairy processors in Ireland
- ❖ Have received some SRC-containing dairy powders and cheese (as well as colonies of agar plates)
- ❖ Tested them according to protocols provided
- ❖ Isolated pure cultures of SRCs, stocked and identified isolates by sequencing the 16S rRNA gene

Molecular assay for detection of SRCs

- ❖ qPCR based assay targeting genes responsible for this phenotype
- ❖ Isolate and identify more SRCs to help identify common genes
- ❖ Target these genes to make a more rapid and specific phenotypic assay
- ❖ Have identified a possible target gene cluster, but work is on-going
- ❖ Early PCR results



Eliminating thermodurics to improve the quality of powdered dairy ingredients

- ❑ Focused on detection of aerobic spores in powders/milk

- ❑ Objectives
- ❑ **1) Survey the species of spore-forming bacteria present in powdered dairy ingredients generated by Irish Dairy Companies and generate a rapid real-time PCR assay to detect, differentiate between and quantify spore-forming bacteria**
- ❑ **2) Identification of food-grade antimicrobials with activity against spore-forming bacteria**
- ❑ **3) Studies on biofilm formation and control in laboratory scale reactors**
- ❑ **4) Develop approaches to prevent the outgrowth of spore-forming bacteria during secondary processing**

Culture methods

- ❑ Culture methods compared –
- ❑ MYP (Mannitol Egg Yolk Polymyxin Agar) v Bacarra

Analysis

- Raw samples – not pasteurised
- Serially diluted
- Plated on MYP
- Incubated at 32°C for 48 hours
- Confirmation on blood agar – β haemolysis
- Isolated for 16S rRNA identification



Identify 10 milk sample isolates from MYP

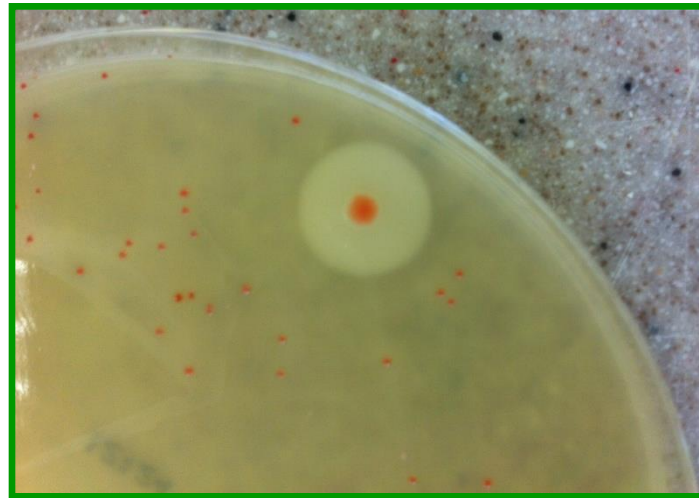
Sample	Identified species (Homology)	BC species (Homology)
1	Macrocooccus sp. KW16 (99%)	Bacillus mycoides strain JP44SK50 (94%)
2	Staphylococcus sp. ChDC B592 (99%)	Bacillus thuringiensis serovar konkukian str. 97-27 (93%)
3	Enterobacter aerogenes (95%)	Bacillus cereus strain M-7 (81%)
4	Pseudomonas gessardii strain AMHSOL259 (99%)	Bacillus weihenstephanensis strain CtST10.5 (88%)
5	Pseudomonas trivialis strain KOPRI 25674 (99%)	Bacillus weihenstephanensis strain CtST10.5 (88%)
6	Yersinia sp. UA-JF0918 (100%)	Bacillus weihenstephanensis strain CtST10.5 (85%)
7	Uncultured bacterium clone S11_049 (95%)	Bacillus anthracis strain TMPTTA CASMB 6 (87%)
8	Enterococcus faecalis strain L3B1K3 (99%)	Bacillus cereus strain AGP-03 (92%)
9	Staphylococcus simulans strain QTR-52 (98%)	Bacillus cereus strain LCB46 (93%)
10	Lactococcus lactis subsp. cremoris strain RU36-7 (99%)	Bacillus anthracis strain HDDMM10 (86%)



No isolate was identified as a *Bacillus cereus* species.

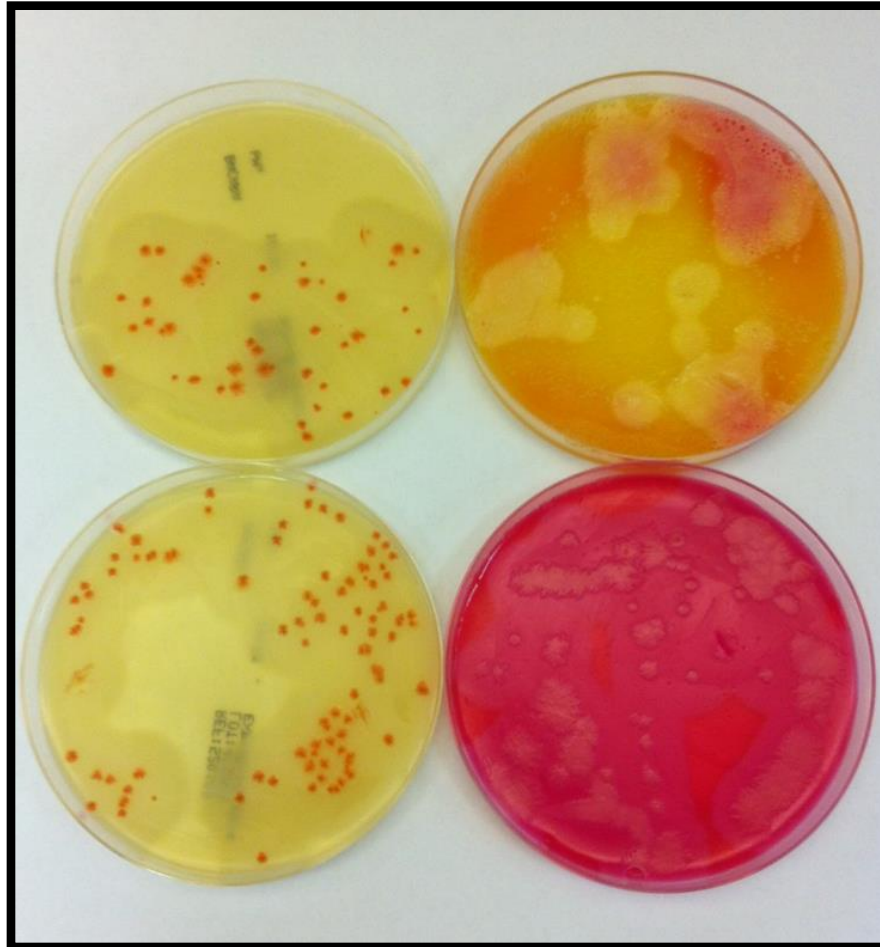
Compare MYP to BACARA agar

- Bacara agar – FDA recommended
- Pre-poured plates are bought from Biomerieux
- Incubated at 32°C for 24 hours



Compare MYP to BACARA agar

Raw



Pasteurised

Compare MYP to BACARA agar

Sample	Bacara identified species	MYP identified species
1	<i>Bacillus cereus</i> strain	<i>Staphylococcus hominis</i> strain
2	<i>Bacillus mycoides</i> strain	<i>Lactococcus lactis</i> strain
3	<i>Bacillus thuringiensis</i>	<i>Bacillus cereus</i> strain BE4-1 <i>Bacillus cereus</i> strain HKS1-1
4	<i>Bacillus cereus</i> strain	Bacterium M133-5
5	Not enough DNA	<i>Bacillus mycoides</i> strain
6	<i>Enterococcus</i>	<i>Lactococcus</i>

Conclusion – this section

- ❑ Bacara agar is more selective for *Bacillus cereus* group, especially for raw milk samples

Non-culture methods

Several existing PCR methods based on toxin detection.

- ❑ Duopath® Cereus enterotoxins - confirms the presence of the diarrhoeal enterotoxins of *B. cereus*. The kit has a detection limit of 100 CFU/g
- ❑ *B. cereus* detection kit - target the *B. cereus*-specific gene GroEL.
- ❑ *B. cereus* real-time PCR kit - real-time PCR kit detects the presence of an amplified *B. cereus* DNA fragment and toxins are identified

Develop a multiplex assay combining these method

Will detect *B. cereus* but not other *B. cereus* group bacteria.



Thank you for your attention