Detection of spore-forming bacteria in dairy products

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Harvesting and ensiling

Silage consumption

Silage $<10^2$ - 10^7

Cow

Defecation

Faeces $<10^2$ -10^8

Udder contamination

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

Manure fertilization

Soil $<10^2$ -10^5

Grass $<10^2$ - 10^4

Milk $<10^1$ - 10^2

Eliminating thermodurics 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.

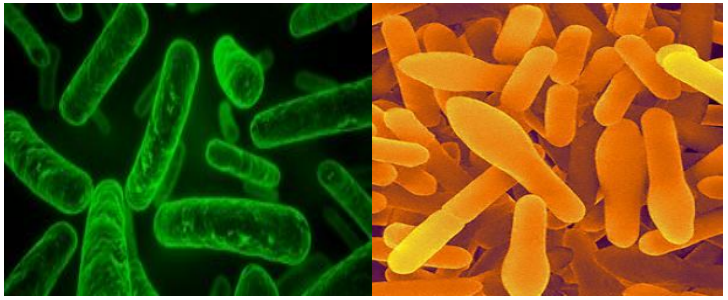
Thermoduric bacteria

- *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 beijerinkii

- 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

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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

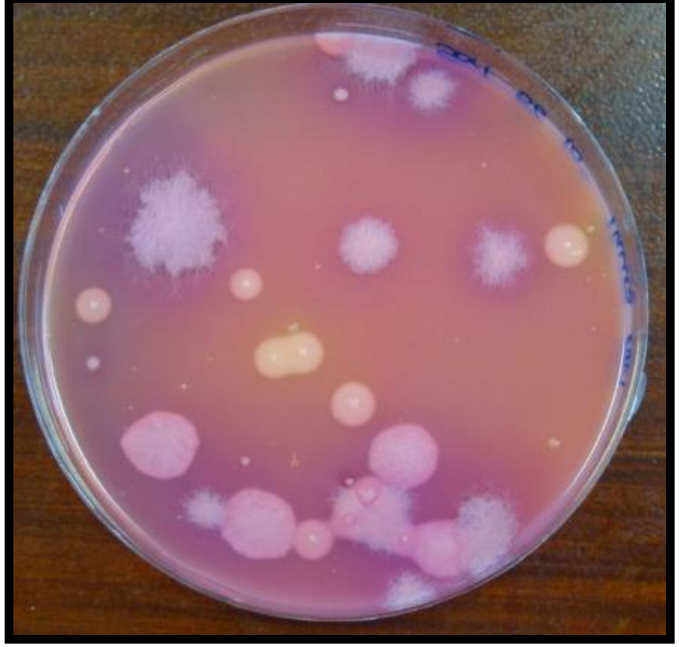
- Focused on detection of aerobic spores in powders/milk

- 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

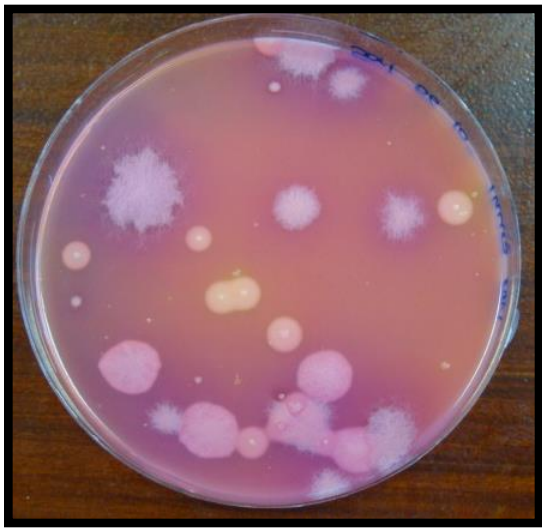
Analysis



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

Identify 10 milk sample isolates from MYP

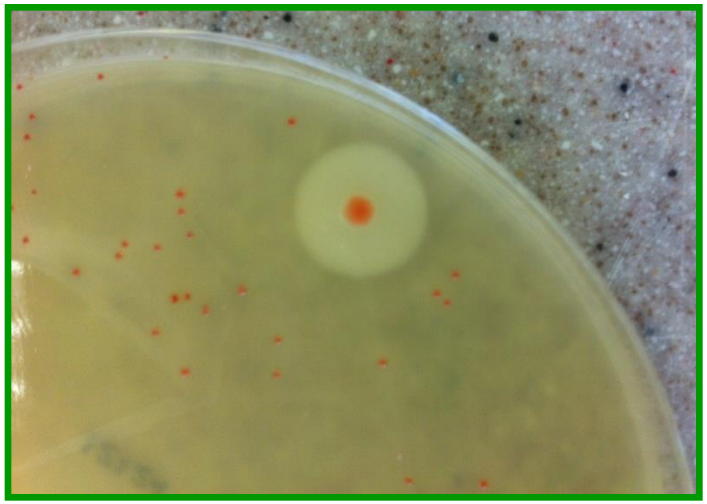
Sample	Identified species (Homology)	BC species (Homology)
1	Macrococcus sp. KW16 (99%)	Bacillus mycoides strain JP44SK50 (94%)
2	Staphylococcus sp. ChDC B592 (99%)	Bacillus thuringiensis serovar konkukian str. 97-27 (93%)
3	Enterobacter aerogenes (95%)	Bacillus cereus strain M-7 (81%)
4	Pseudomonas 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.

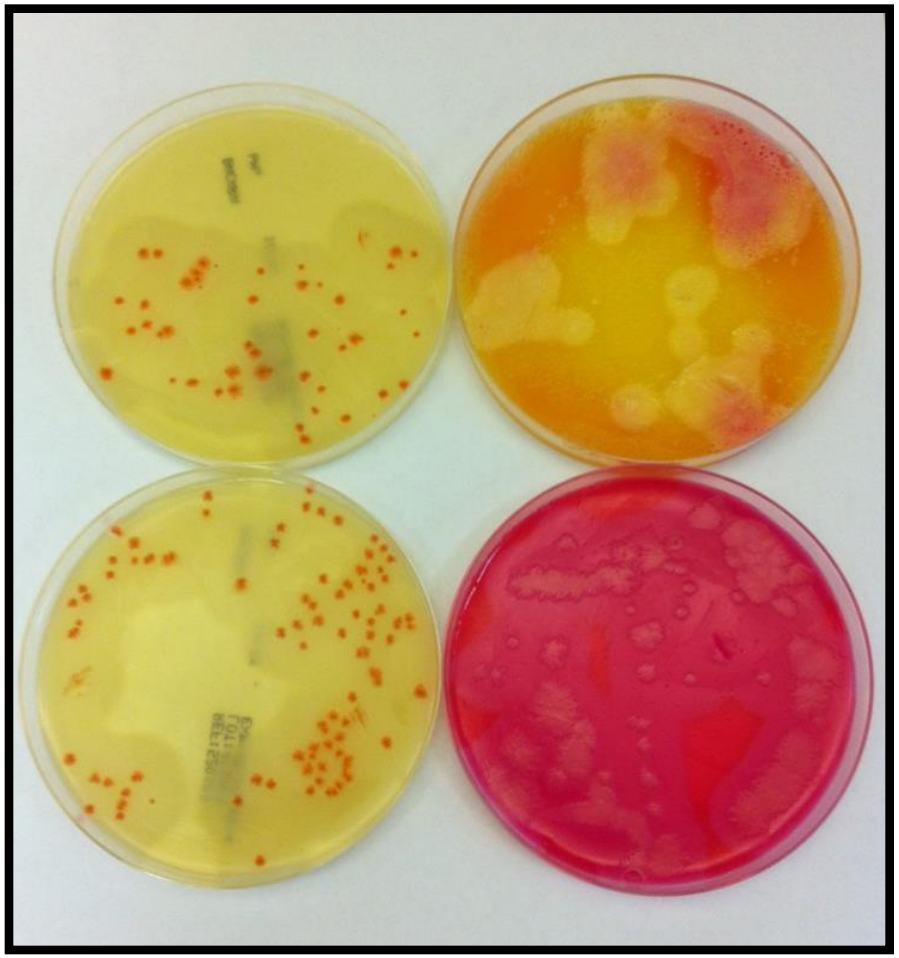
Compare MYP to BACARA agar

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



Compare MYP to BACARA agar

Raw



Pasteurised

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Compare MYP to BACARA agar

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

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