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Risk characterisation of *Bacillus cereus* in Extended Shelf Life (ESL) milk

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B. cereus

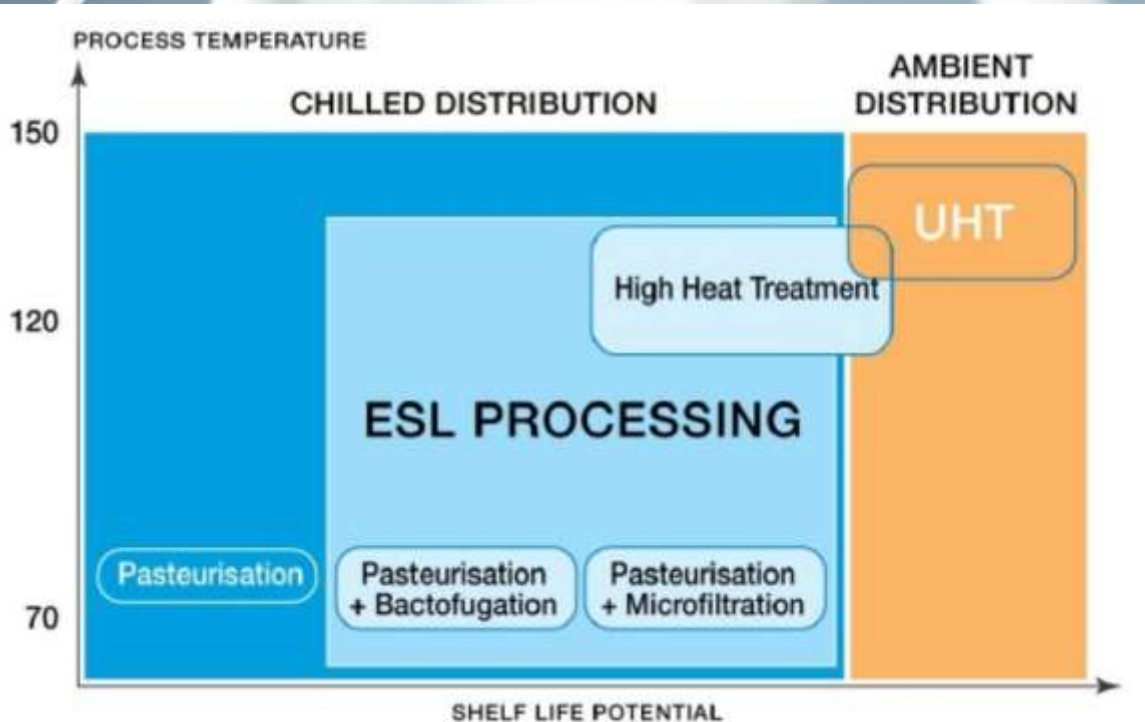
- Characteristics of *B. cereus*
- Sources
- Mesophilic vs Psychrotrophic strains
- Gastrointestinal disease
 - Diarrheal
 - Emetic syndrome

B. cereus outbreaks

- CDC - 1 in 6 Americans get sick, 128,000 are hospitalized, and 3,000 die of foodborne diseases
- Naranjo *et al*, (2011) -*B. cereus* food poisoning case that claimed a life in America
- Al-Abri *et al* (2011) an outbreak
- Public Health England, July 2014 - *B. cereus* implicating hospital in the Food and Drug Administration (FDA) outbreak implicated in intravenous liquid (Total Parenteral Nutrition, TPN)
- Dierick *et al* cases across Europe
- 23 cases were reported in 9 were confirmed same 17 who died in the year at a number of day
- Argentina the Health Ministry reported 19 cases of food-borne disease linked to *B. cereus* poisoning in Padartha in Germany were life of the youngest after consumption of a contaminated rice pudding from the same caterer

What is the risk pertaining to *B. cereus* in South Africa ?????

Case study: ESL milk processing



- Ultra-pasteurisation
- Non thermal methods
 - Bactofugation
- Cold filling
- Recontamination

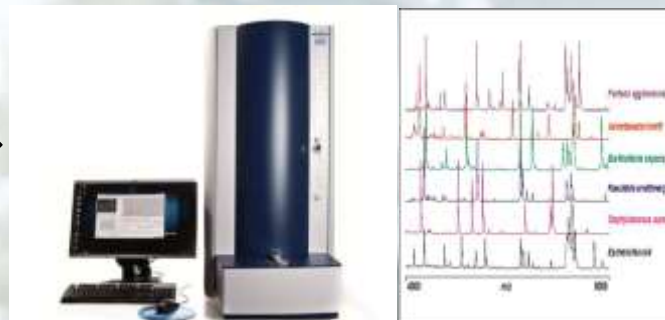
- Bacterial diversity
 - *Bacillus* spp. & *Paenibacillus* spp.

Research objective

- Characterise the risk of *B. cereus* in ESL milk processing

Research Approach

Isolation of aerobic spore forming bacteria from Raw milk, Milk after pasteurisation, ESL milk & Filler nozzles



Identification of Isolates using MALDI TOF MS

B. Cereus isolates

1. Rep PCR (GTG₅)
2. End point PCR

Discrimination of psychrotrophs & mesophiles

1. Growth rate at 7°C

2. Enzyme Activity

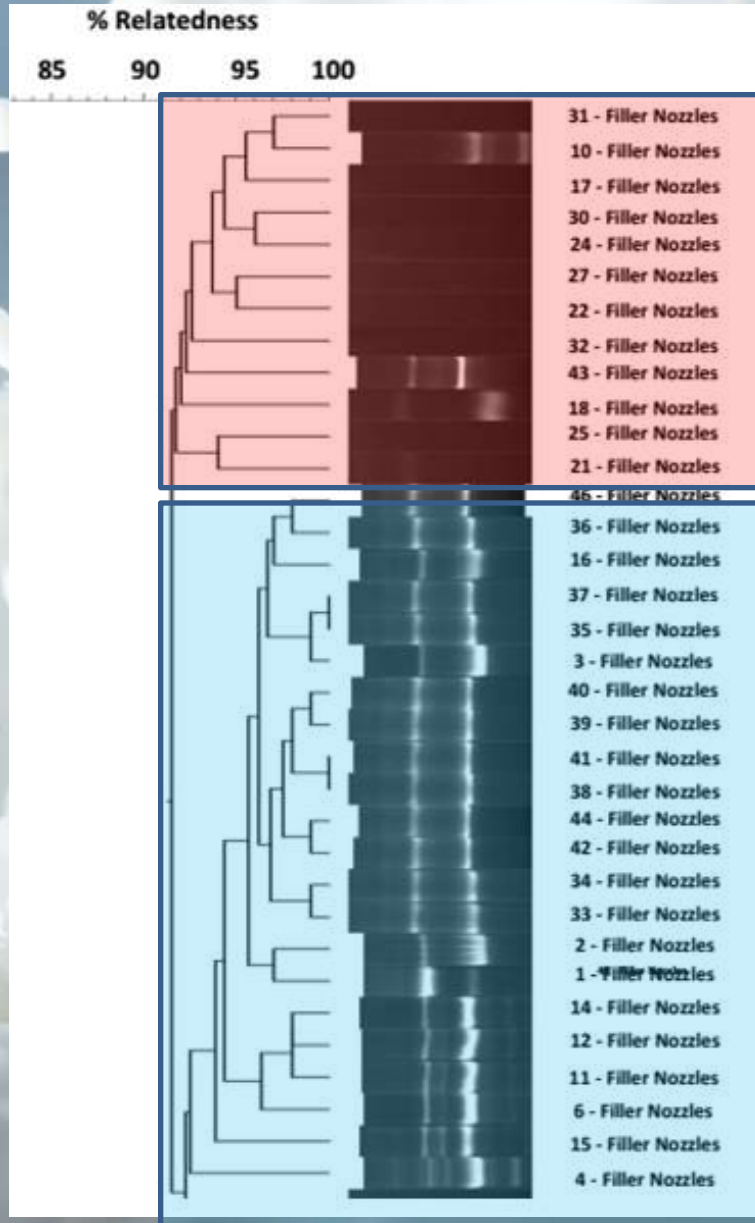
- Lipolysis
- Proteolysis

3. Haemolysis

B. cereus prevalence & enzyme activity

- 49 Isolates were identified as *B. cereus*
- There was 9.7 % *B. cereus* prevalence in the milk samples
- All *B. cereus* isolates were positive for proteolytic activity
- All *B. cereus* isolates were negative for lypolytic activity
- **All *B. cereus* were positive for haemolysis**

B. cereus (GTG₅) fingerprint patterns



- Filler nozzles only

Fig 1: Dendrogram of Rep PCR (GTG₅) fingerprint patterns in *B. cereus* isolates obtained from Extended Shelf Life milk filler nozzles after CIP process.

B. cereus (GTG₅) fingerprint patterns

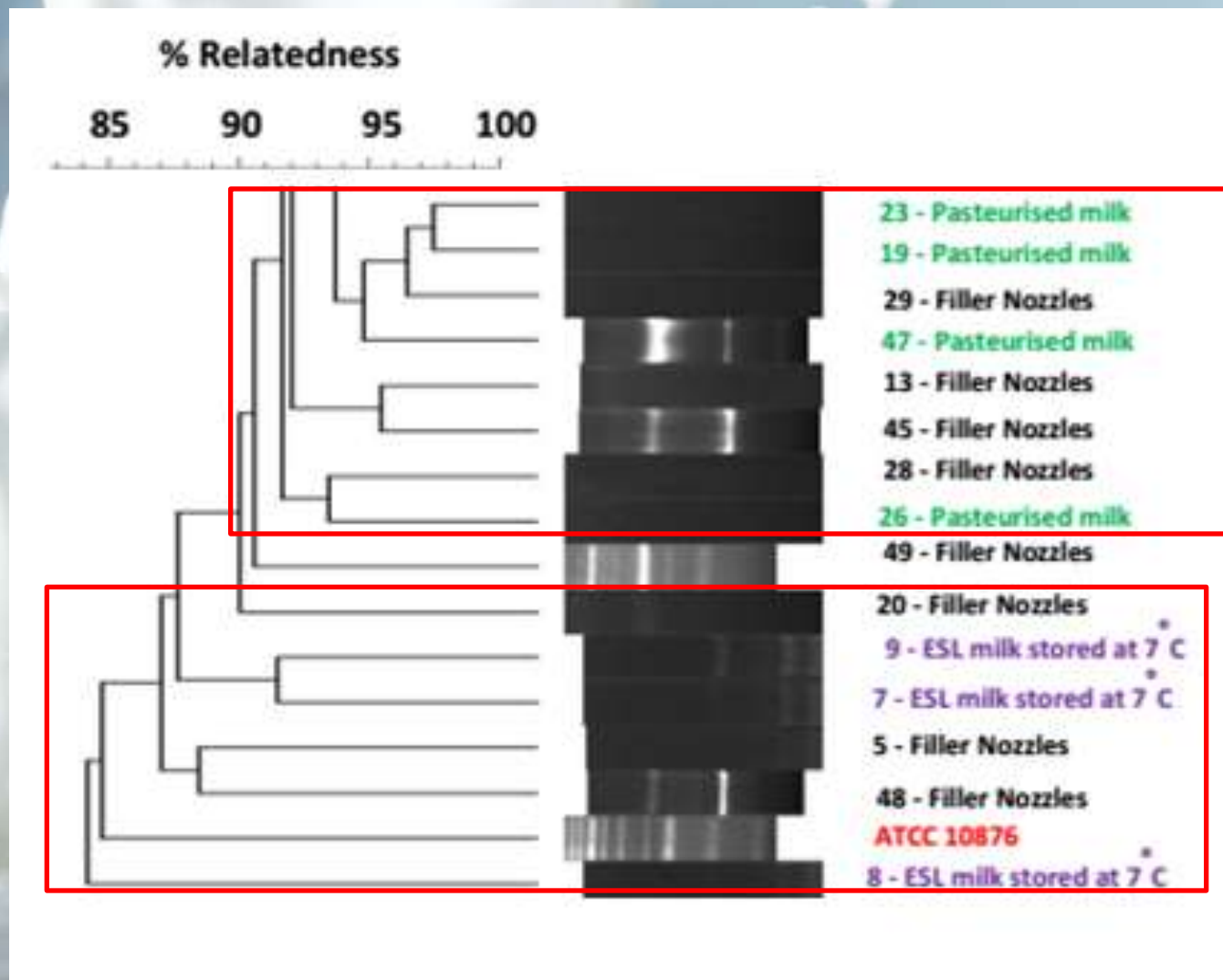


Fig 2: Dendrogram of Rep PCR (GTG₅) fingerprint patterns in *B. cereus* isolates obtained from Extended Shelf Life milk filler nozzles after CIP process, Extended Shelf Life milk during processing and storage at 7 °C.

Discrimination of psychrotrophic and mesophilic *B. cereus* strains

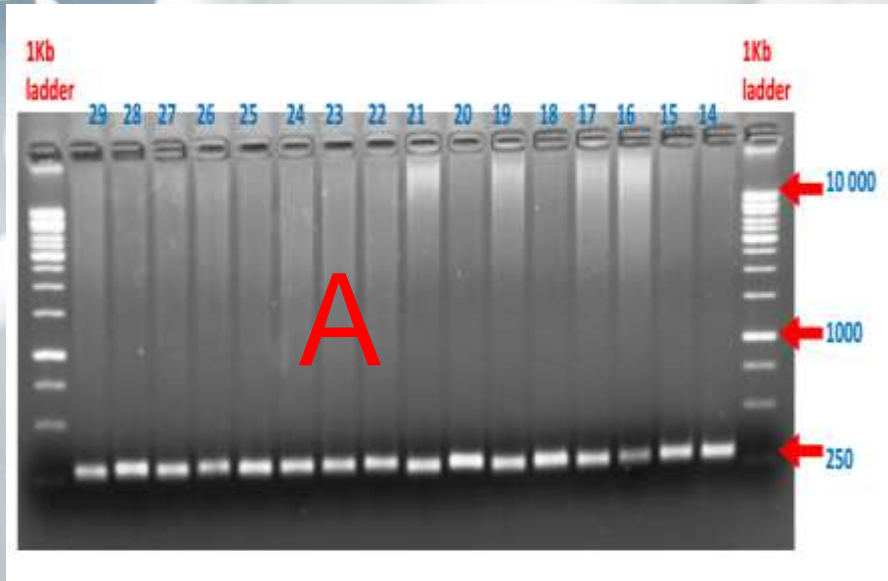


Fig 3: Agarose gel electrophoresis showing the detection of mesophilic *Bacillus cereus* strains by targeting the 250-bp mf-ur 16S-rDNA signature.

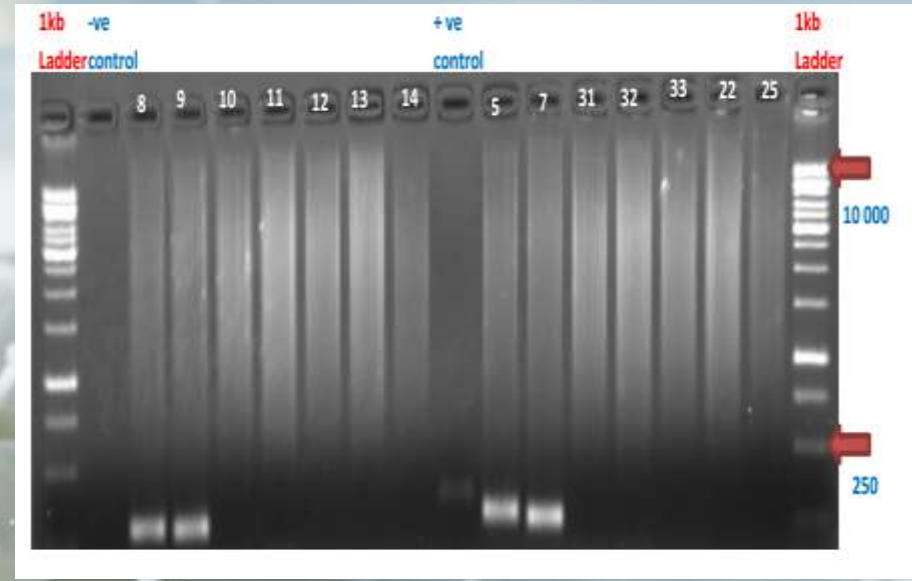


Fig 4: Agarose gel electrophoresis showing the detection of mesophilic *Bacillus cereus* strains by targeting the 130 – 150 bp uf-pr 16S-rDNA signature.

- All *B. cereus* isolates had amplification with primer pair mf-ur which amplifies the mesophilic 16s signature
- Only 4 Isolates of *B. cereus* under study had amplification with primer pair uf-pr which amplifies the psychrotrophic 16s signature

Growth of *B. cereus* in milk at 7°C

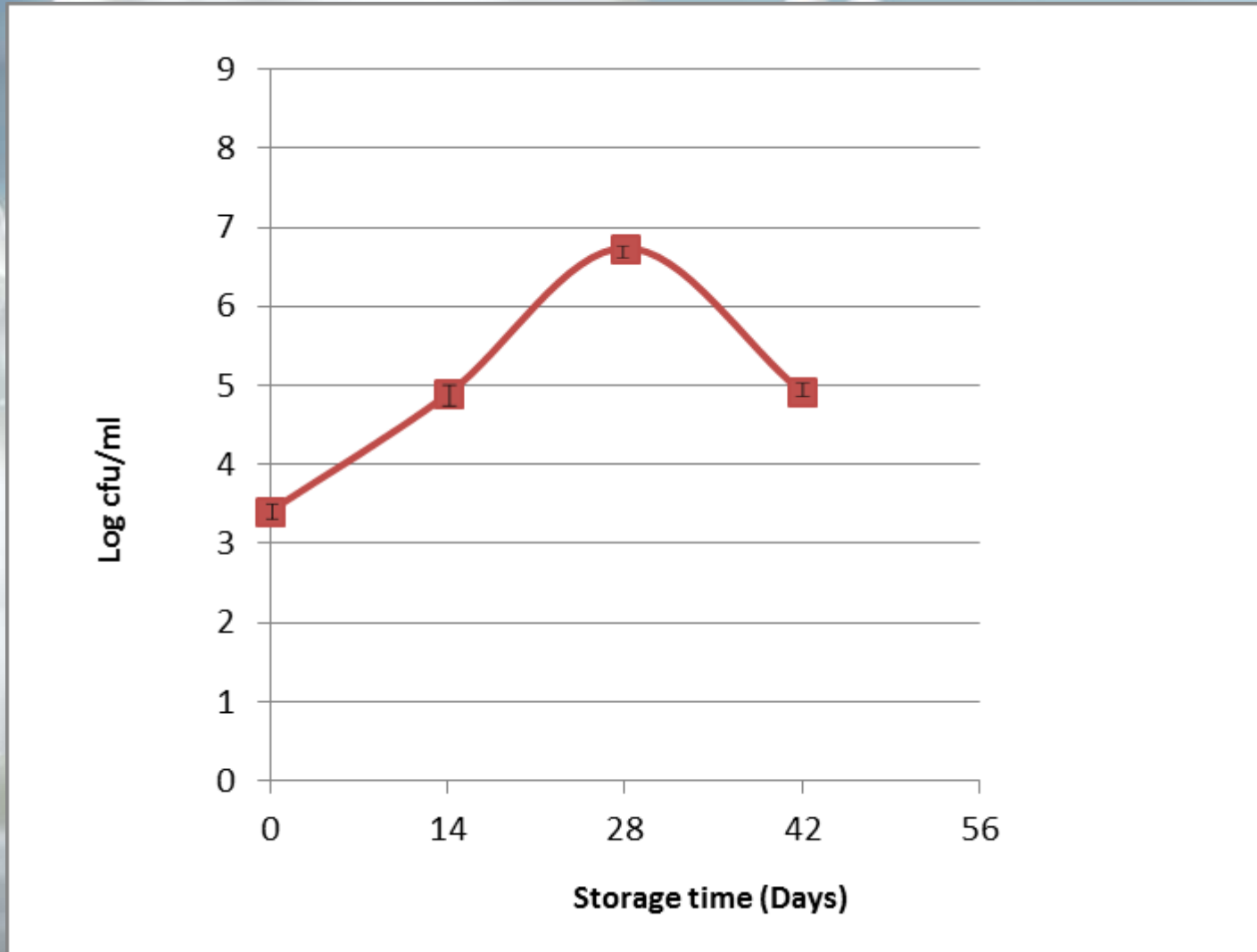


Fig 5: Growth of *B. cereus* strain in milk at 7 °C over 42 days

Growth rate of *Bacillus* & *Paenibacillus* spp. in milk at 7°C

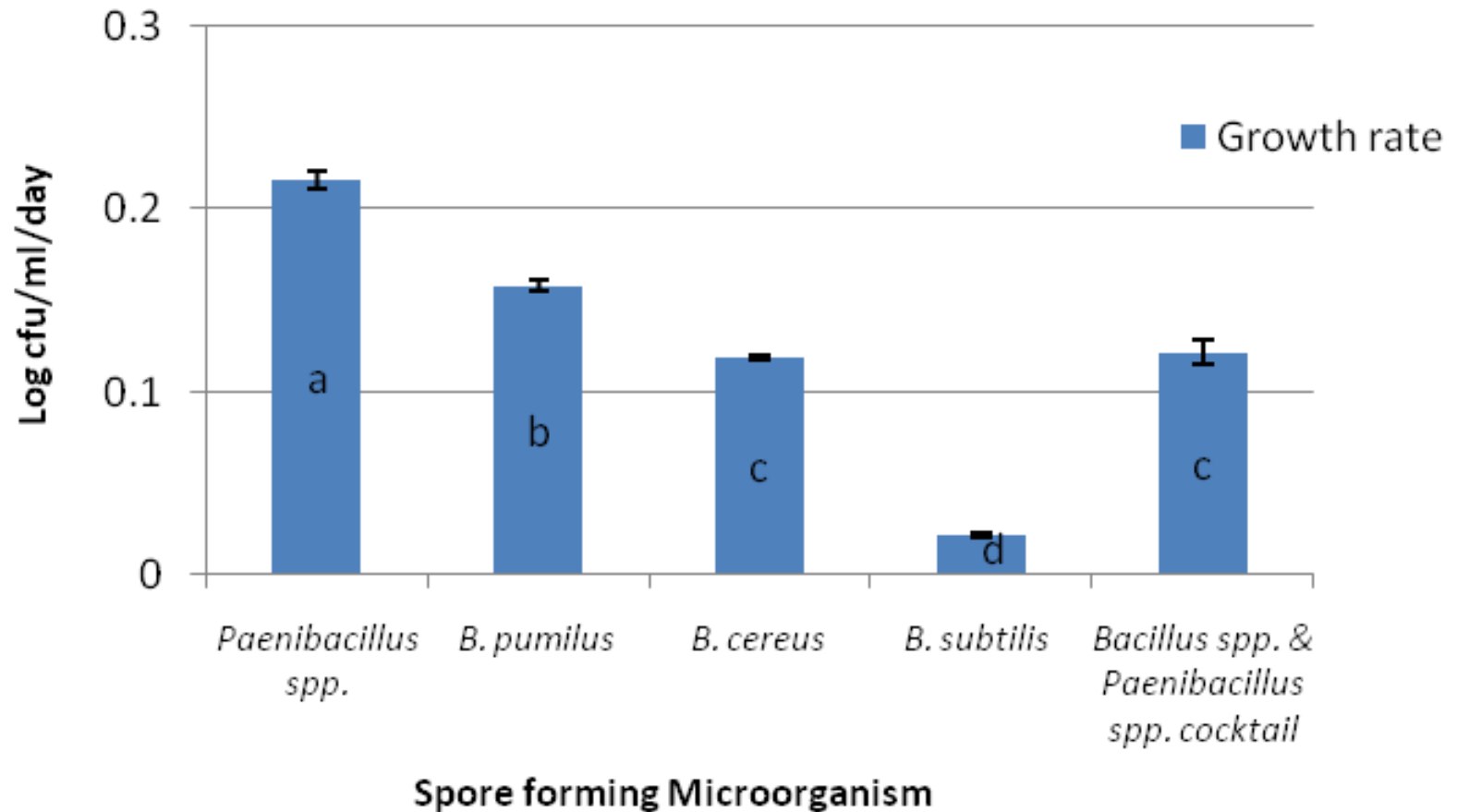


Fig 6: Growth of rate of *B. cereus*, *B. pumilus*, *B. subtilis*, *Paenibacillus* spp. and *Bacillus* - *Paenibacillus* spp. cocktail s in milk at 7 °C

Bacterial species with different letter labels have mean growth rates that are significantly different

Hazard Exposure Summary

Product type

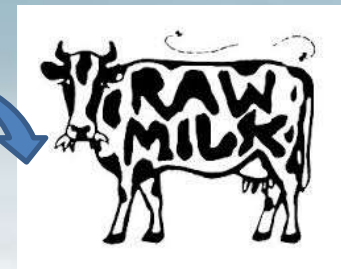


Microbiological hazard



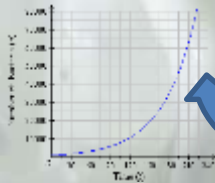
B. cereus

Raw material contamination



- Frequency up to 100%
- Contamination levels
< 100 cfu/ml
< 10 000 cfu/m – spore-formers

Storage, consumer use & bacterial growth



Packaging & decontamination



- No decontamination

Process & contamination



- Isolated in low levels
< 100 cfu/cm²
- Attachment to Stainless steel
Khoza & Buys 2014

- Temperature abuse
 - Shopping habits
 - Power cuts
- Storage time
- *B. cereus* population after 14 days at 7°C storage

Acknowledgements

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gracias

Tatenda

ありがとう

Dankie

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Siyabonga

Thank you

Merci



Ke a leboga

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References

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