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

Desmond T. Mugadza

Supervisor : Prof Elna M. Buys

Department of Food Science University of Pretoria

21st SAAFoST International Congress and Exhibition

Durban, South Africa. 7 - 9 September 2015

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 implicating hospital kitchen food affecting 58 people
- Dierick *et al*, (2005) - 5 children from the same family who were [illegible] B. *cereus* poisoning incident that claimed the life of the youngest girl

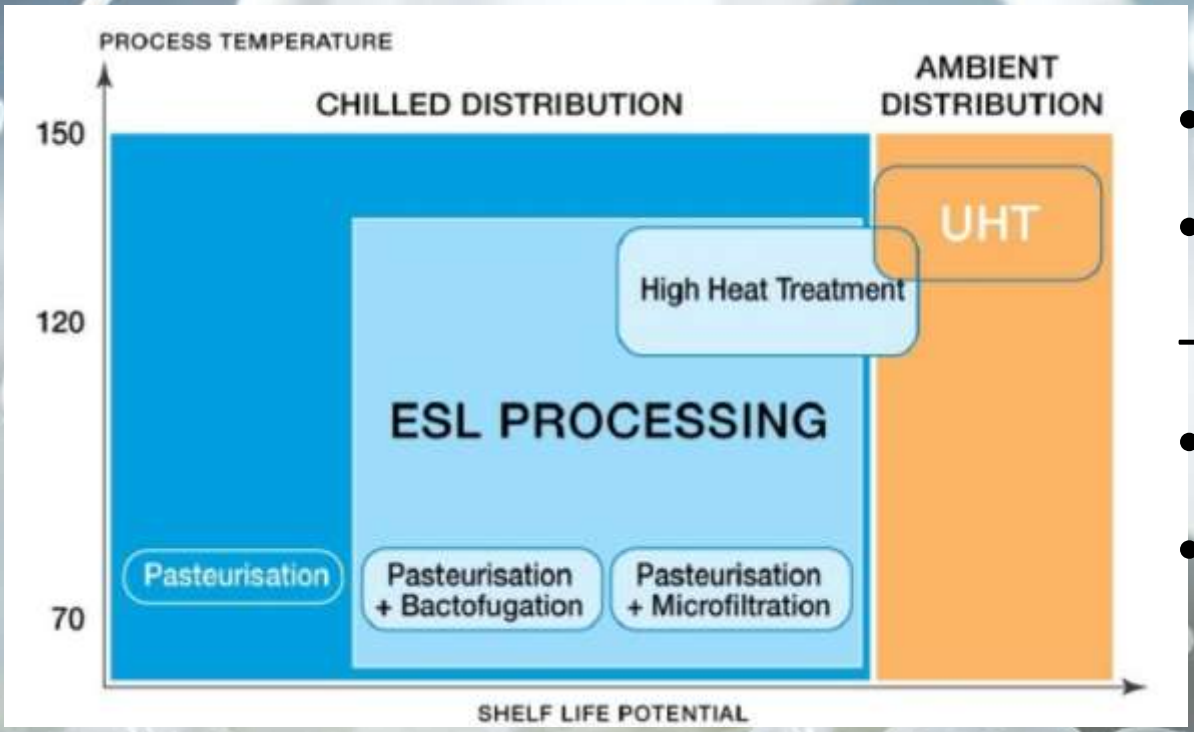
- Public Health England, July 2014 - *B. cereus* outbreak implicated in intravenous liquid (Total Parenteral Nutrition, TPN).
- 23 cases were reported, 19 were confirmed while 3 children died and the whole batch of the product recalled.

- In April 2015 the Infection Control Today reported an increase in the *B. cereus* infection cases across Europe,
- [illegible] children and staff at a number of day care centres near Paderborn in Germany were infected after consumption of a contaminated rice pudding from the same caterer

Argentina the Health Ministry reported 1778 cases of food-borne disease between [illegible] and 2013, of which 26 were positive for *B. cereus*.

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

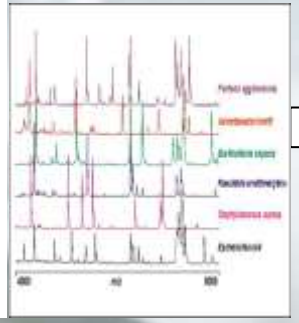
- Bacterial diversity
  - *Bacillus* spp. & *Paenibacillus* spp.

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 (GTG_5)**
2. **End point PCR**

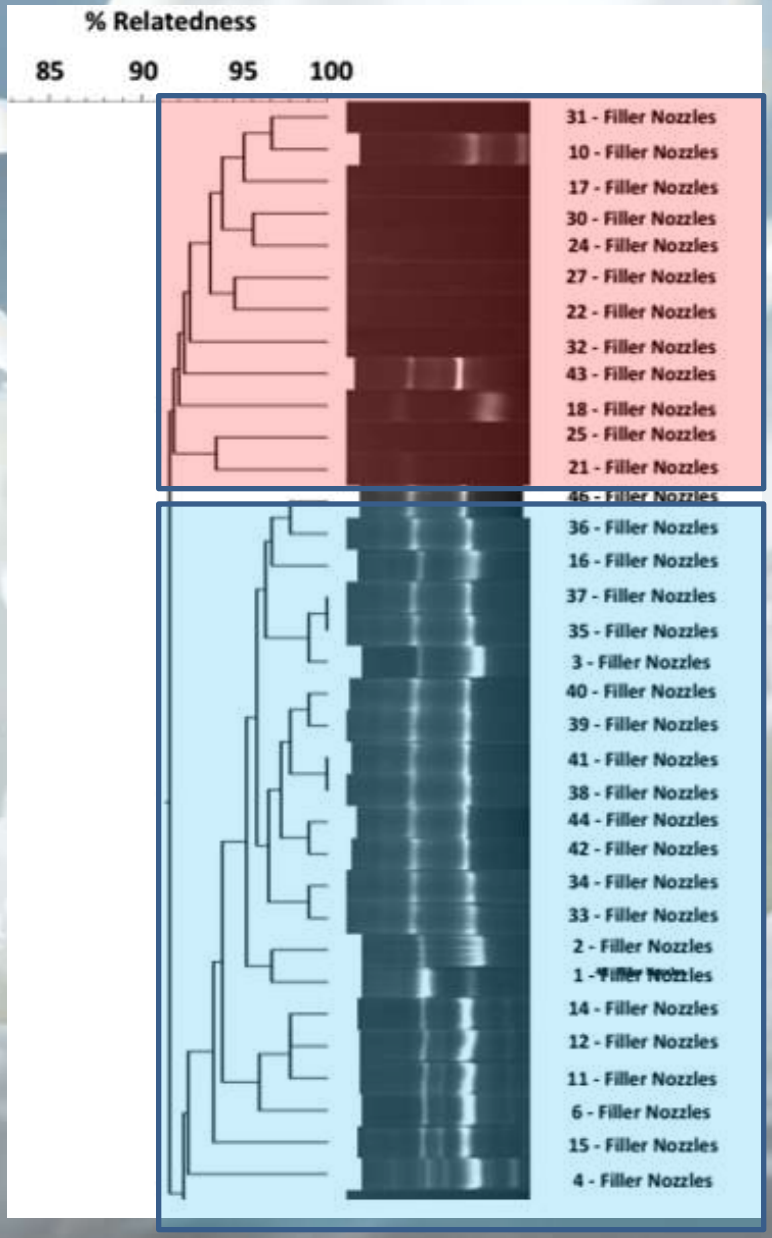
Discrimination of psycrotrophs & mesophiles

1. **Growth rate at 7^{O}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 (GTG_5) fingerprint patterns



- Filler nozzles only

Fig 1: Dendrogram of Rep PCR (GTG_5) fingerprint patterns in *B. cereus* isolates obtained from Extended Shelf Life milk filler nozzles after CIP process.

B. cereus (GTG_5) fingerprint patterns

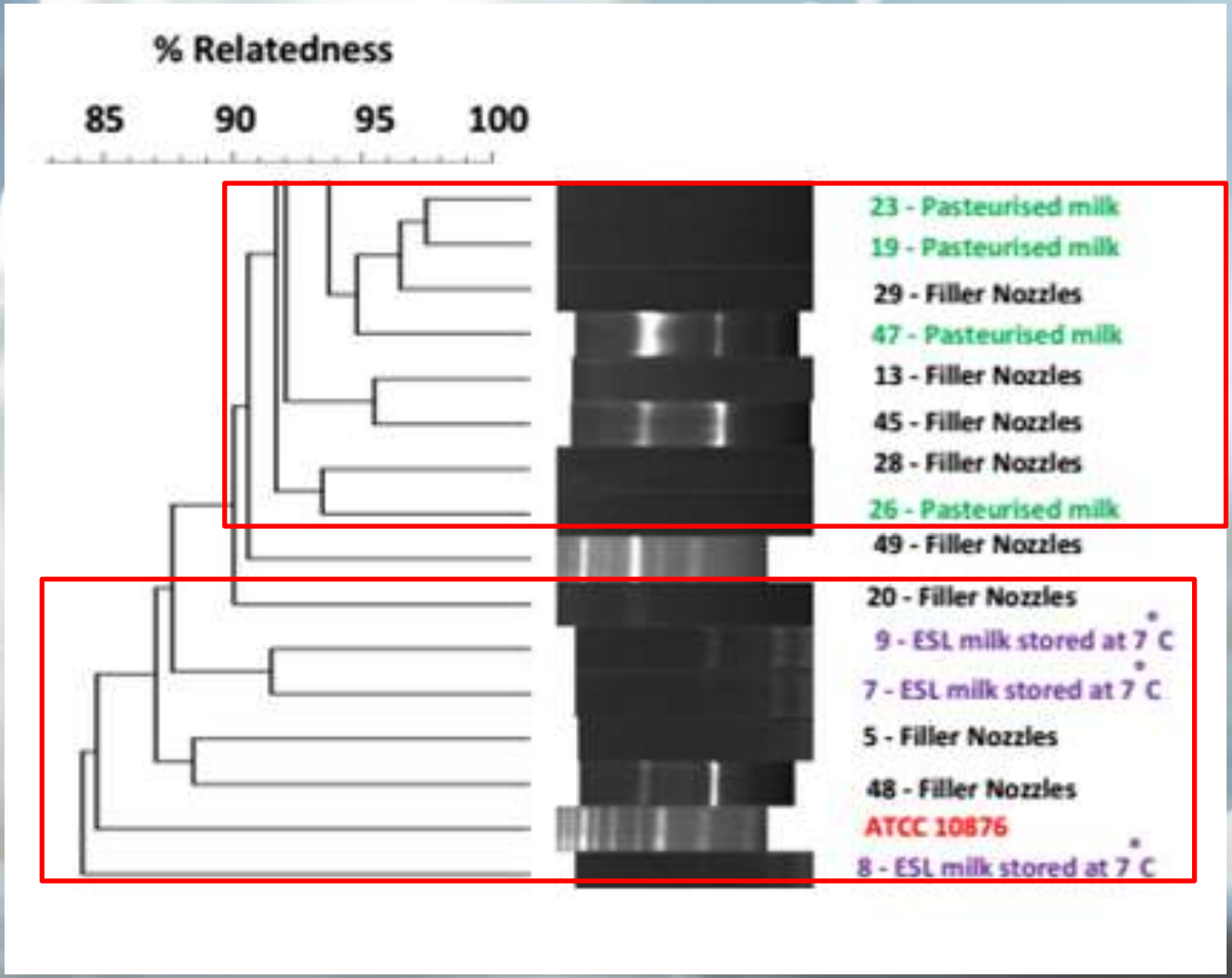


Fig 2: Dendrogram of Rep PCR (GTG_5) fingerprint patterns in *B. cereus* isolates obtained from Extended Shelf Life milk filler nozzles after CIP process, Extended Shelf Life milk during processing and storage at 7 °C.

Discrimination of psychrotrophic and mesophilic *B. cereus* strains

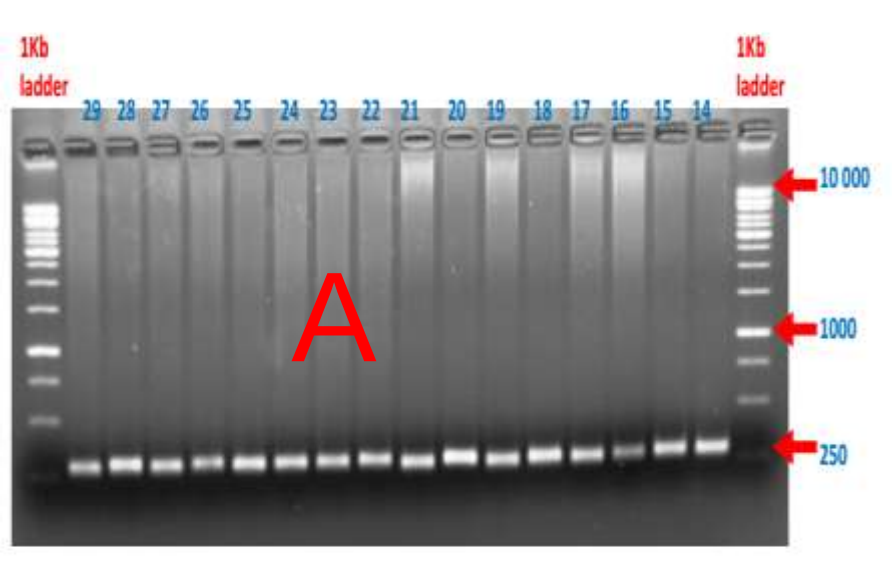


Fig 3: Agarose gel electrophoresis showing the detection of mesophilic *Bacillus cereus* strains by targeting the 250-bp mf-ur 16S-rDNA signature.

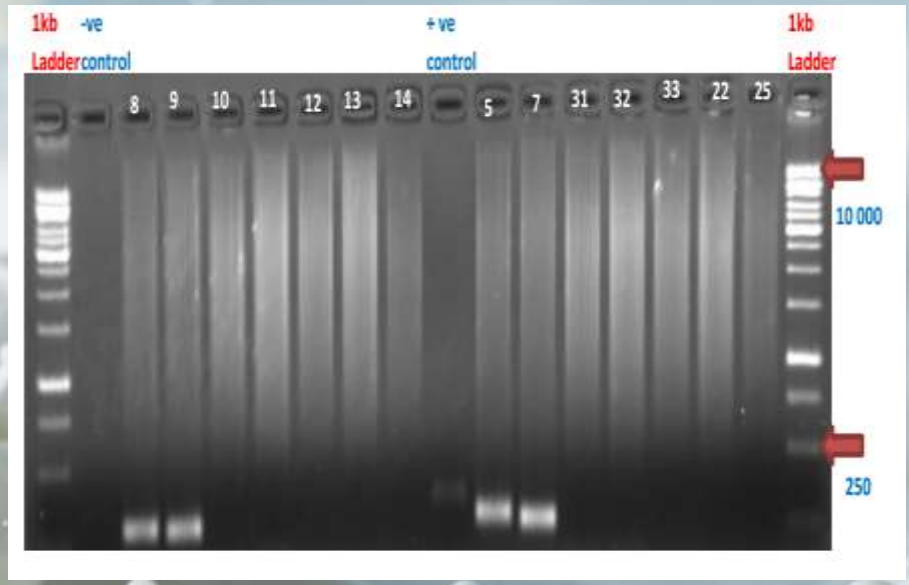


Fig 4: Agarose gel electrophoresis showing the detection of mesophilic *Bacillus cereus* strains by targeting the 130 – 150 bp uf-pr 16S-rDNA signature.

- **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

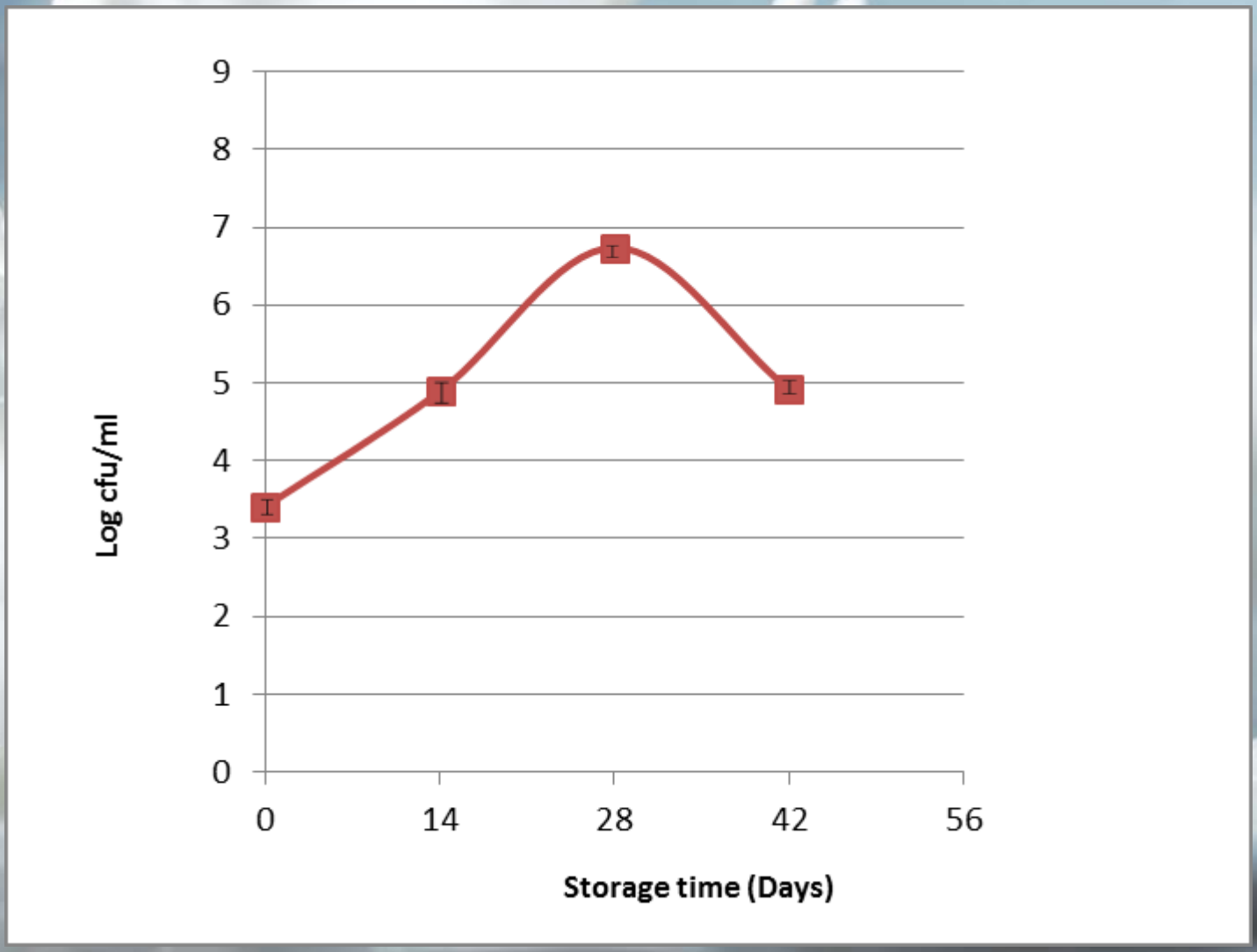


Fig 5: Growth of *B. cereus* strain in milk at 7 °C over 42 days

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

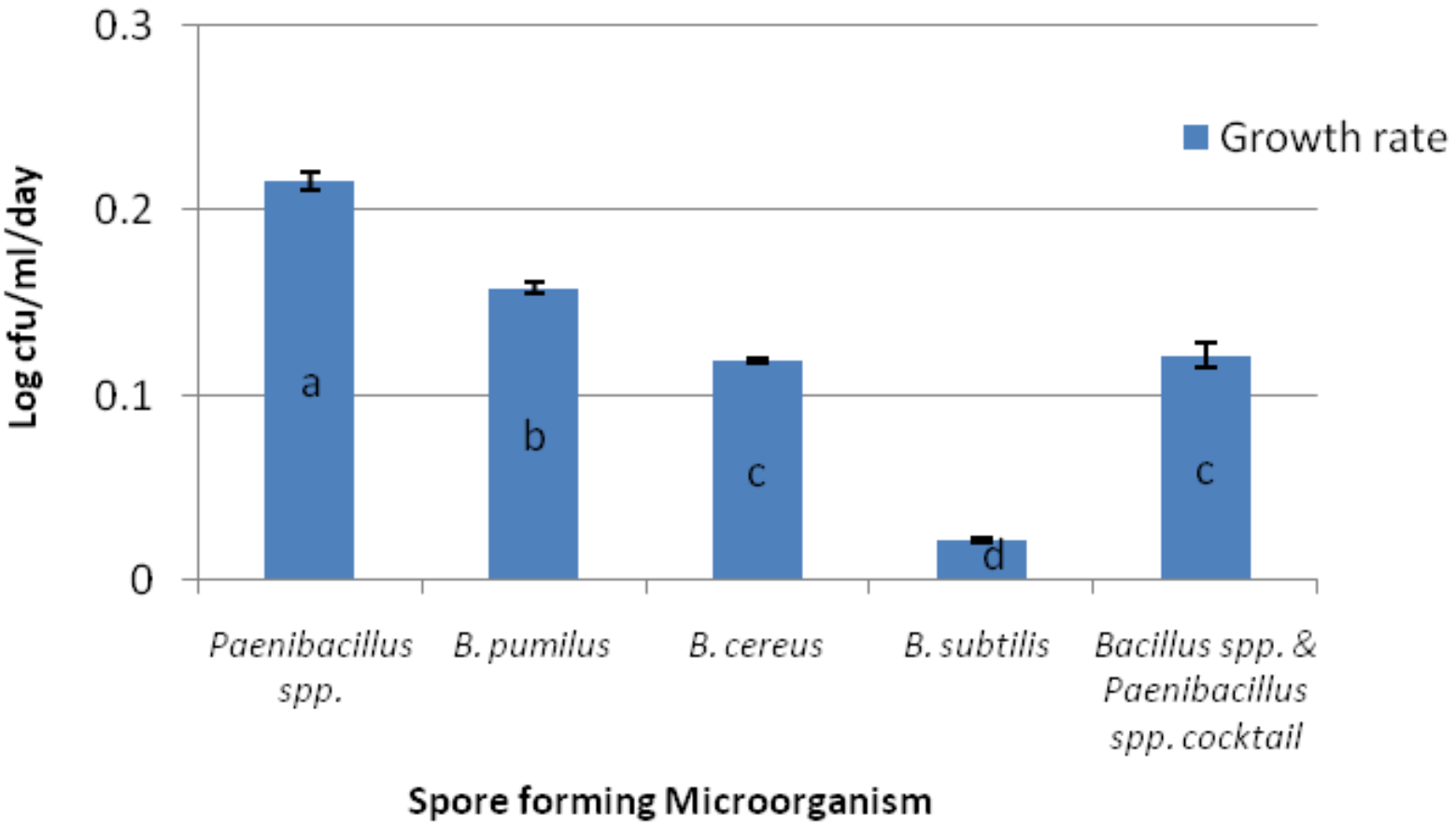


Fig 6: Growth of rate of *B. cereus, B. pumilus, B. subtilis, Paenibacillus* spp. and *Bacillus - Paenibacillus* spp. cocktail s in milk at 7 °C

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

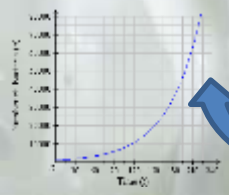
Process & contamination

- Isolated in low levels
  < 100 cfu/cm^2
- Attachment to Stainless steel
  Khoza & Buys 2014

Packaging & decontamination

- No decontamination

Storage, consumer use & bacterial growth



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

Acknowledgements

DST - NRF Centre of Excellence in Food Security

UNIVERSITY of the WESTERN CAPE

NRF National Research Foundation | RISA Research and Innovation Support and Advancement

University of Pretoria

science & technology
Department:
Science and Technology
REPUBLIC OF SOUTH AFRICA

A place of quality,
a place to grow, from hope
to action through knowledge

gracias

Tatenda

ありがとう

Dankie

Arigatou

Siyabonga

Thank you

Merci

Ke a leboga

Grazie.

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