Source tracking of *Bacillus cereus* in an Extended Shelf Life (ESL) milk processing factory

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ESL milk processing

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

- Bacterial diversity
  - Bacillus spp. & Paenibacillus spp.
B. cereus

- Characteristics of B. cereus
- Sources
- Mesophilic vs Psychrotrophic strains
- Gastrointestinal disease
  - Diarrheal
  - Emetic syndrome
B. cereus contamination routes
B. cereus contamination routes
Research objective

- Characterise *B. cereus* isolates obtained from ESL milk processing and during shelf life under refrigeration.
- Source tracking of *B. cereus*
Research Approach

1. Rep PCR (GTG₅)
2. End point PCR
   - Virulent genes,
   - Discrimination of psychrotrophs & mesophiles
3. 16S Sequencing
4. rpoB Sequencing
5. MLST
**B. cereus** (GTG)$_5$ fingerprint patterns

- Both groups comprise isolates from filler nozzles and raw milk only.
B. cereus (GTG₅) fingerprint patterns
Hierarchical clustering of *B. cereus* using virulent & temperature genes
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B. cereus principal component analysis of virulent and temperature genes

Raw Milk – 1
Pasteurised Milk – 2
Filler nozzles – 15
**B. cereus** principal component analysis of virulent and temperature genes

![Biplot (axes F1 and F2: 65.95 %)](image)

- Pasteurised Milk – 1
- Filler nozzles – 10

- **Filler nozzles (11)**
B. cereus principal component analysis of virulent and temperature genes
16S rRNA maximum likelihood phylogenetic tree of *B. cereus*
*rpoB* Dendrogram representing the phylogenetic relationships of *Bacillus cereus* isolates obtained by the neighbor-joining method.
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Relationships between *B. cereus* isolates using concatenated sequences from five housekeeping alleles.
Conclusions

- Despite the high diversity of the *B. cereus* under study, there is evidence that filler nozzles and raw milk are a source of contamination in ESL milk.

- The presence of psychrotrophnic 16S signature in some of the isolates together with the presence of at least two virulence genes in majority of the isolates, is a cause for concern in ESL milk.
Acknowledgements

South African Society For Dairy Technology
IT'S TIME TO

B. CEREUS

But
Then

You can’t
B. cereus