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

Desmond T. Mugadza

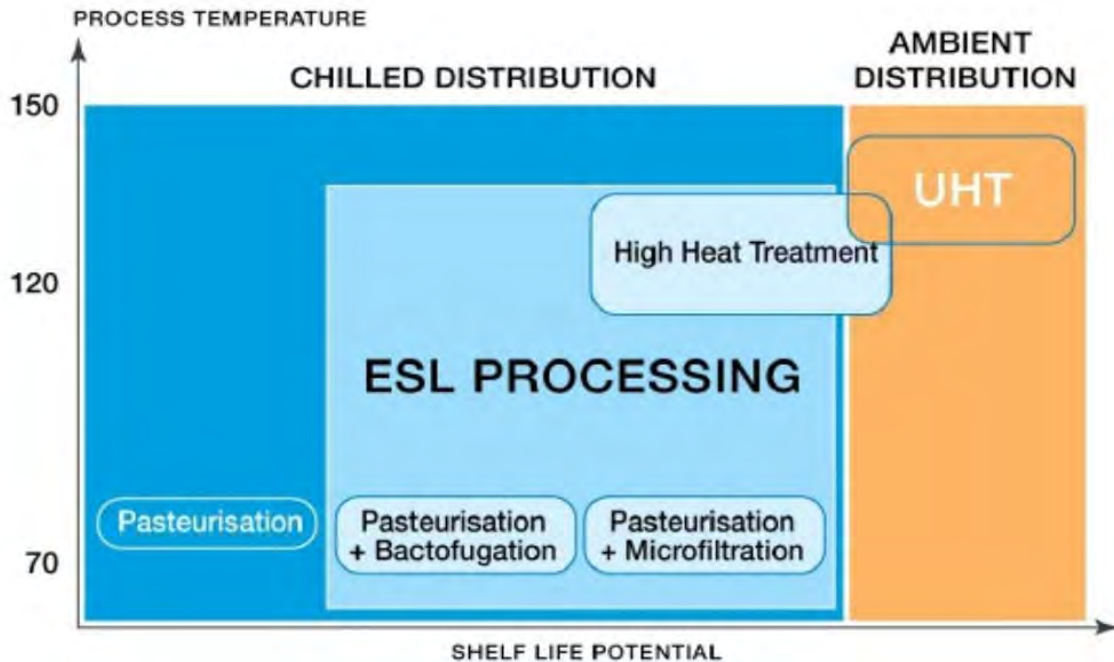
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University of Pretoria



ESL milk processing



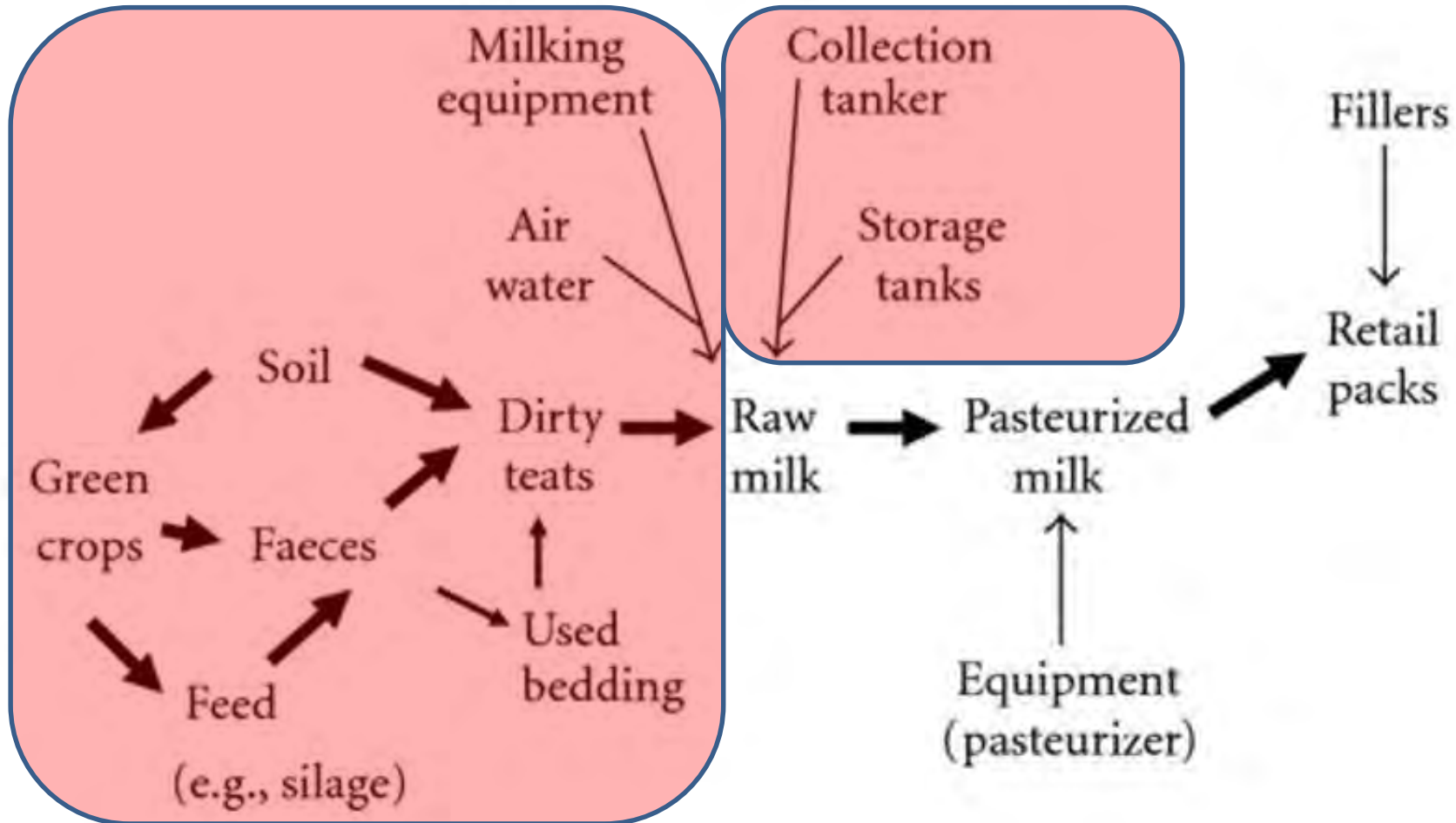
- Ultra-pasteurisation
- Non thermal methods
 - Bactofugation
- Cold filling
- Recontamination

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

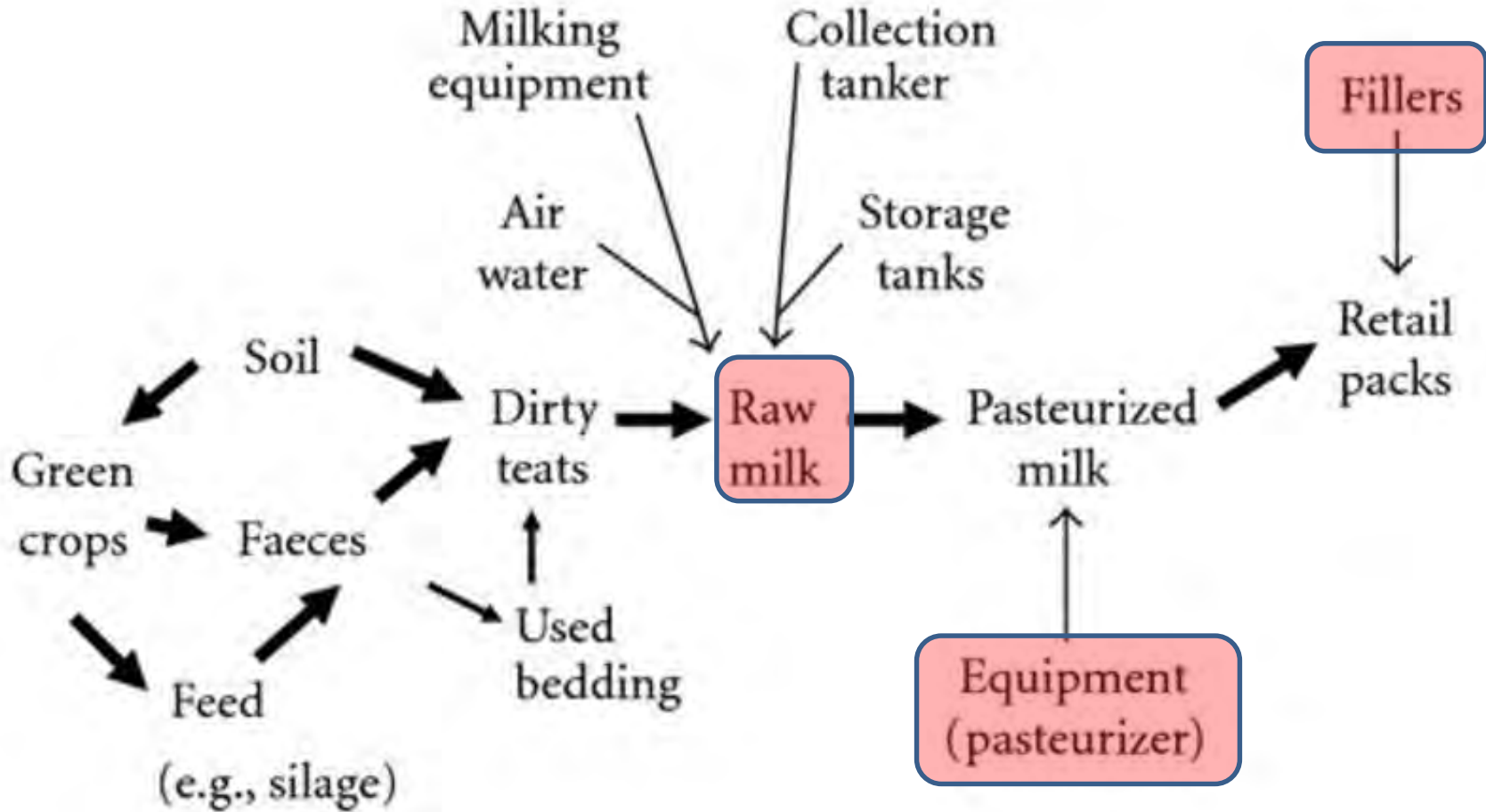
B. cereus

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

B. cereus contamination routes



B. cereus contamination routes

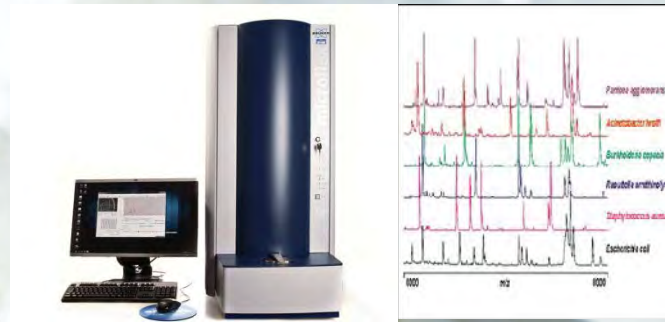


Research objective

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

Research Approach

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

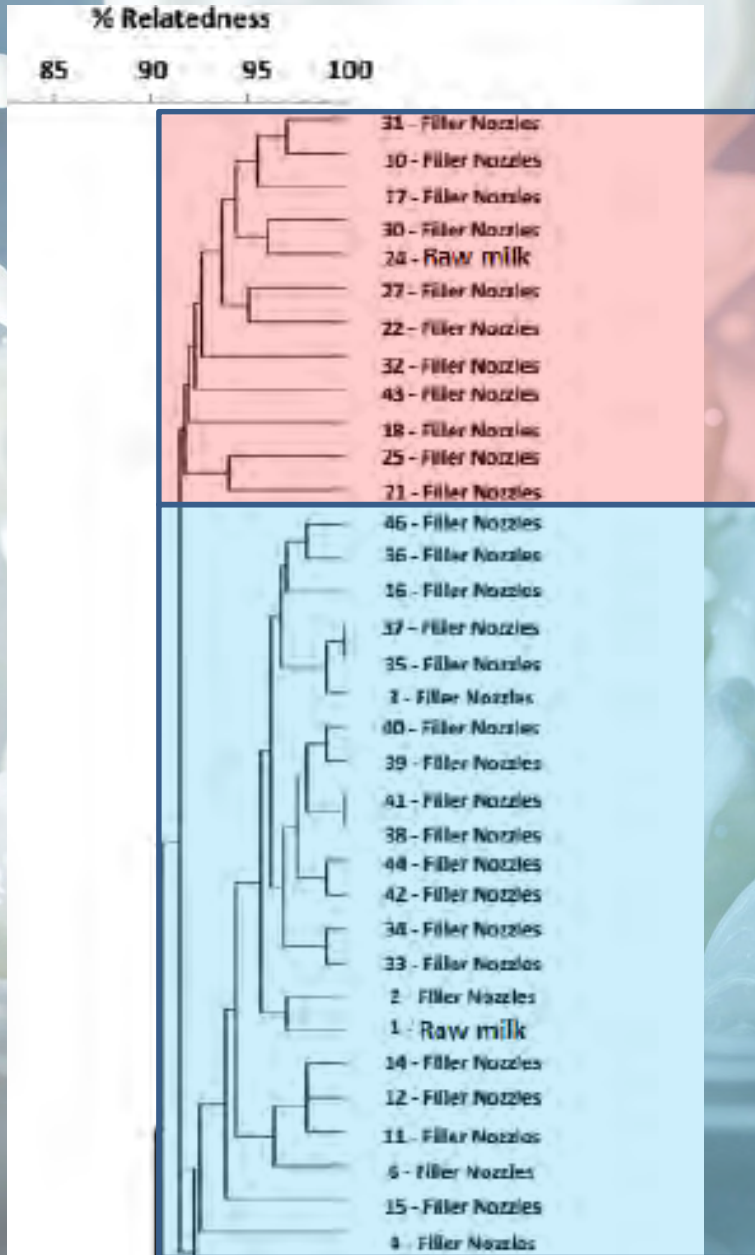


Identification of Isolates using MALDI TOF MS

B. Cereus isolates

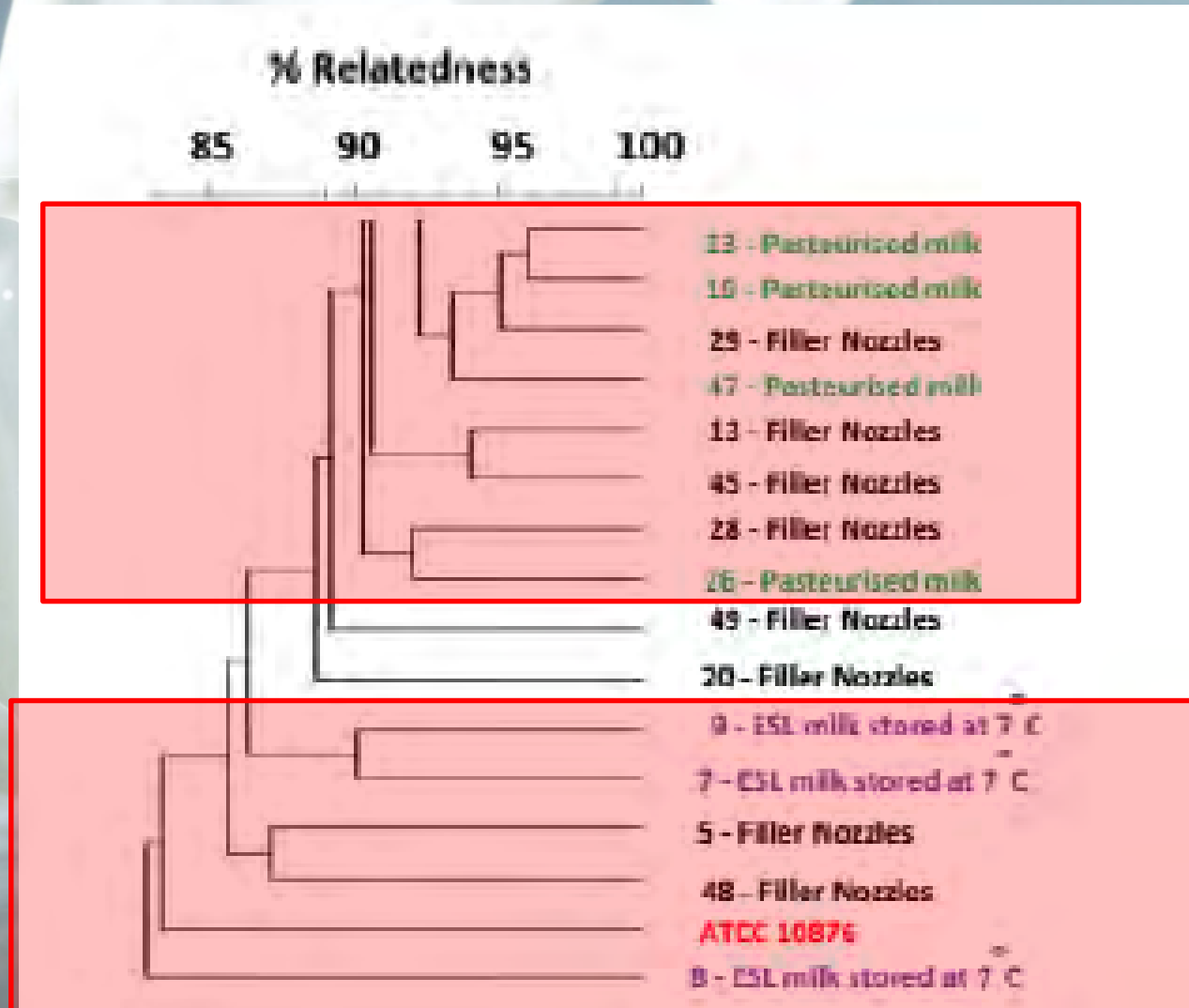
1. Rep PCR (GTG₅)
2. End point PCR
 - Virulent genes,
 - Discrimination of psychrotrophs & mesophiles
3. 16S Sequencing
4. *rpoB* Sequencing
5. MLST

B. cereus (GTG)₅ fingerprint patterns

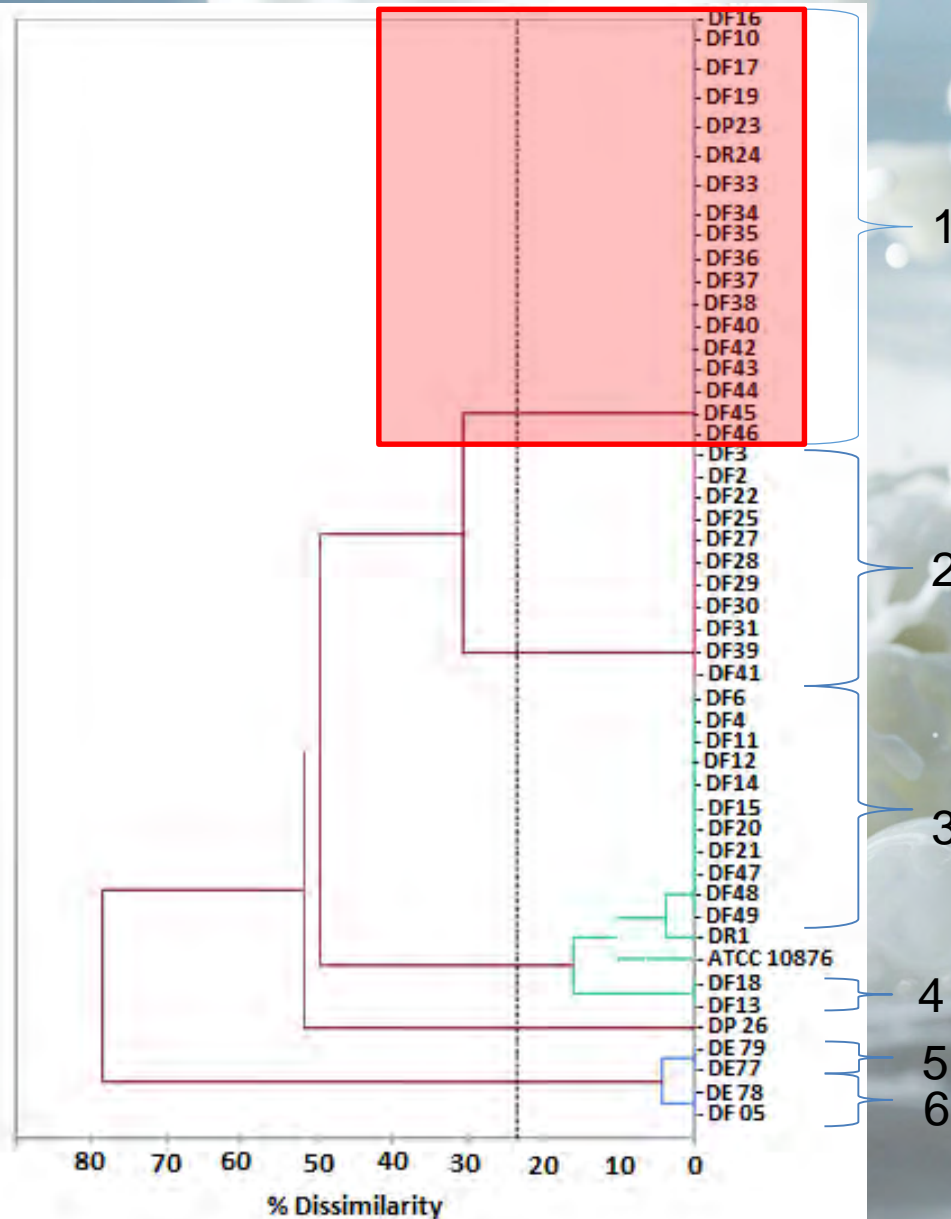


- Both groups comprise of isolates from filler nozzles and raw milk only

B. cereus (GTG₅) fingerprint patterns

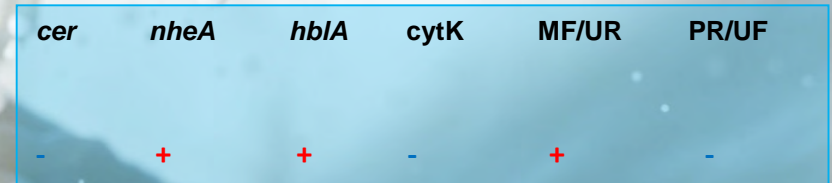
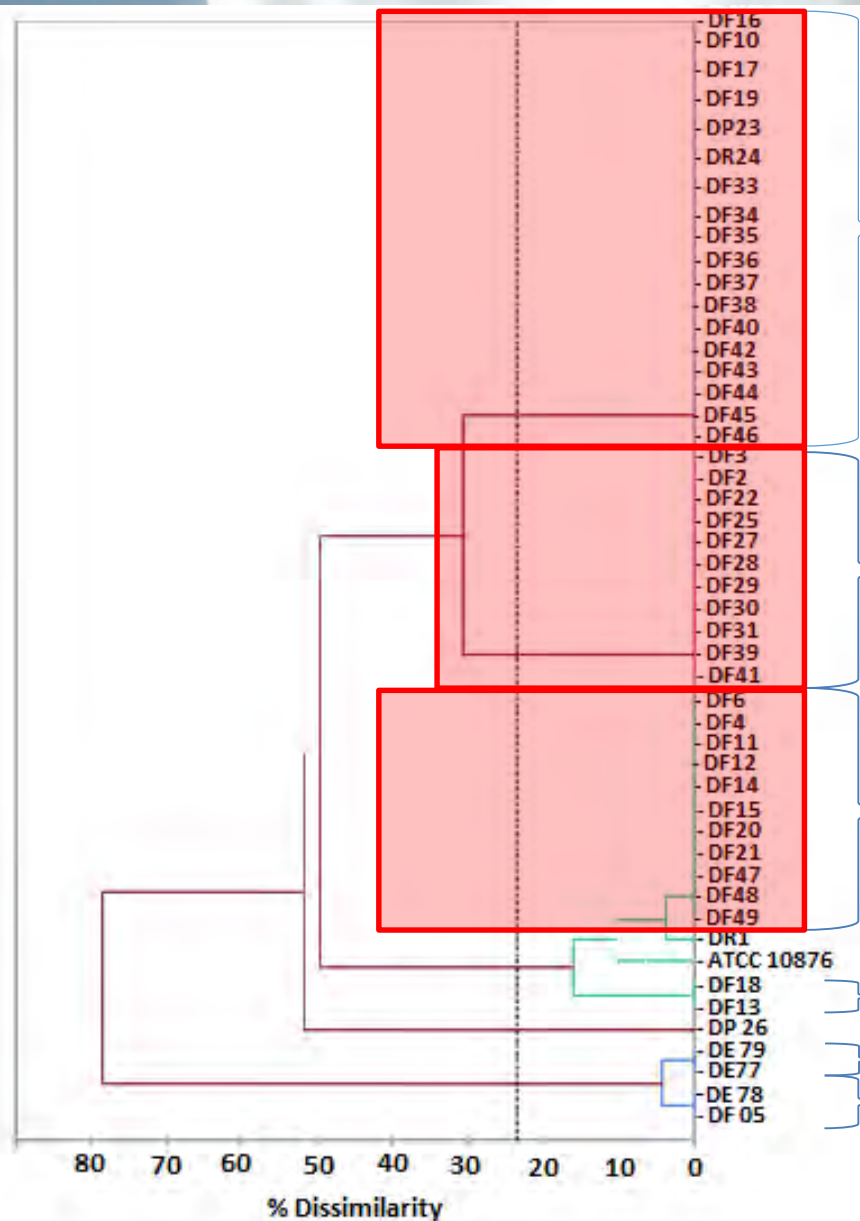


Hierarchical clustering of *B. cereus* using virulent & temperature genes



<i>cer</i>	<i>nheA</i>	<i>hblA</i>	<i>cytK</i>	MF/UR	PR/UF
+	+	+	-	+	-

Hierarchical clustering of *B. cereus* using virulent & temperature genes

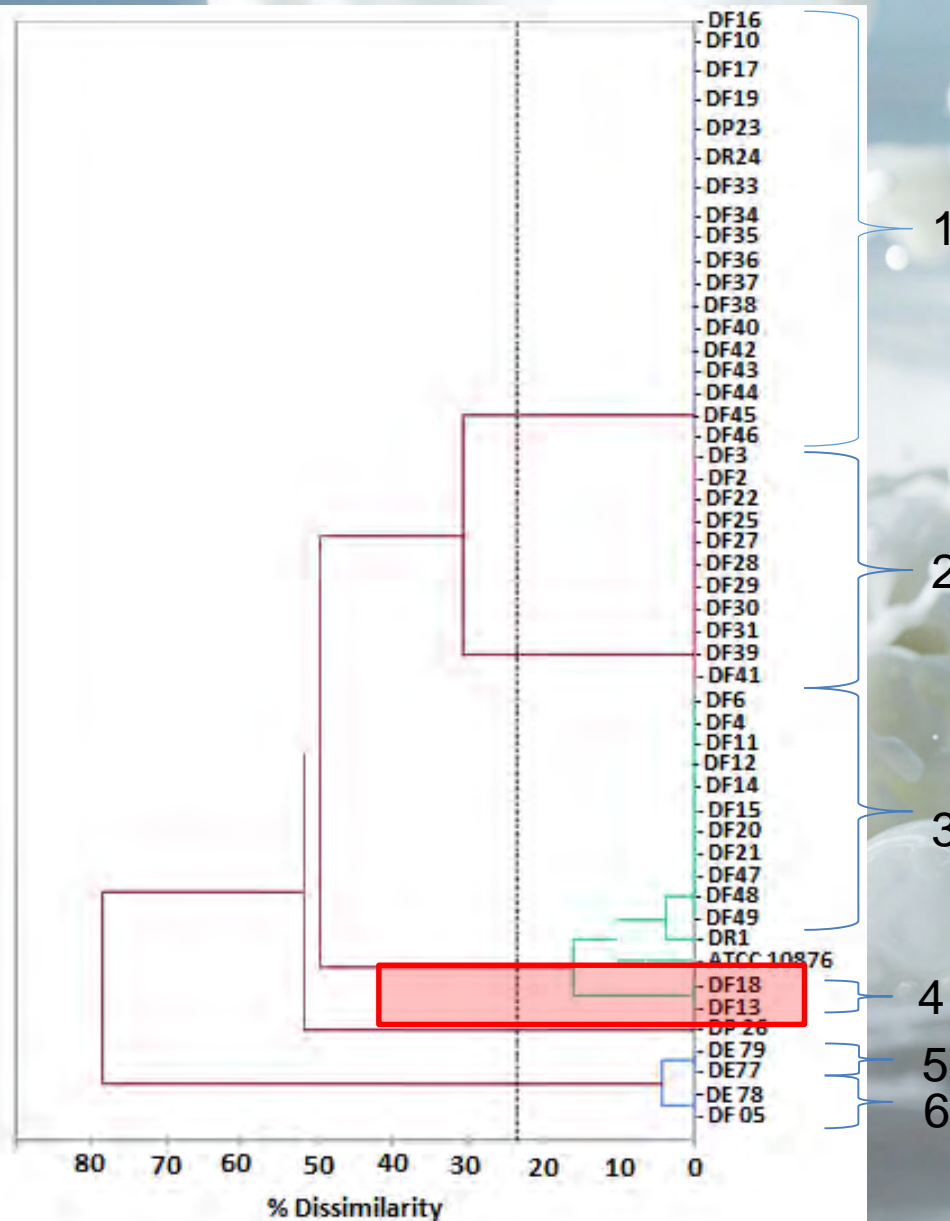


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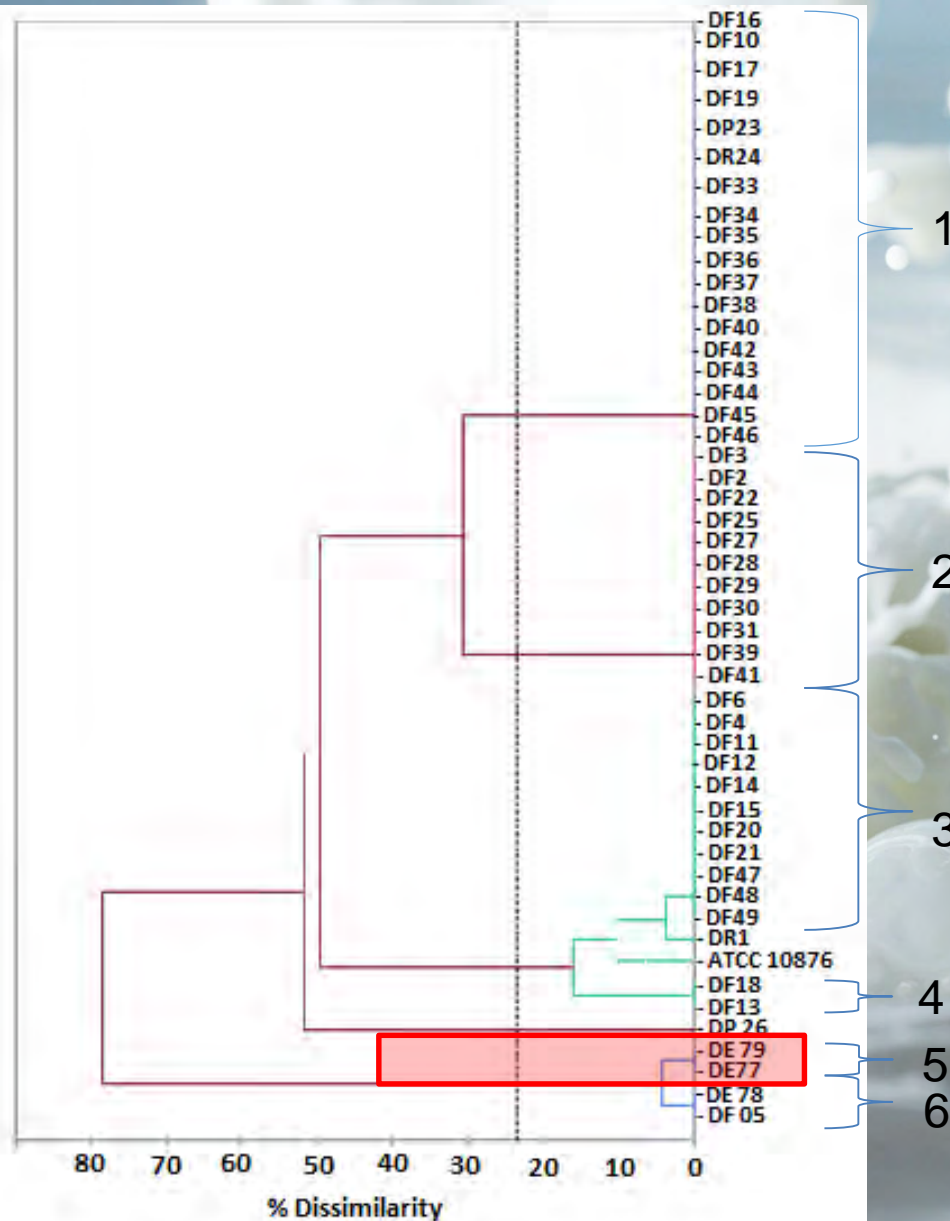
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Hierarchical clustering of *B. cereus* using virulent & temperature genes



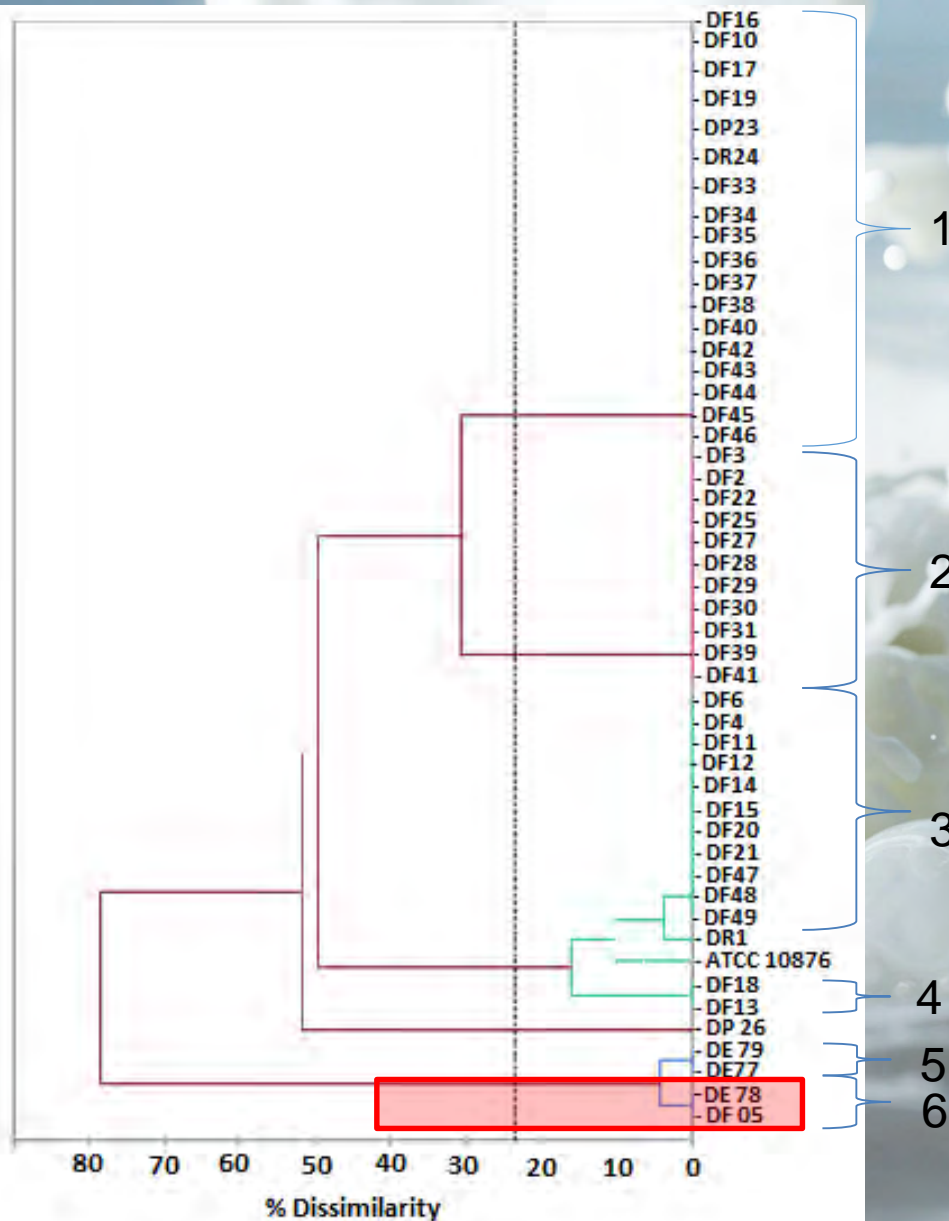
	<i>cer</i>	<i>nheA</i>	<i>hblA</i>	<i>cytK</i>	MF/UR	PR/UF
	+	+	-	+	+	-

Hierarchical clustering of *B. cereus* using virulent & temperature genes



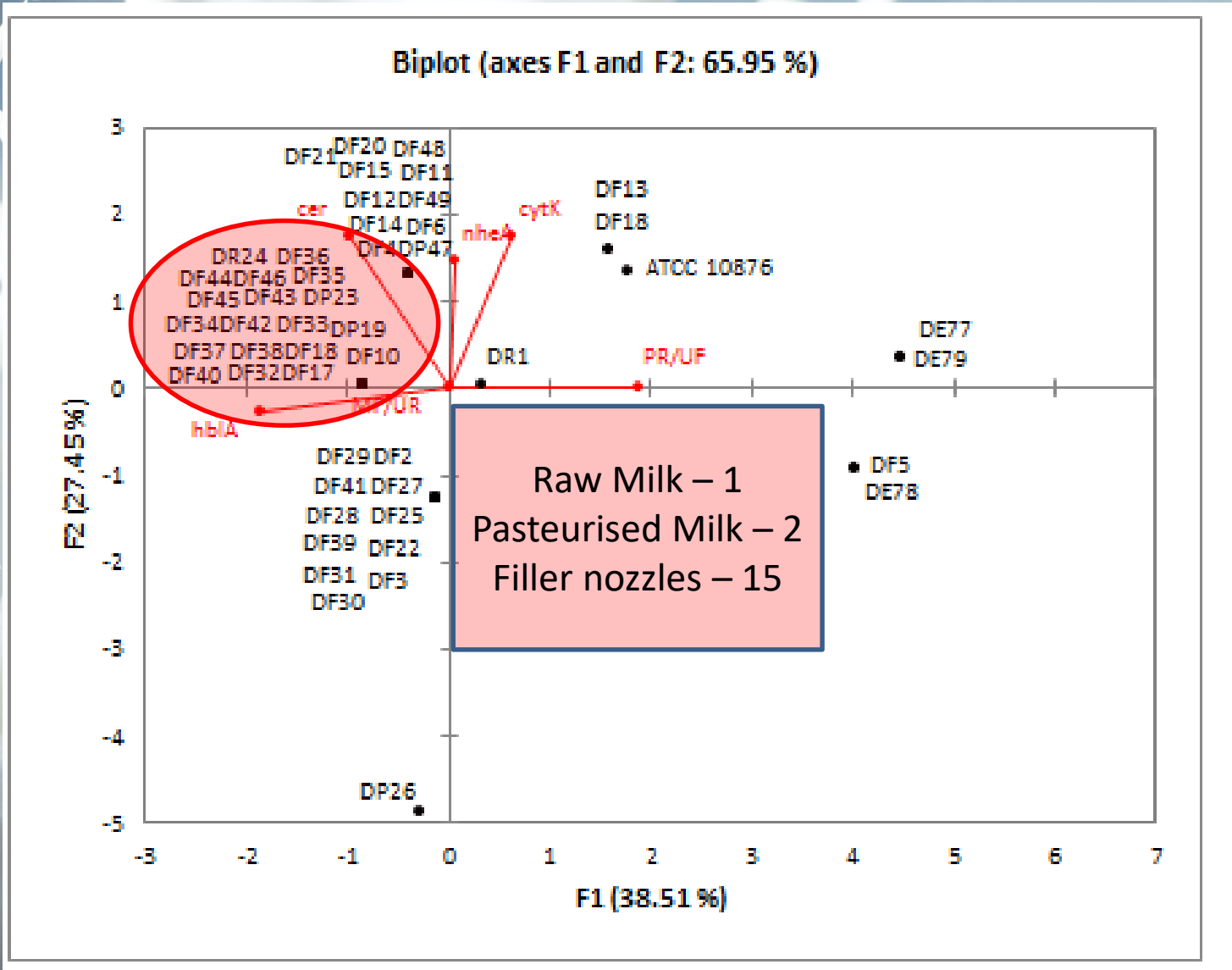
	<i>cer</i>	<i>nheA</i>	<i>hblA</i>	<i>cytK</i>	MF/UR	PR/UF
	-	+	-	+	+	+

Hierarchical clustering of *B. cereus* using virulent & temperature genes

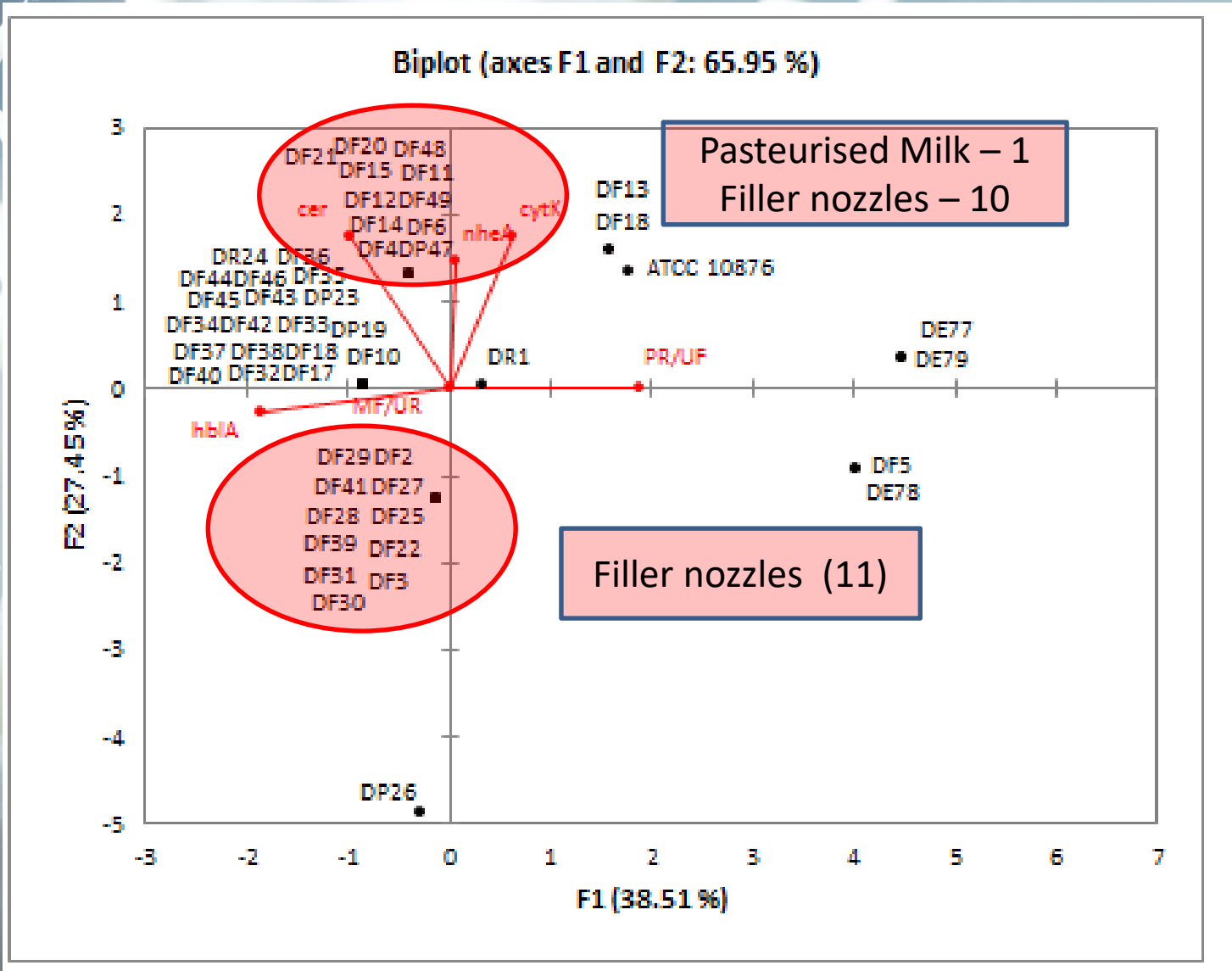


Gene	<i>cer</i>	<i>nheA</i>	<i>hblA</i>	<i>cytK</i>	MF/UR	PR/UF
Strains in Cluster 6	-	+	-	-	+	+

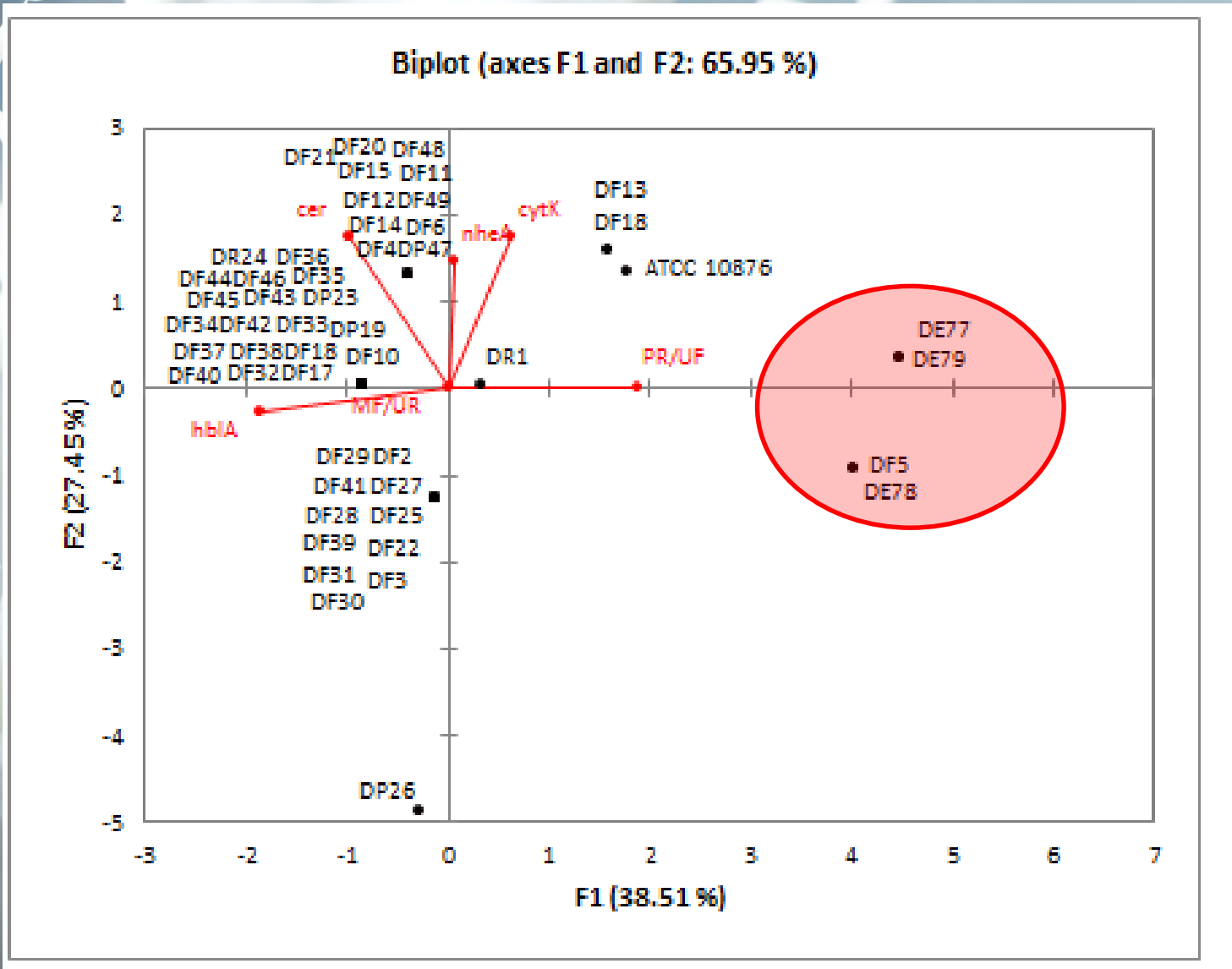
B. cereus principal component analysis of virulent and temperature genes



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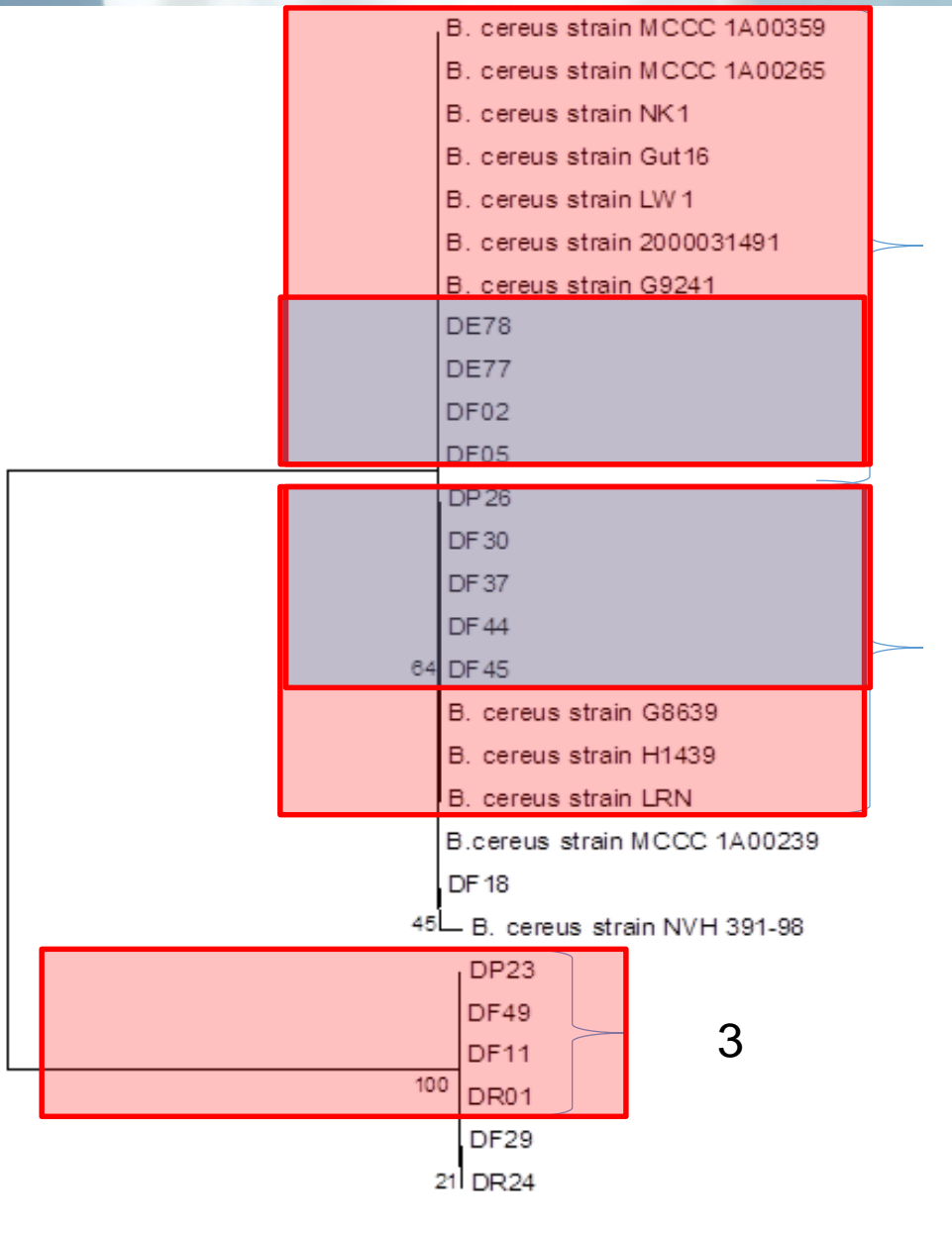


16S rRNA maximum likelihood phylogenetic tree of *B. cereus*

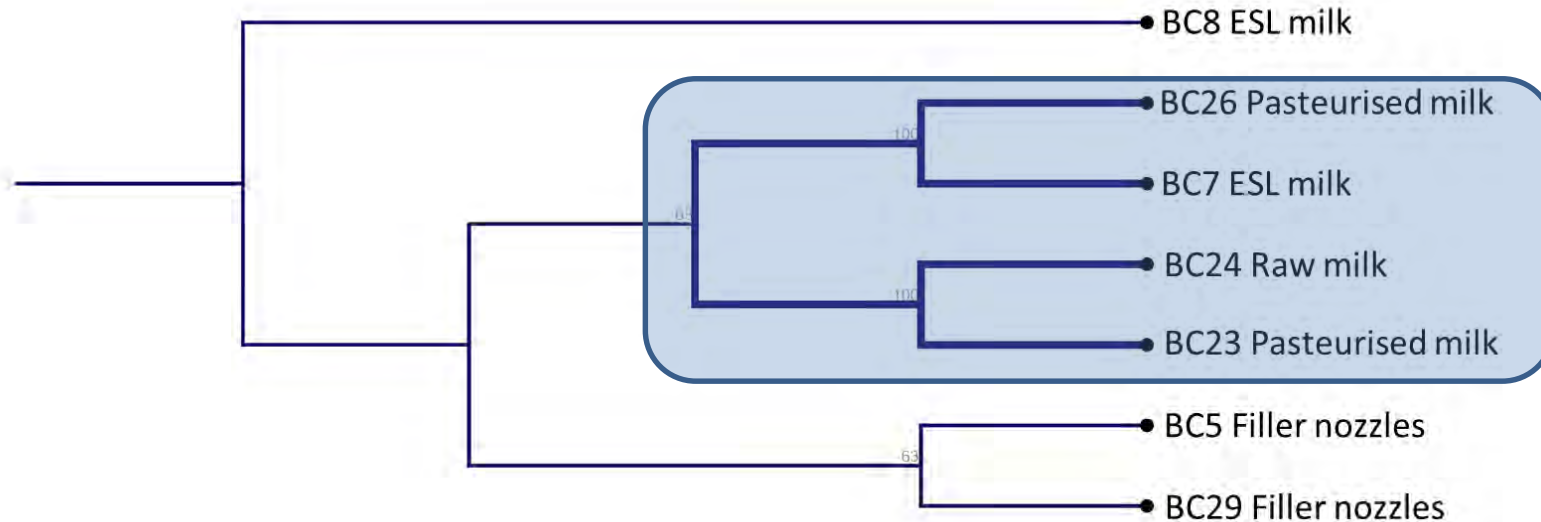
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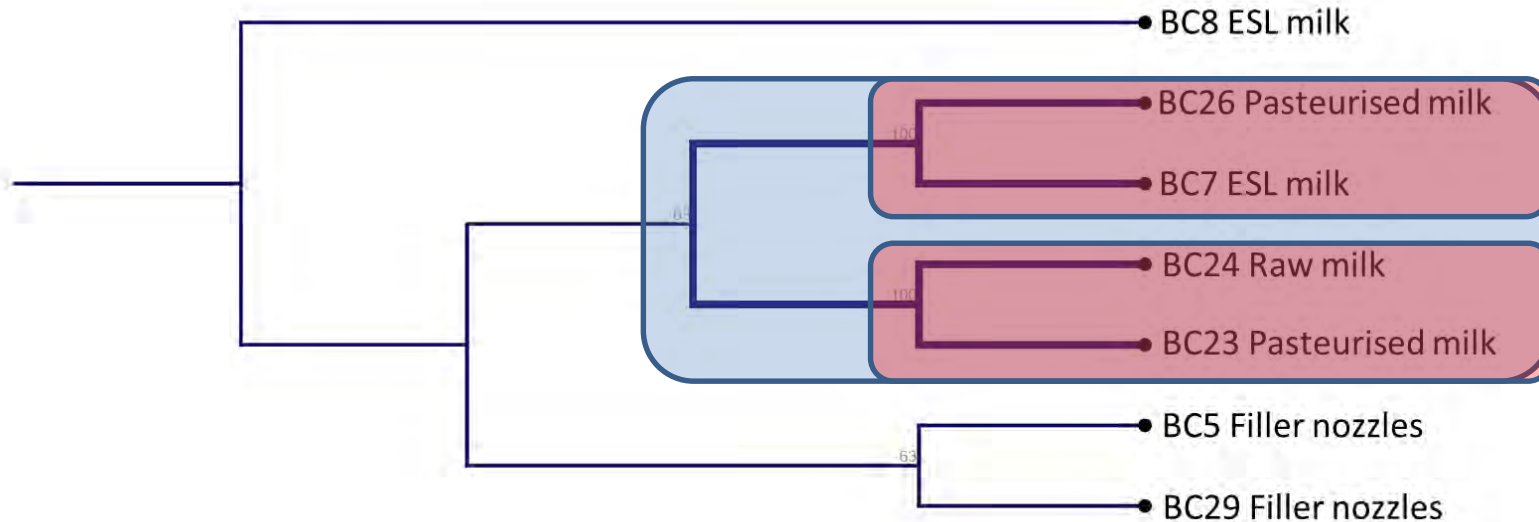
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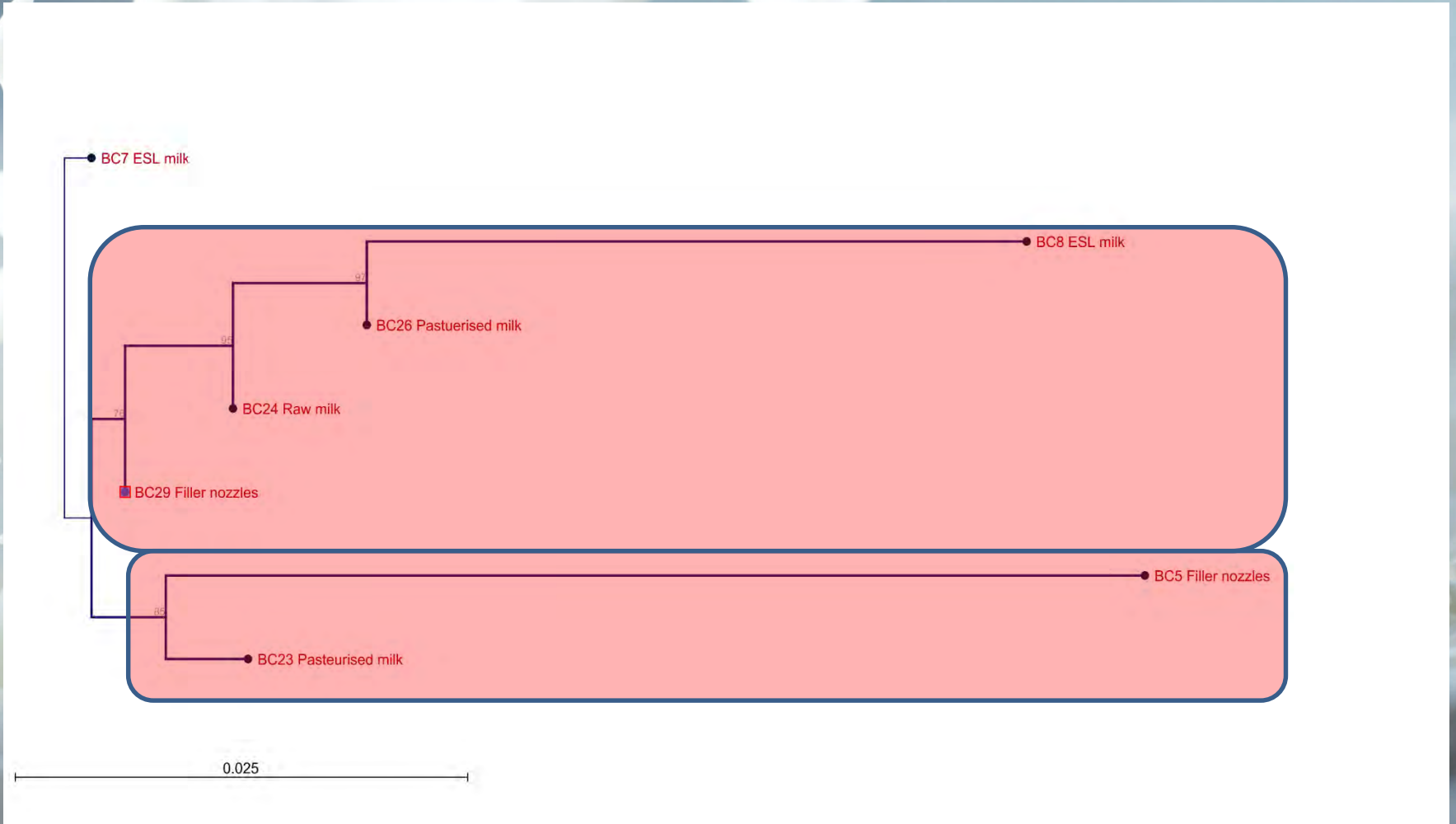
rpoB Dendrogram representing the phylogenetic relationships of *Bacillus cereus* isolates obtained by the neighbor-joining method



rpoB Dendrogram representing the phylogenetic relationships of *Bacillus cereus* isolates obtained by the neighbor-joining method



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 psychrotrophic 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. CERREUS



*But
Then*

You can't
B. cereus



gracias

Tatenda

ありがとう

Dankie

Asante

Arigatou

Siyabonga

Thank you

Merci

Ke a leboga

شكرا

Obrigado

Grazie

Daalu