Bacterially induced cheese blowing defects with particular attention to butyric acid bacteria

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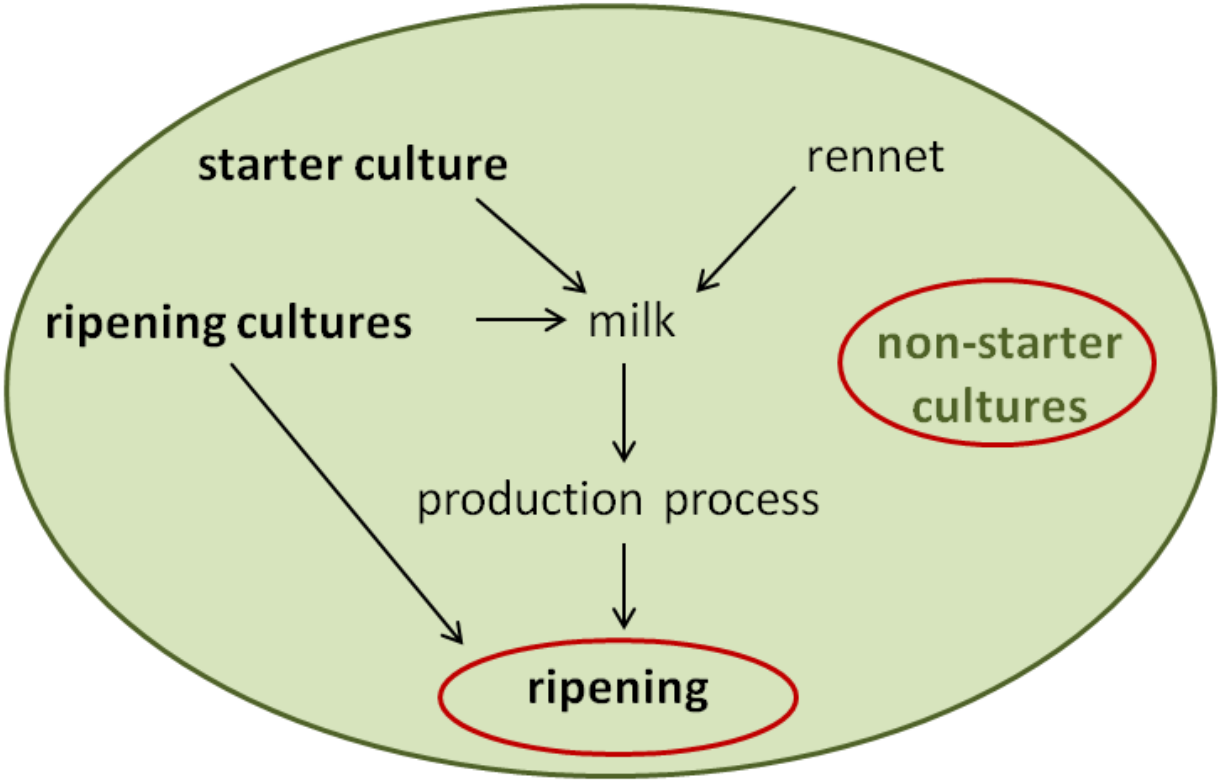
Bacterial influence on cheese quality



- Cheese quality:

  ´Cheese must conform to the expected characteristics of the variety´

  Fox et al. 2004

Cheese blowing defects

- Slits, cracks, irregular eye formation
- Propionibacteria, facultatively heterofermentative lactobacilli, clostridia
- Analysis of 13 cheese samples

→ High numbers of facultatively heterofermentative lactobacilli and propionibacteria but no clostridia

Clostridia in hard cheese

- Cheese spoilage: late blowing defect
- Ripening process: lactic acid → butyric acid + CO_2 + H_2

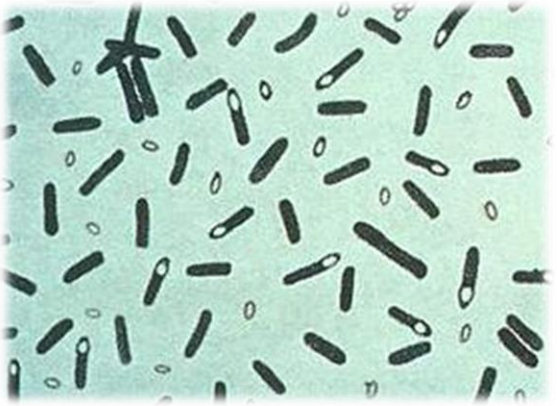
- Taste and blowing defects
- Unsaleable cheeses: economic losses

- Butyric acid bacteria:
  - *C. tyrobutyricum*
  - *C. butyricum*
  - *C. beijerinckii*
  - *C. sporogenes*

Detection and enumeration of clostridia

- Endospore-forming bacteria
- Contaminated raw milk
- Farm management, milking practices

  → raw milk quality! → low spore count



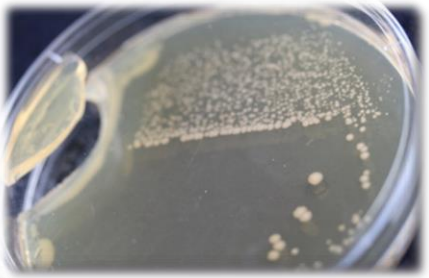
- No selective culture medium
- Detection and enumeration are complex
- time-consuming, labour-intensive
- No standard method

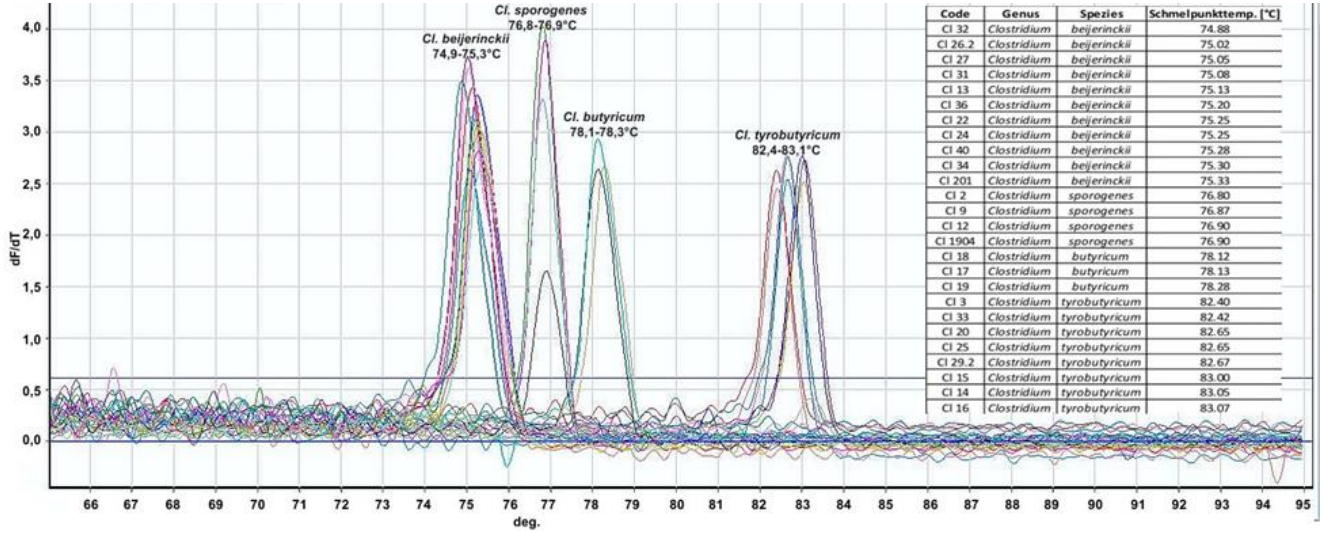
Aim: Development of a method for the detection of cheese-damaging clostridial spores in raw milk

Collection and identification of clostridia

- Activation of strains of the university strain library
- Optimisation of isolation procedure from milk and cheese
- Analysis of 35 cheeses suspected of late-blowing
- Identification of isolates

  PCR, sequencing, Real-Time PCR + melting curve analysis





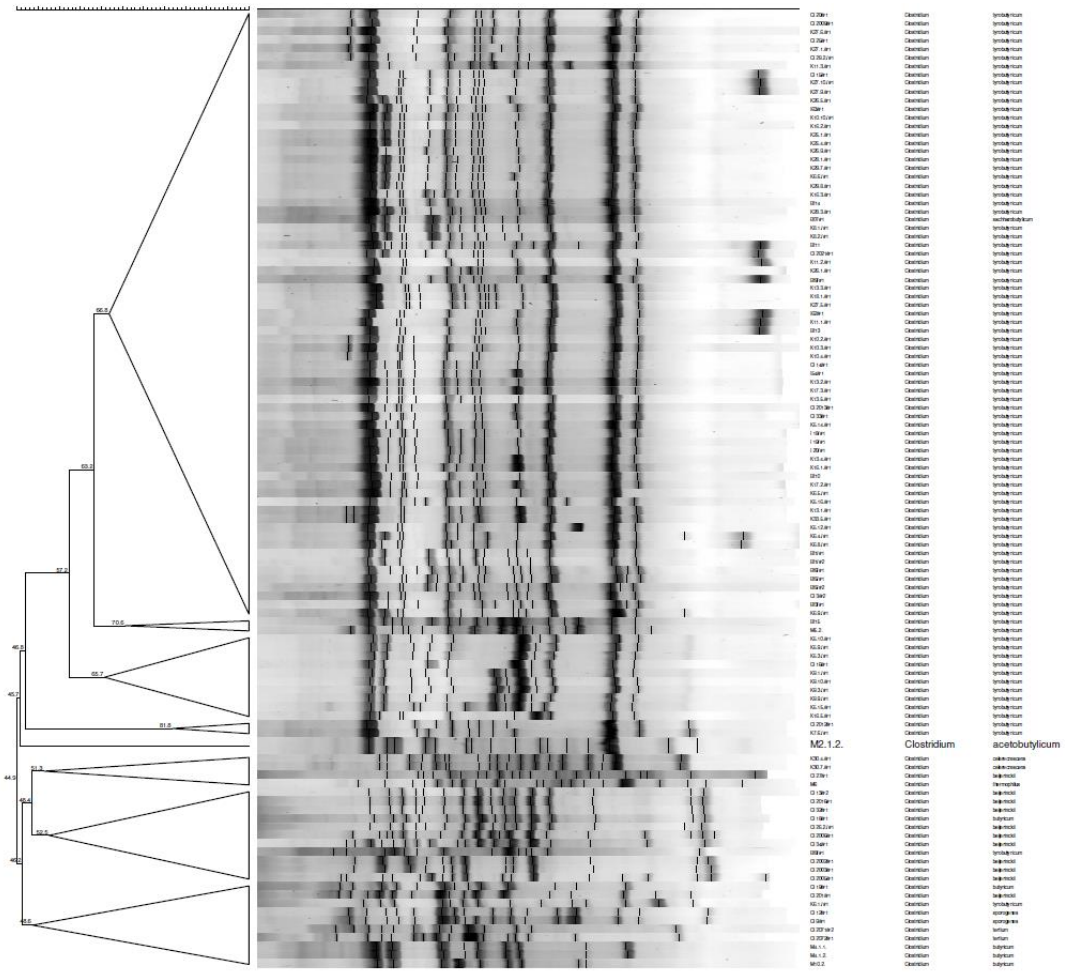
Code	Genus	Spezies	Schmelpunkttemp. [°C]
Cl 32	Clostridium	beijerinckii	74.88
Cl 26.2	Clostridium	beijerinckii	75.02
Cl 27	Clostridium	beijerinckii	75.05
Cl 31	Clostridium	beijerinckii	75.08
Cl 13	Clostridium	beijerinckii	75.13
Cl 36	Clostridium	beijerinckii	75.20
Cl 22	Clostridium	beijerinckii	75.25
Cl 24	Clostridium	beijerinckii	75.25
Cl 40	Clostridium	beijerinckii	75.28
Cl 34	Clostridium	beijerinckii	75.30
Cl 201	Clostridium	beijerinckii	75.33
Cl 2	Clostridium	sporogenes	76.80
Cl 9	Clostridium	sporogenes	76.87
Cl 12	Clostridium	sporogenes	76.90
Cl 1904	Clostridium	sporogenes	76.90
Cl 18	Clostridium	butyricum	78.12
Cl 17	Clostridium	butyricum	78.13
Cl 19	Clostridium	butyricum	78.28
Cl 3	Clostridium	tyrobutyricum	82.40
Cl 33	Clostridium	tyrobutyricum	82.42
Cl 20	Clostridium	tyrobutyricum	82.65
Cl 25	Clostridium	tyrobutyricum	82.65
Cl 29.2	Clostridium	tyrobutyricum	82.67
Cl 15	Clostridium	tyrobutyricum	83.00
Cl 14	Clostridium	tyrobutyricum	83.05
Cl 16	Clostridium	tyrobutyricum	83.07

Characterisation of clostridia

- Typing: Rep-PCR
- Clustering according to band patterns

C. tyrobutyricum

Other Clostridia



Future strategy

- Creation of a reference strain library
- Selection of isolates
- Development of a high-throughput method for the detection and enumeration of clostridia in raw milk
- Avoidance of cheese spoilage and economic losses

Thank you for your attention!

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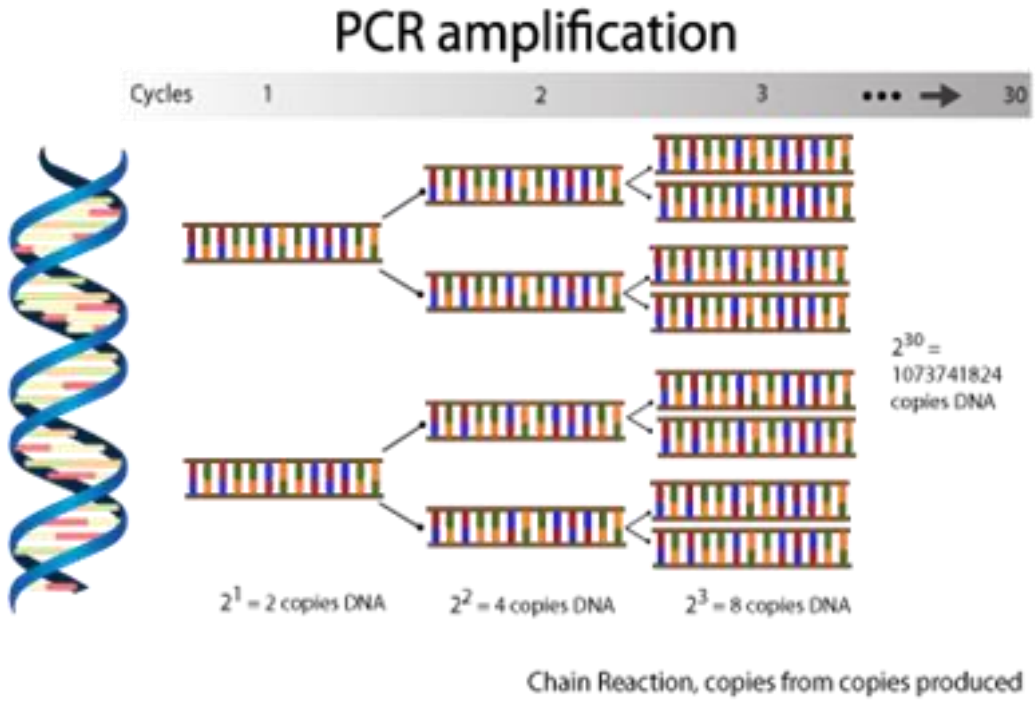
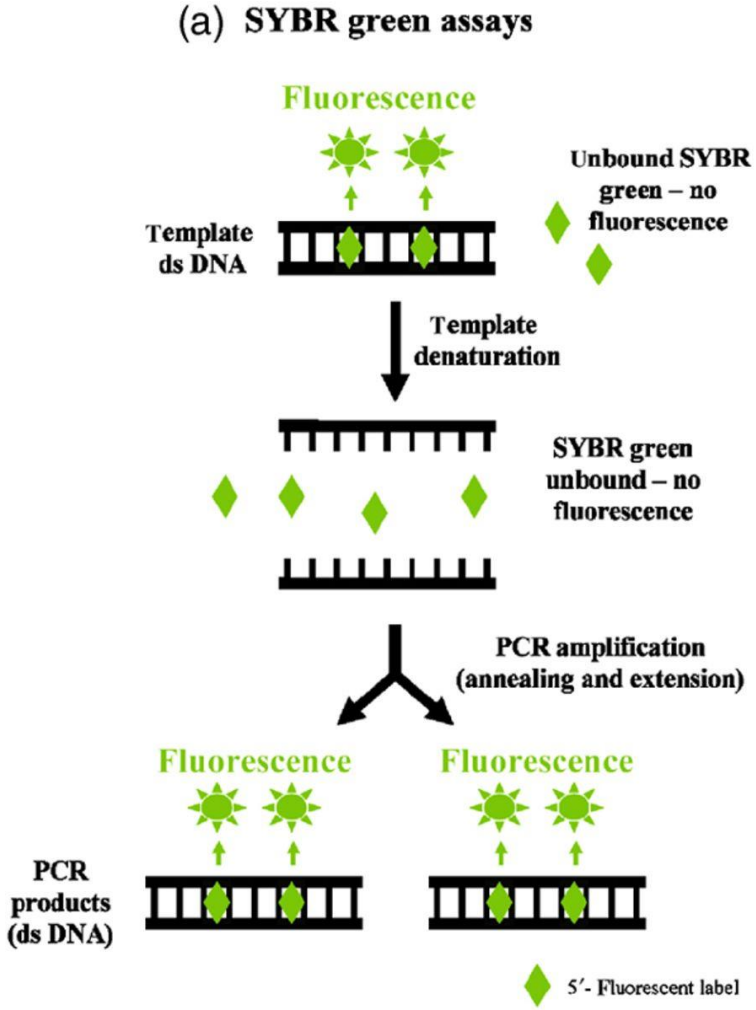
Real Time PCR Sybr® Green





Contamination pathway of clostridia