The challenge of detecting cheese-damaging clostridia in milk

J. Brändle, K.J. Domig and W. Kneifel

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Comet K-Projekt ADDA – Advancement of Dairying in Austria

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Late-blowing







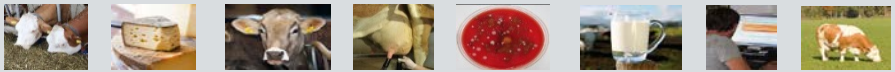

Late-blowing

butyric acid
producing clostridia

Irregular cheese eyes
Strong off-flavour

H_2 CO_2

butyric acid



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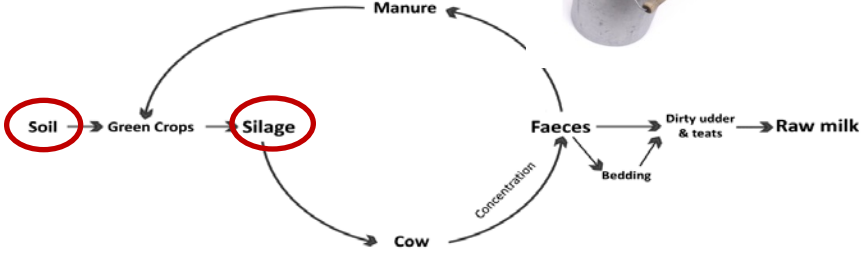








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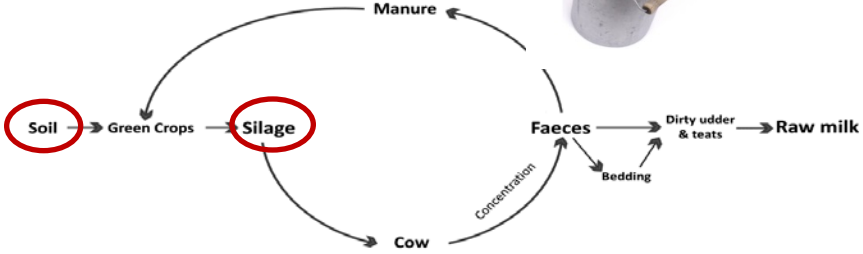
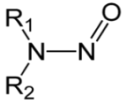


bactofugation

lysozyme

bacteriocins

nitrate







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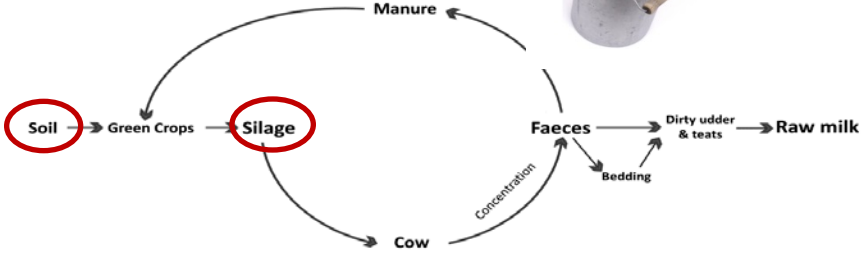

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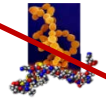

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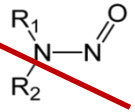








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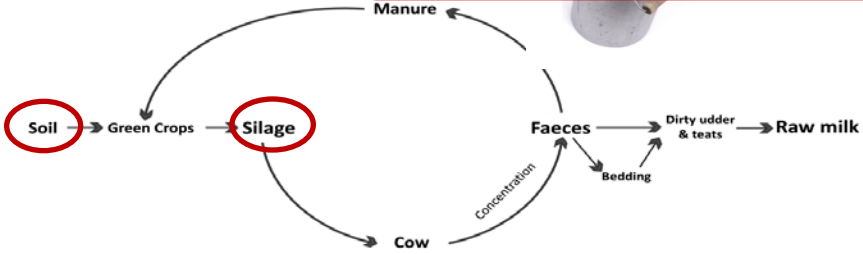

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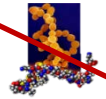

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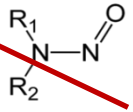




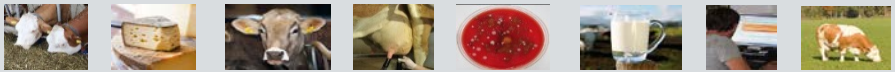




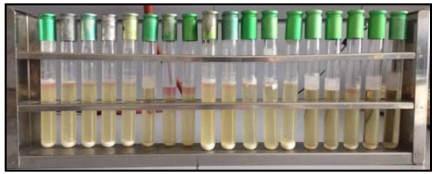

Detecting clostridia in milk

- hard and semi-hard cheeses
- endospores
- extremely low detection limit
- no international standard method

→ Routine application ?



MPN methods



MPN methods

Study design

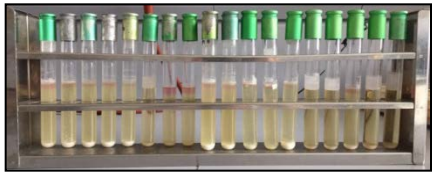
Comparison of 4 MPN methods

- 70 milk samples
- duplicates of each method
- routine application

Differences in

- media composition
- pH
- procedure
- incubation time (3-10 days)

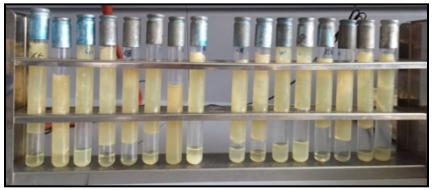
1
Bryant und Burkey

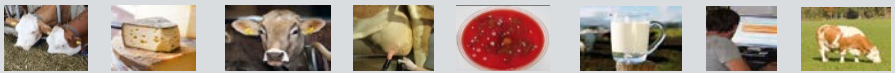


2
NIZO

3
RCM-lactate (VDLUFA)

4
RCM (LAAF)



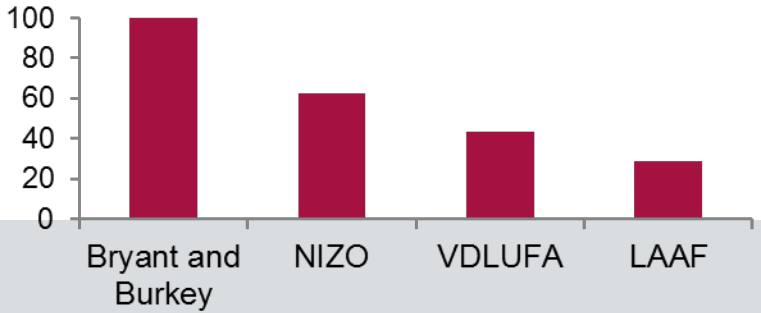


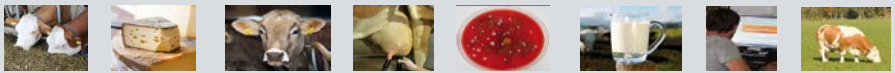
Results

- Wilcoxon-matched-pairs test
- **Highly significant differences** between the methods

- Hodges-Lehmann-estimator (median difference)

	single values	mean values
BB(1) vs. NIZO (2)	1.6x	1.6x
BB (1) vs. VDLUFA (3)	2.3x	2.4x
BB (1) vs. LAAF (4)	3.5x	3.7x





Isolates

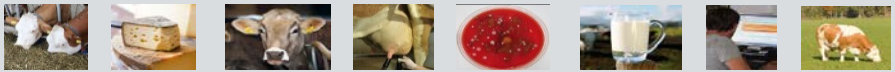
- spreading of positive tubes on non-selective medium

- identification by 16S rDNA sequencing

→ ***Clostridium tyrobutyricum***
C. butyricum
C. beijerinckii
C. sporogenes

Other clostridial species

Bacillus paralicheniformis
B. coagulans
B. sonorensis
Other Bacilli



Conclusion

- labour-intensive
- long analysis time
- lack of selectivity

- significant **differences** between the methods

- detected **spore numbers** are extremely **method-specific**

→ threshold value for clostridia in milk?








Future outlook

Standardisation!

- development of a **faster**, **semi-automated** method to detect butyric acid producing clostridia in milk
- potential **routine application** and **international standard method**








Thank you!

PD Dr. Konrad J. Domig
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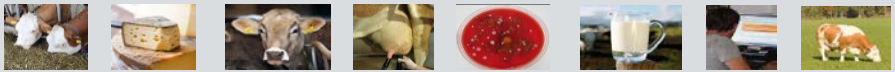

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