Impact of microbial biodiversity on the quality of Danish cheeses

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Content

• Microbial diversity at the dairy level
• Origin of microorganisms
• Isolation and identification of the cheese microbiota (the hidden identities)
• Matrix interactions, small-talk and technological properties
• Conclusion
• Questions for the audience
Why study microbial biodiversity at the dairy level?

- Performance of the microbial cultures gives the quality of the cheeses
- Increased biodiversity can lead to increased diversity (new brands)
- Optimised microorganisms result in lean production, standardised quality and less waste
- The technological conditions at the dairy significantly influence the microorganisms (establishment and performance)
- Microorganisms influence each other through a number of interactions (competition for nutrients, production of inhibitory compounds, “small talk” etc.)
- “The bad guys will be outcompeted by the good ones” (enhanced food safety)
Microbial diversity

It is not the strongest of the species that survive, but the one most responsive to change.

-CHARLES DARWIN

If the production change – most likely the microbial diversity will change as well
Origin of microorganisms in dairy products

- Raw milk (raw-milk cheeses)
- Starter cultures (DL-starters and adjunct cultures)
- Brine/NaCl
- Equipment and processing environments, biofilms
- Back-slopping – reinoculation
- Ingredients, herbs etc.

*Lactococcus lactis/Streptococcus thermophilus/Leuconostoc mesenteroides/Brevibacterium spp./Corynebacterium spp. a.o.*

*Penicillium roqueforti/Penicillium camemberti/Penicillium commune a.o.*

*Debaryomyces hansenii/Saccharomyces cerevisiae/Yarrowia lipolytica/Galactomyces geotrichum a.o.*
Isolation of bacteria at cheese surfaces
Danish farm house cheeses

TSA (3.5% (w/v) NaCl)

Cheese A

- Corynebacterium casei
- Brevibacterium linens
- Brachybacterium spp.
- Leucobacter spp.
- Miscellaneous

Cheese B

- Corynebacterium casei
- Staphylococcus saprophyticus
- Brevibacterium linens
- Miscellaneous
- Corynebacterium variabilis

Cheese C

- Corynebacterium casei
- Staphylococcus equorum
- Microbacterium gubbeneense
- Alcaligenes faecalis
- Brevibacterium linens
- Miscellaneous

Don’t forget the yeasts!
Untangling the complex ecology of Danish cheeses

- Farm house cheeses had a more complex microbiota compared to more industrially produced cheeses.
- Non-culturable halotolerant bacteria might account for up to 20% of the surface microbiota as identified by culture-independent technologies.
- Some species as e.g. *Marinilactibacillus psychrotolerans* are hardly detected by culture dependent technologies.
DNA based identification of microorganisms in the dairy

DGGE

1. Cheese A
2. Cheese B
3. Cheese C
4. Standard 1 (lactic acid bacteria)
5. Standard 2 (smear bacteria)

**Staphylococcus equorum** 15097

Corynebacterium casei 15097

Brevibacterium linens BL2

16S/26S/ITS sequencing

Rep-PCR (amplification of repetitive sequences)
Going in-depth with the indigenous microflora of surface-ripened cheeses

Sequencing the surface microbiota of Danbo cheese

**Surface yeasts**


**Surface bacteria**

Understanding the basic mechanisms behind cheese ripening
NaCl content influences growth, death and autolysis of *Lactococcus lactis*

- The cheese NaCl content had a significant culture-dependent influence on proliferation, viability and autolysis of the DL starter cultures.
- During ripening, loss of viability and autolysis were most pronounced for bacteria in groups of ≥ 4 bacteria (is autolysis under QS control?)
Figure 4. Response surfaces of the doubling time as a function of pH and NaCl concentration analyzed by the use of multiple linear regression. A: Isolate with mtDNA RFLP profile G (starter culture). B: Isolate with mtDNA RFLP profile H (dominant isolate).
Cheese flavour might be influenced by yeast species on the cheese surface.

**Compounds associated with blank cheese agar:**
Hexanal, heptanal, octanal, nonanal, benzaldehyde, 1-pentanol, hexanol, 1-octanol, 3-hydroxy-2-butanone.

**S. cerevisiae ass. compounds:**
Esters (ethylacetate, isoamylacetate, ethylpropiionate, ethylbutanoate), decanal.

**Y. lipolytica ass. compounds:**
Sulfides (disulfide, dimethyl, trisulfidedimethyl), furanes (2-pentylfurane, hexylfurane), short-chain ketones (2-propanone, 2-butanone, 2-pentanone, 3-methyl-2-pentanone), alcanes, benzenes and limonene.

**D. hansenii ass. compounds:**
Branched chain aldehydes (2-methylpropanal, 2-methylbutanal, 3-methylbutanal), branch-chain alcohols (2-methyl-1-propanol, 2-methylbutanol, 3-methylbutan-1-ol, 3-methyl-3-buten-1-ol)
Conclusion

✓ The microbial biodiversity at the dairies is huge – larger than you imagine!
✓ Understanding microbial ecology is of outmost importance for production of delicious and safe dairy products of consistent quality
✓ Microbial biodiversity gives the magic touch – identification to species and strain level is important
✓ Performance can vary significantly – usually the fittest wins the battle!
✓ Understanding microbial establishment and communication at the single cell level can improve quality and be a sustainable way to conquer spoilage and pathogenic microorganisms
Thank you for your attention ....
Diskussionsoplæg

• Er der behov for øget viden om mejeriprodukters mikrobiologi? Hvis ja – hvad mangler vi viden om?

• Kan produktionsforholdene på mejeriet styres, så man i øget grad sikrer fremvækst af ønskede mikroorganismer/kulturer?

• Hvordan kan forholdene på mejeriet optimeres, så den mikrobielle diversitet bliver optimal?

• Har mikroorganismer i f.eks. saltlagen betydning for produktkvalitet?