Faculty of Science

Impact of microbial biodiversity on the quality of Danish cheeses

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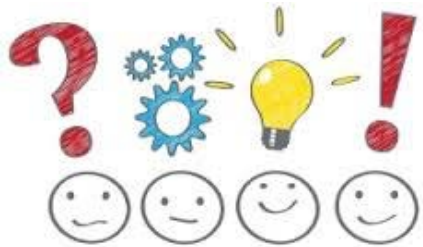
Mejeriforskningens dag, 2nd March 2017, Billund

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 audients



Why study microbial biodiversity at the dairy level?

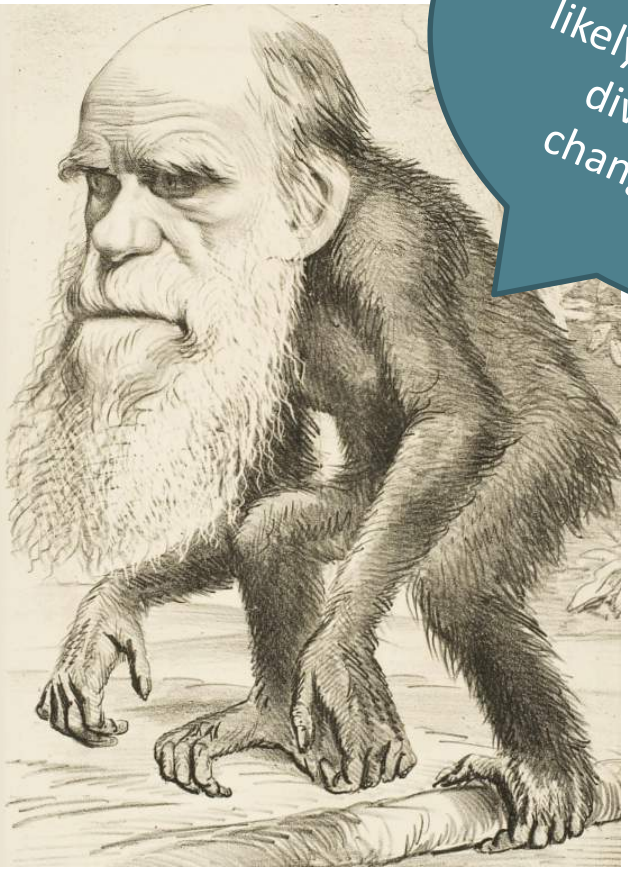
- Performance of the microbial cultures gives the quality of the cheeses
- Increased biodiversity can lead to increased diversity (new brands)
- Optimised microorganisms result in lean production, standardised quality and less waste
- The technological conditions at the dairy significantly influence 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

If the production change – most likely the microbial diversity will change as well

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

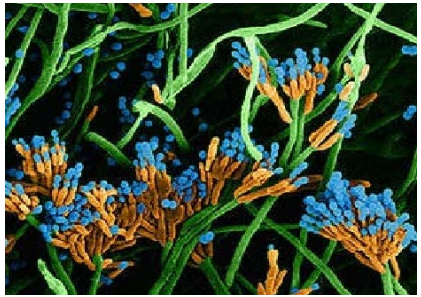
-CHARLES DARWIN



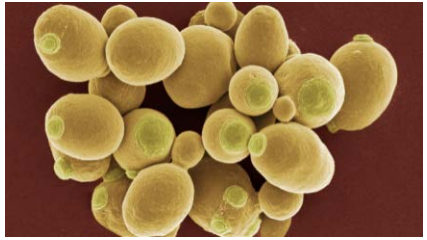
Origin of microorganisms in dairy products

- 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.

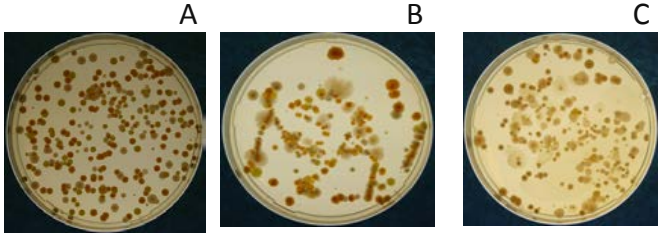


Debaryomyces hansenii/ Saccharomyces cerevisiae/ Yarrowia lipolytica/Galactomyces geotrichum a.o.

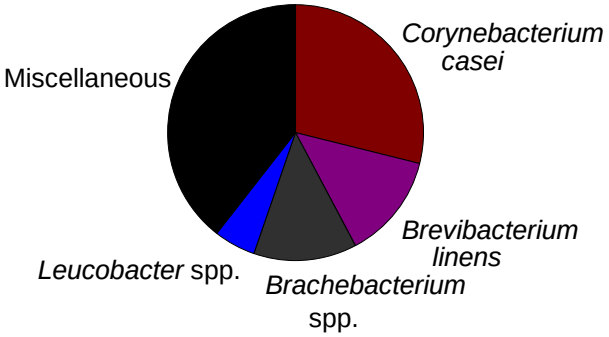
Isolation of bacteria at cheese surfaces

Danish farm house cheeses

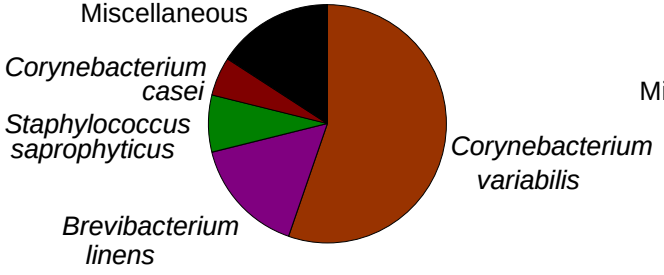
TSA (3.5% (w/v) NaCl)



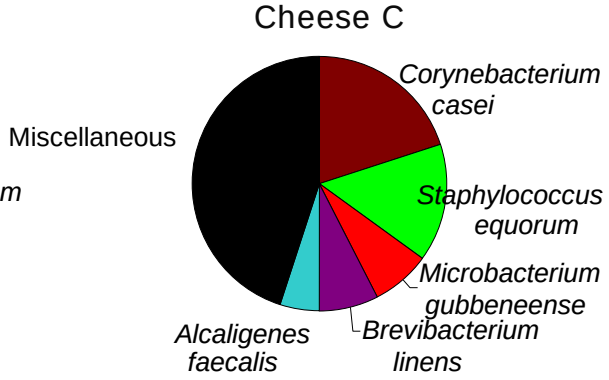
Cheese A

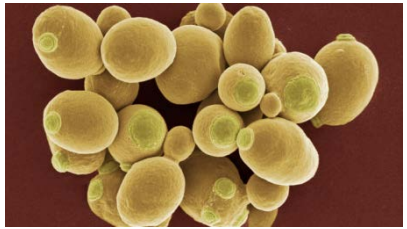


Cheese B



Cheese C





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

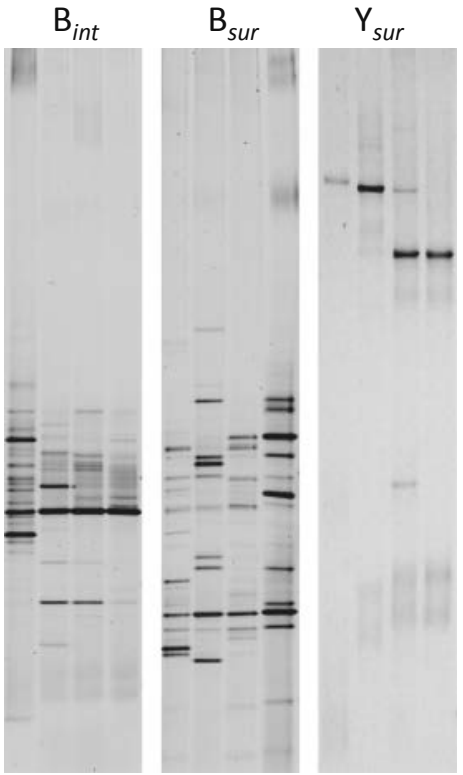
Microb Ecol (2013) 65:602–615
DOI 10.1007/s00248-012-0138-3

ENVIRONMENTAL MICROBIOLOGY

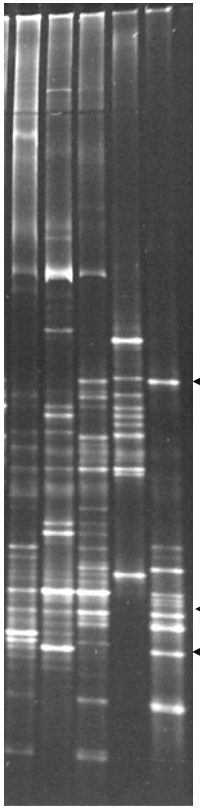
Isolation and Identification of the Microbiota of Danish Farmhouse and Industrially Produced Surface-Ripened Cheeses

Klaus Gori · Mia Ryssel · Nils Arneborg · Lene Jespersen

Danish farm house cheeses



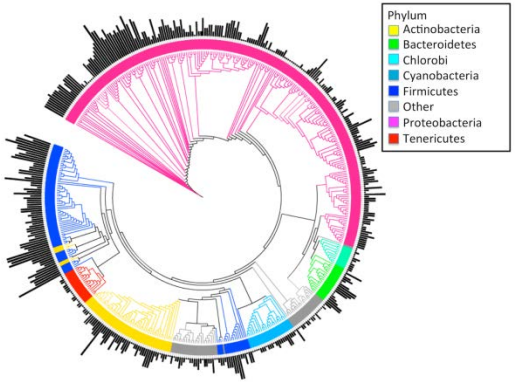
DNA based identification of microorganisms in the dairy



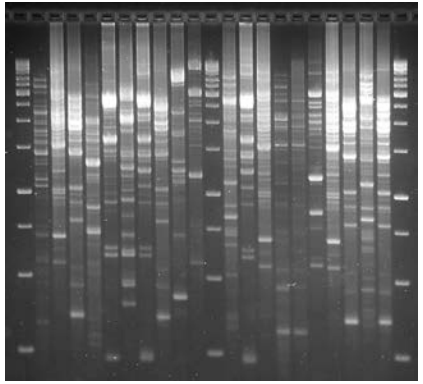
DGGE

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

16S/26S/ITS sequencing



Rep-PCR
(amplification of repetitive sequences)



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Going in-depth with the indigenous microflora of surface-ripened cheeses

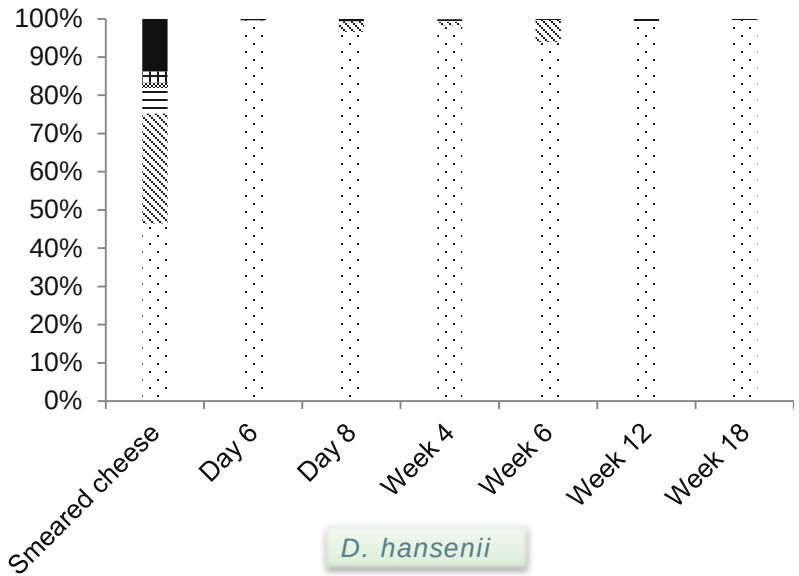
Sequencing the surface microbiota of Danbo cheese

Contents lists available at ScienceDirect
International Journal of Food Microbiology
journal homepage: www.elsevier.com/locate/ijfoodmicro

Microbial diversity and dynamics throughout manufacturing and ripening of surface ripened semi-hard Danish Danbo cheeses investigated by culture-independent techniques

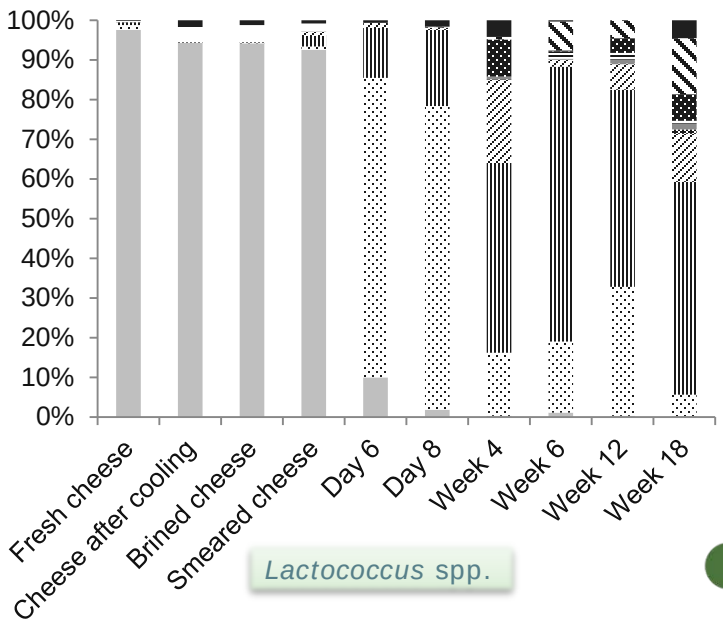
Mia Ryssel [a,1], Pernille Johansen [a,*,1], Waleed Abu Al-Soud [b], Søren Sørensen [b], Nils Arneborg [a], Lene Jespersen [a]
[a] Department of Food Science, University of Copenhagen, Rolighedsvej 26, DK-1958 Frederiksberg C, Denmark
[b] Department of Biology, University of Copenhagen, Universitetsparken 15, DK-2100 København Ø, Denmark

Surface yeasts



D. hansenii

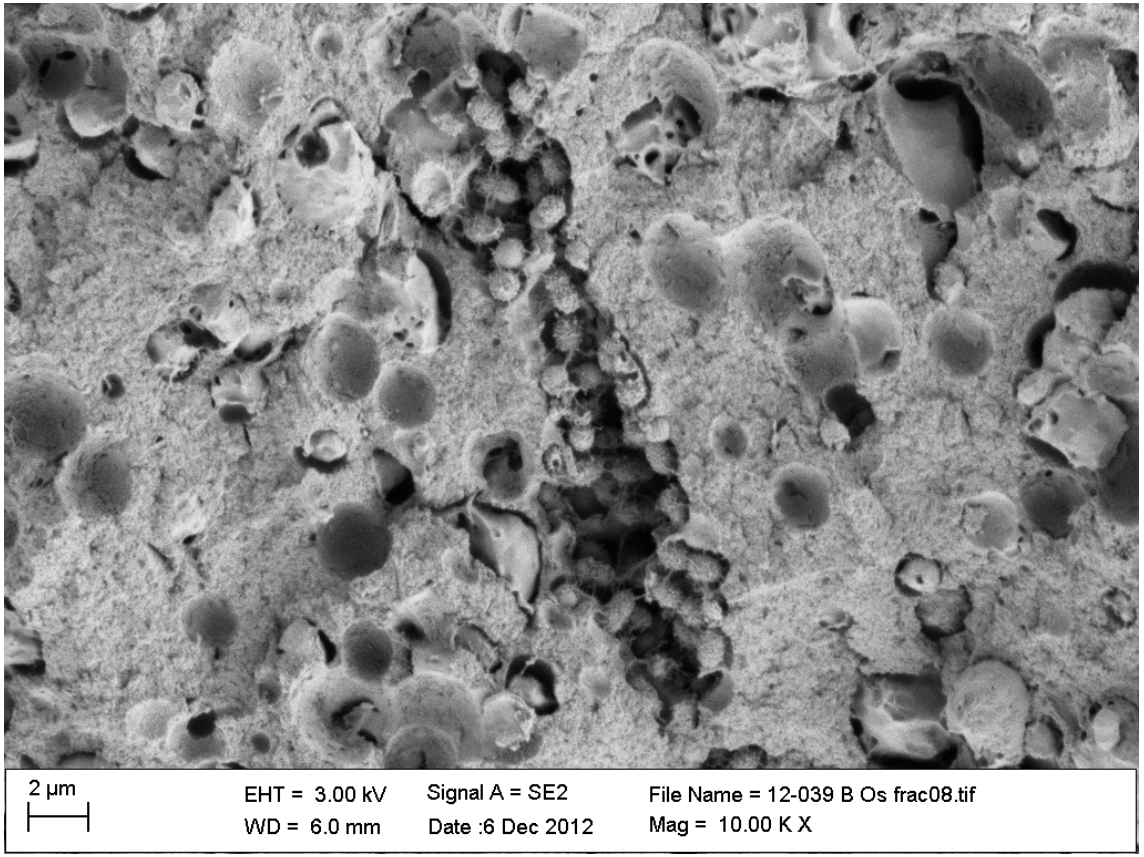
Surface bacteria



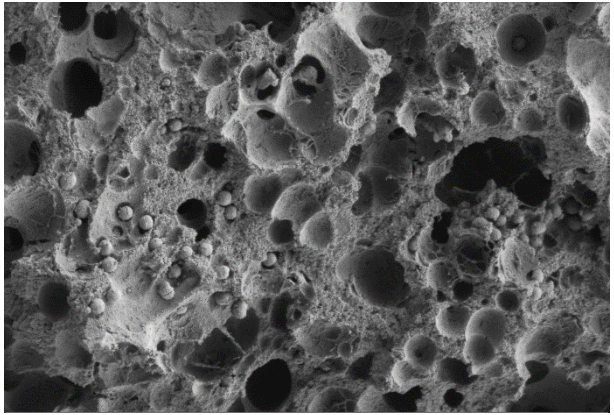
Lactococcus spp.

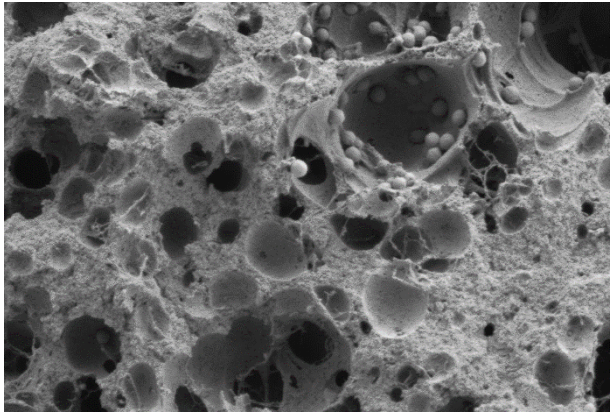
Yeast: *Debaryomyces* spp., *Trichosporon* spp., *Candida* spp., *Torulaspora* spp., *Pichia* spp.
Bacteria: *Lactococcus* spp., *Lactobacillales, Staphylococcus* spp., *Corynebacterium* spp., *Brevibacterium* spp., *Pseudoclavibacter* spp., *Alkalibacterium* spp., *Marinilactibacillus* spp., *Clostridiisalibacter* spp., *Acinetobacter* sp

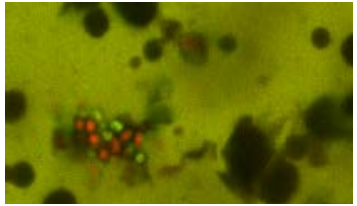
Understanding the basic mechanisms behind cheese ripening



NaCl content influences growth, death and autolysis of *Lactococcus lactis*







International Journal of Food Microbiology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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Impact of NaCl reduction in Danish semi-hard Samsoe cheeses on proliferation and autolysis of DL-starter cultures

Lise Søndergaard [a,*], Mia Ryssel [a], Carina Svendsen [a], Erik Høier [b], Ulf Andersen [c], Marianne Hammershøj [d], Jean R. Møller [d,1], Nils Arneborg [a], Lene Jespersen [a]

[a] Department of Food Science, Faculty of Science, University of Copenhagen, 1958 Frederiksberg C, Denmark
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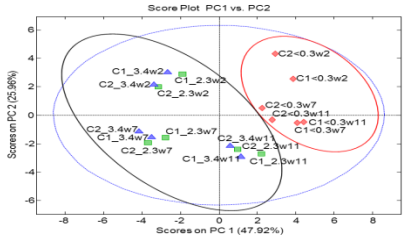
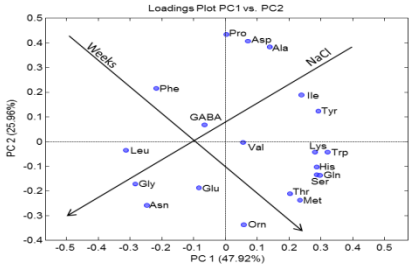
[d] Department of Food Science, Aarhus University, Blichers Allé 20, Postboks 50, 8830 Tjele, Denmark





- 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?)

Strain variation in NaCl tolerance

D. hansenii strains from surface ripened cheeses

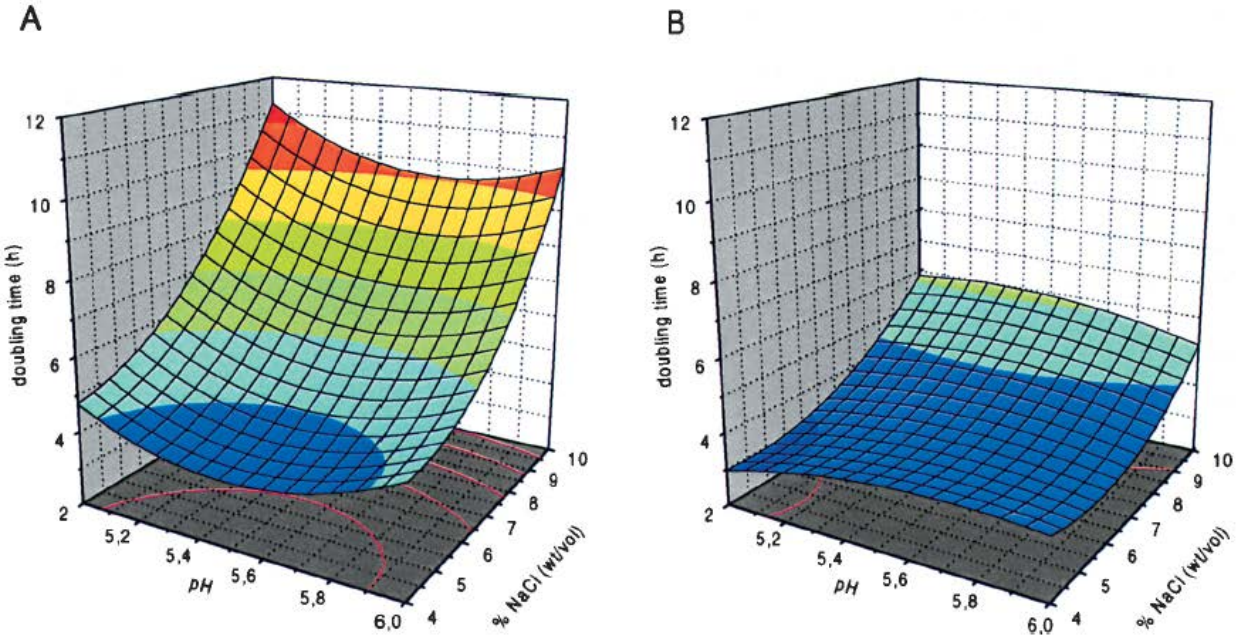


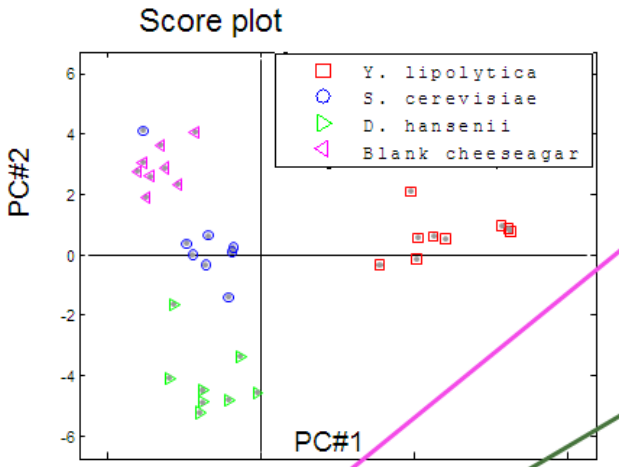
Figure 4. Response surfaces of the doubling time as a function of pH and NaCl concentration analyzed by the use of multiple linear regression. A: Isolate with mtDNA RFLP profile **G** (starter culture). B: Isolate with mtDNA RFLP profile **H** (dominant isolate).

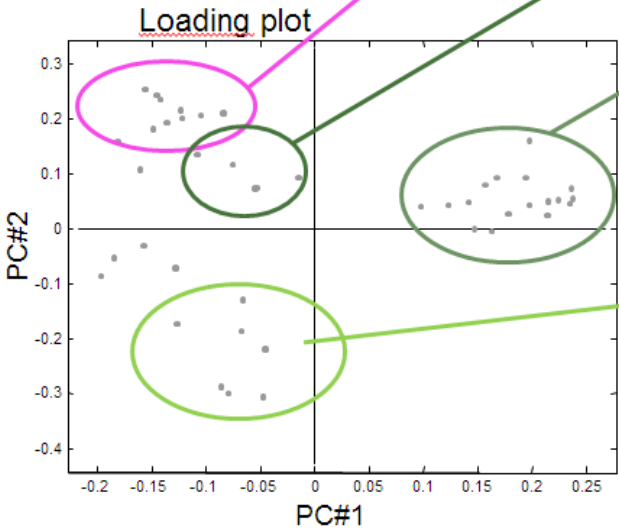
J. Dairy Sci. 85:478–486

Microbial Succession of *Debaryomyces hansenii* Strains During the Production of Danish Surfaced-Ripened Cheeses

K. M. Petersen,* S. Westall,† and L. Jespersen*
*The Royal Veterinary and Agricultural University.

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 (ethylacetat, isoamylacetat, ethylpropionat, ethylbutanoat), 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)

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***Debaryomyces hansenii* strains differ in their production of flavor compounds in a cheese-surface model**

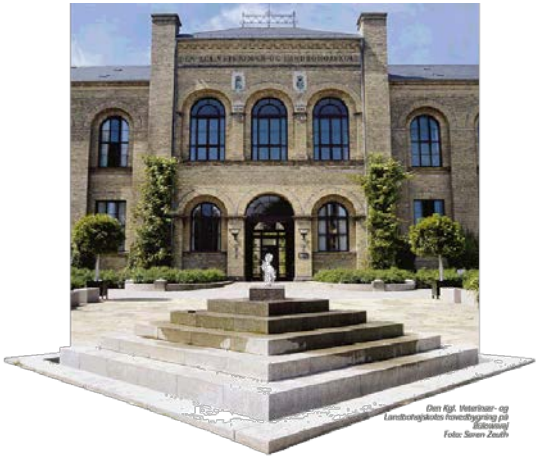
Klaus Gori[1], Louise Marie Sørensen[1], Mikael Agerlin Petersen[2], Lene Jespersen[1] & Nils Arneborg[1]

[1]Department of Food Science, Food Microbiology, Faculty of Life Sciences, University of Copenhagen, Rolighedsvej 30, DK-1958, Frederiksberg C, Denmark
[2]Department of Food Science, Quality and Technology, Faculty of Life Sciences, University of Copenhagen, Rolighedsvej 30, DK-1958, Frederiksberg C, Denmark

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 ....



Rolighedsvej 26, 4. sal – lj@food.ku.dk

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?