Mejeriforskningens Dag

Potential for Micro-organisms in Dairy Products

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2. marts 2017, Billund

Chr. Hansen A/S in brief

- Founded in 1874 in Copenhagen by Danish pharmacist Christian D.A. Hansen
- Products include microbial cultures for Dairy, Meat, Wine, Animal and Human applications, Dairy Enzymes and Natural Colors
- Producing starter cultures since the 1890s
- Turnover in 2015/16 of €949 million
- Spend ~7% of turnover on R&D
- ~400 employees in R&D
- State-of-the-art production facilities on five continents
- Estimate >1.2 billion people consume our products on any given day

UNITED STATES PATENT OFFICE.

VILHELM STORCH, OF COPENHAGEN, DENMARK, ASSIGNOR TO CHRISTIAN D. A. HANSEN, OF SAME PLACE.

FERMENT FOR RIPENING MILK, &c.

SPECIFICATION forming part of Letters Patent No. 561,291, dated June 2, 1896.

Application filed August 9, 1890. Serial No. 361,540. (No specimens.)

The worlds largest factory for starter cultures, Avedøre, DK

Micro-organisms in Dairy products - their function

Prokaryotes:

- **Lactic Acid Bacteria:**
  - *Lactococcus* spp.
  - *Lactobacillus* spp.
  - *Leuconostoc* spp.
  - *Streptococcus thermophilus*
- *Bifidobacterium* spp.
- **NS LABs:**
  - *Propionibacterium* spp. *Brevibacterium* spp. *Pediococcus* spp. *Enterobacterium* spp. *Hafnia alvei*

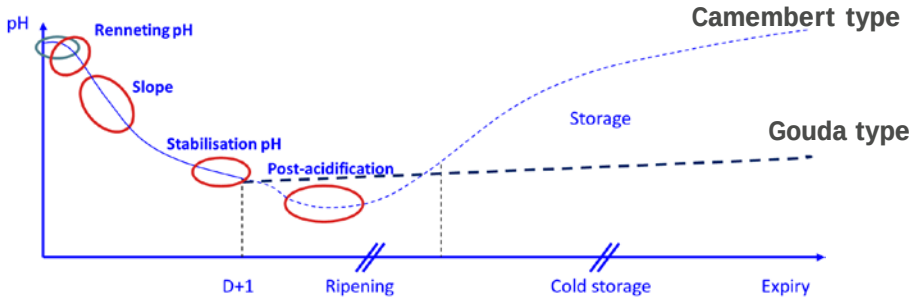
Eukaryotes:

- **Moulds**:
  - *Penicillium* spp.
- **Yeasts**:
  - *Saccharomyces* spp, *Kluyveromyces* spp, *Candida* spp, *Geotrichum candidum*. *Debaryomyces hansenii*

Function of micro-organisms in Dairy Products:

- **Acidification** (Lactose to Lactate)
- **Aroma formation** (Diacetyl, Acetaldehyde, Acetoin)
- **Ripening** (Protein and Fat degradation)
- **Texture** (production of CPS & EPS)
- **Probiotic effect**
- **Bio-protection** (Microbial ecology)

Deciphering the technological needs - Cheese:



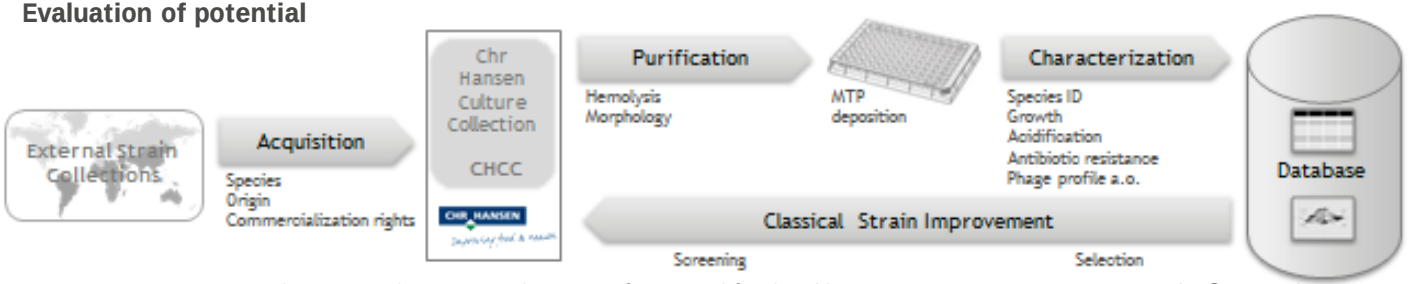
Chr. Hansen's Bacteria Discovery Platform

- The Chr. Hansen Culture Collection CHCC -

- Chr. Hansen strain depository >30.000 deposits (>70% of deposits = LABs)
- Registered culture samples from 1950'
- Worldwide origin
- Sourcing in compliance with Rio Convention on Biological Diversity (1992) and Nagoya Convention on Benefit Sharing (2014)
- Interesting strains are now routinely being genome sequenced in house:
  - Complete identification
  - Safety evaluation
  - Evaluation of potential

Finding & Improving Lactic Acid Bacteria

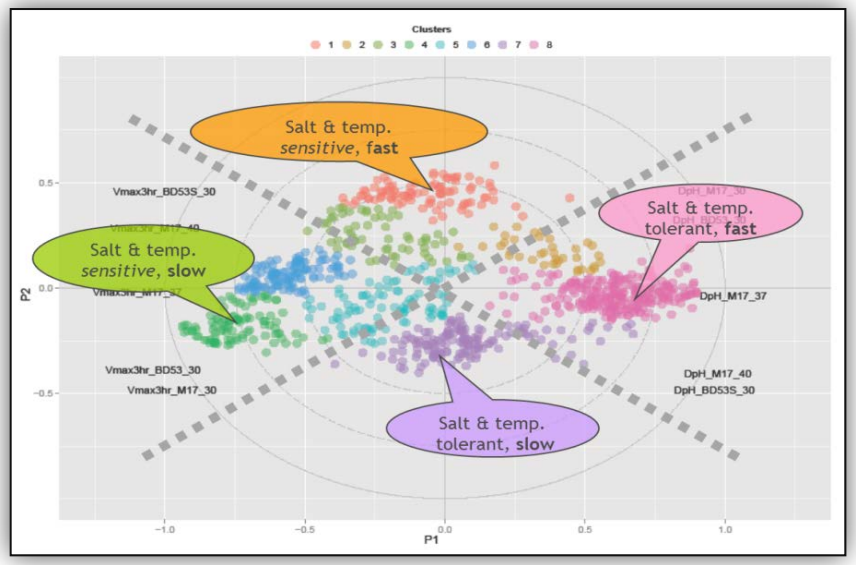
- Natural Diversity
  - Wild types/natural isolates
- Laboratory Strain Improvement
  - No use of recombinant DNA technology
  - Two approaches:
    - Direct Evolution
    - Dominant Selection
- All commercial cultures are evaluated as per EFSA's QPS system
  - Antibiotic resistance genes
  - Virulence genes



Johansen *et al.* 2015. In, Advances in fermented food and beverages, p. 227-248

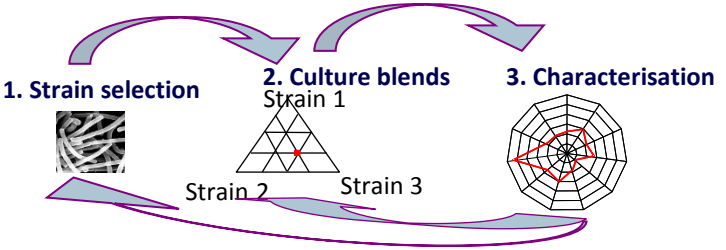
Chr. Hansen's Culture Development Platform

Phenotypic and -omics characterization of individual LAB gives interesting leads

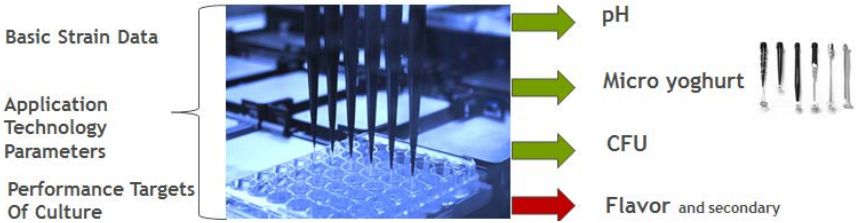


Johansen *et al.* 2015. In, Advances in fermented food and beverages, p. 227-248

Development is an exercise in Combinatorial Microbiology



Automated process for culture development



New Paradigm for Product Development for the Dairy industry

Finding & Improving Lactic Acid Bacteria

Taste
Texture/EPS
Yield
Shelf life
Health benefits

Add

Remove

Sugar
Fat
Protein
Texturizers
Stabilizers
Preservatives

Case #1: How to reduce sugar level in Yogurt and still maintain sweetness level

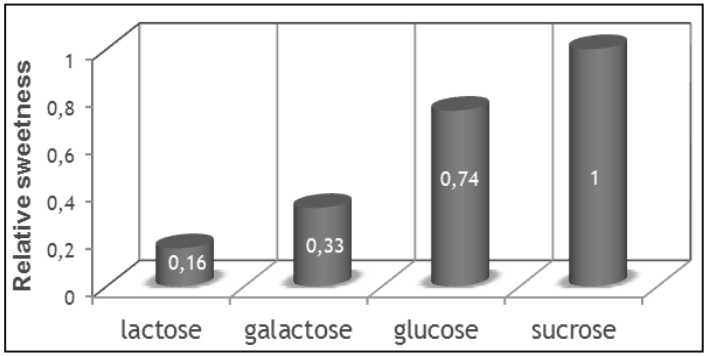
Issue: Yogurt is considered a healthy and Natural food, however contain added sucrose to provide sweetness:

Yoplait Original Yogurt

=

2.7 Krispy Kreme Doughnuts

Not all sugars are created equal with regard to sweetness:

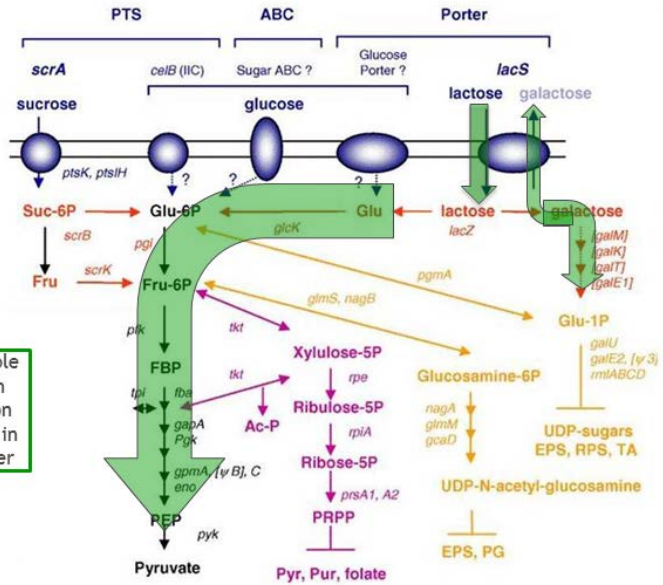


Strategy:

A) Change metabolic pathway in *Streptococcus thermophilus* from consuming glucose to galactose
B) Make *Lactobacillus delbrueckii* subsp. *bulgaricus* defect to consume glucose from the milk.

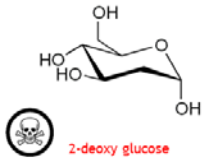
Case #1: Strategy to select the optimal *Streptococcus thermophilus* strain

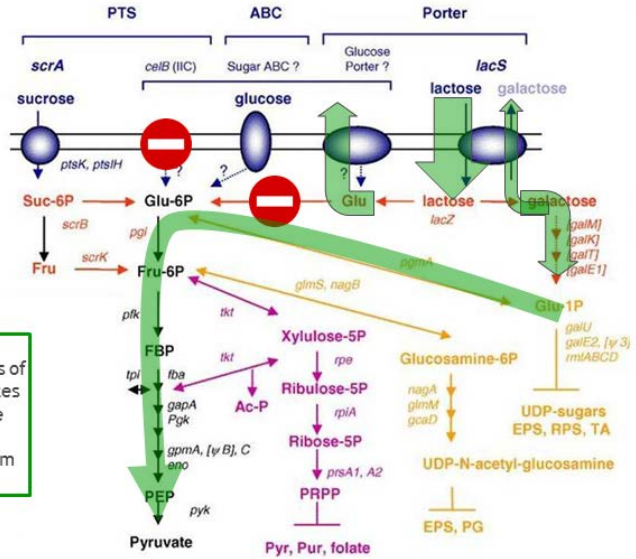
Galactose- fermenting *S. thermophilus* growing on lactose



Selection of mutants able to grow on plates with galactose as sole carbon source gives mutations in the *gal* operon promoter

Glucokinase-negative galactose-fermenting PTS-negative *S. thermophilus* growing on lactose



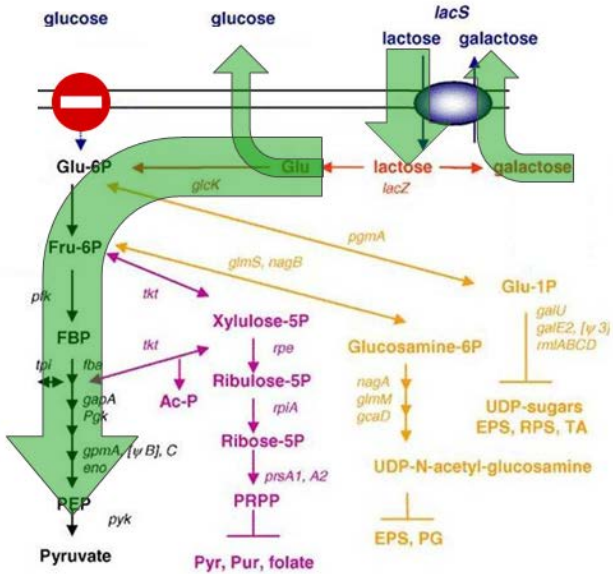


Selection of mutants resistant to higher levels of 2-deoxy-glucose on plates with galactose as sole carbon source gives mutations in PTS system transporting glucose

Sørensen *et al* (2016) *Appl. Environ. Microbiol*. **82**:3283-3692

Case #1: Selection of right *Lactobacillus delbrueckii* subsp *bulgaricus*

Non-glucose absorbing *Lactobacillus delbrueckii* subsp. *bulgaricus* growing on lactose



Selection of mutants resistant to 2-deoxy-glucose on plates with lactose as sole carbon source gives mutations in PTS system transporting glucose

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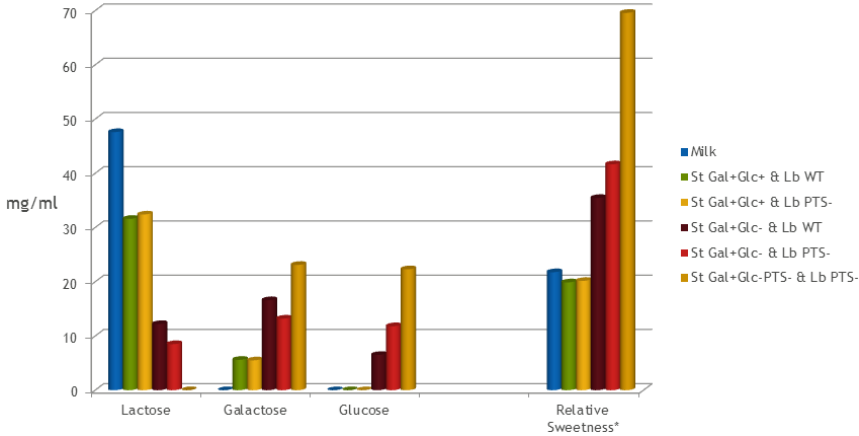
Sugar content of yogurt fermented with various combinations of strains

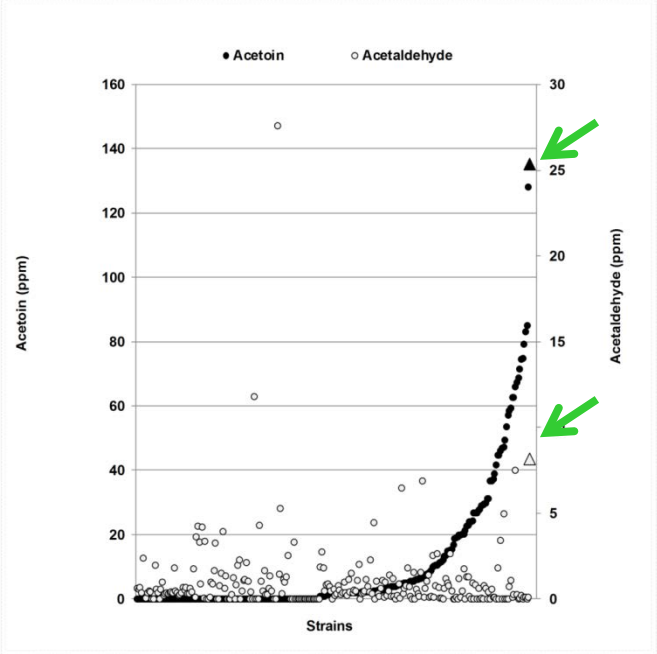


When using the "Sweety" cultures it is possible to reduce addition of sucrose by 10-25% .

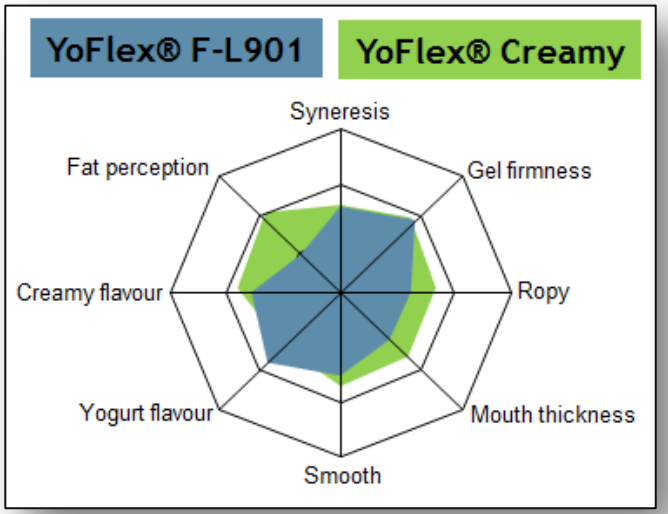
Case #2 : Giving low-fat yogurt great taste

Low-fat yogurt is generally lacking “creamy” flavor notes.

Screening for strains with right flavor production



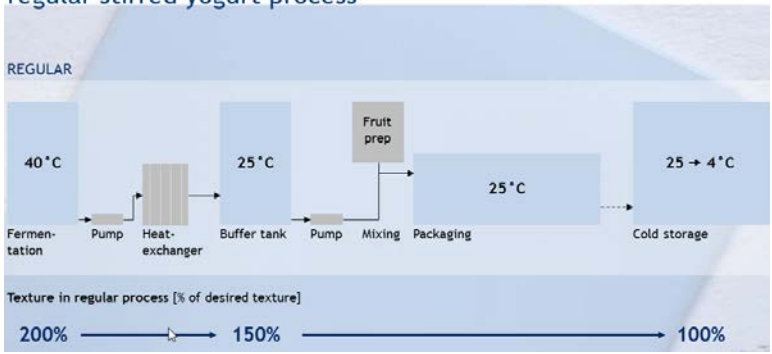
Diacetyl and acetoin increase the sensation of “fat” in yogurt



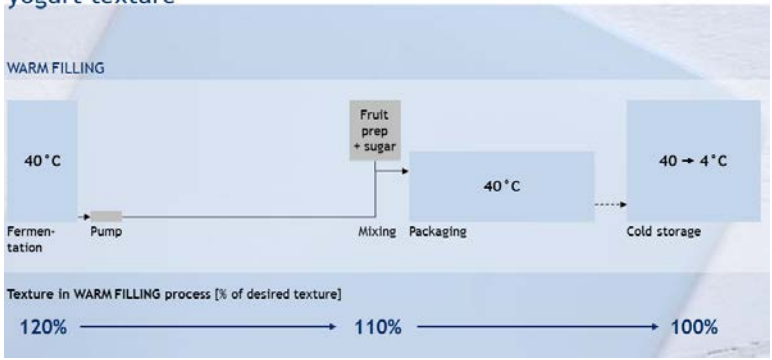
Jiminez *et al.*, 2012 WO/2012/136832

Case #3 : Acidifix: How to preserve texture

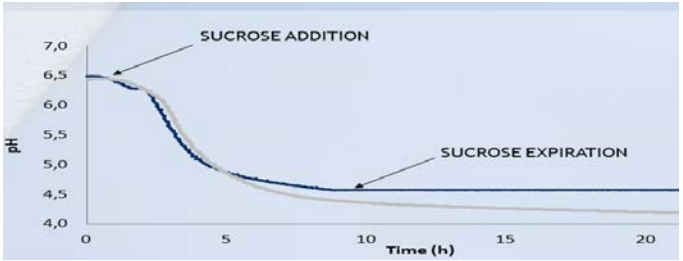
Up to 50% of texture is lost by pumping and shearing in a regular stirred yogurt process



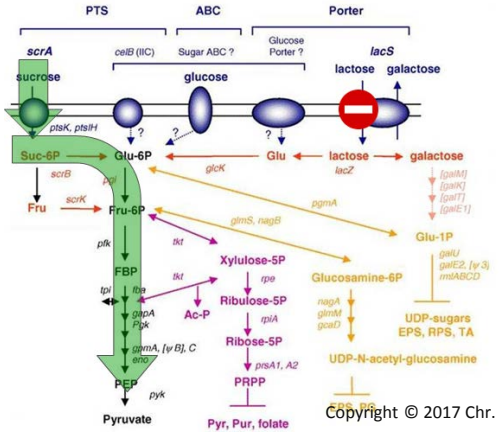
The WARM FILLING yogurt process is the best way to preserve yogurt texture



The key to success is to control the final pH of the yogurt.

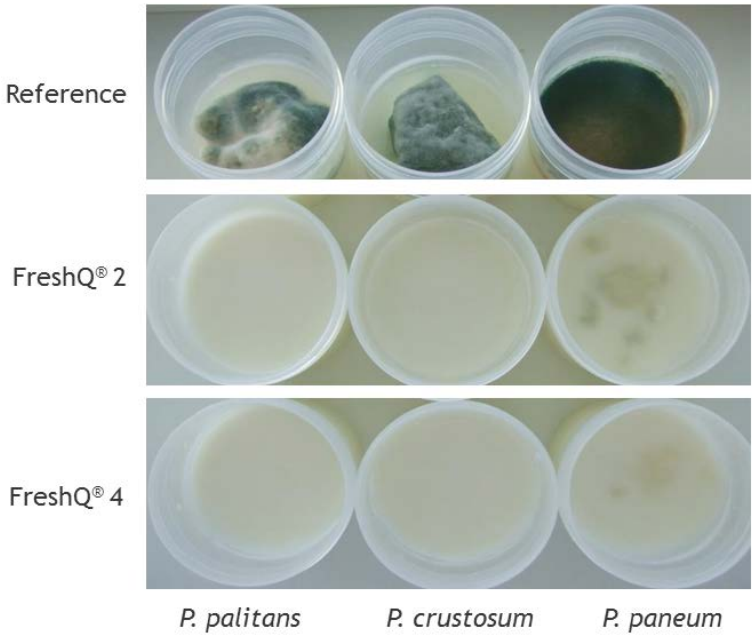


How we solved this challenge:

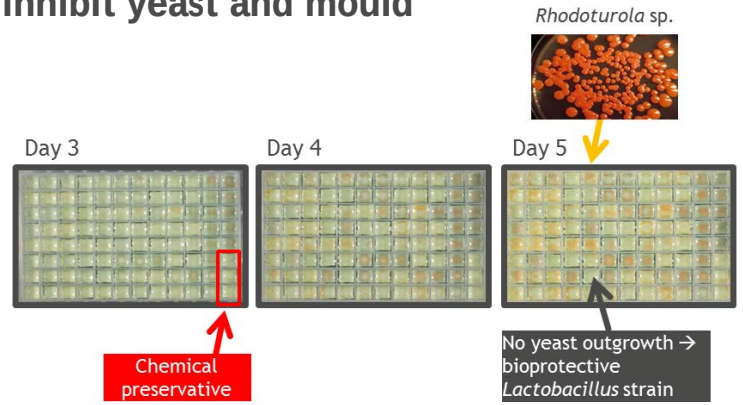


Case #4 : How to Keep it Great - FreshQ

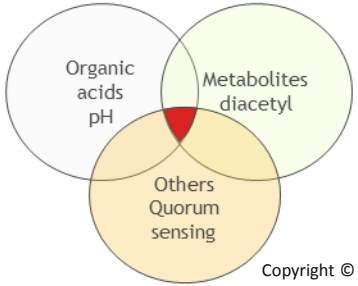
FreshQ: How Microbial Ecology works in a yogurt.



Screening for *Lactobacillus* strains that inhibit yeast and mould

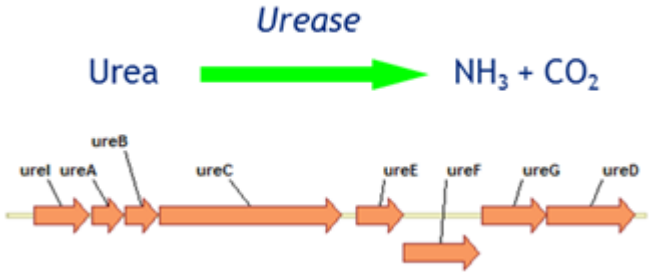


Defining the multifactorial bio-protection hot spot

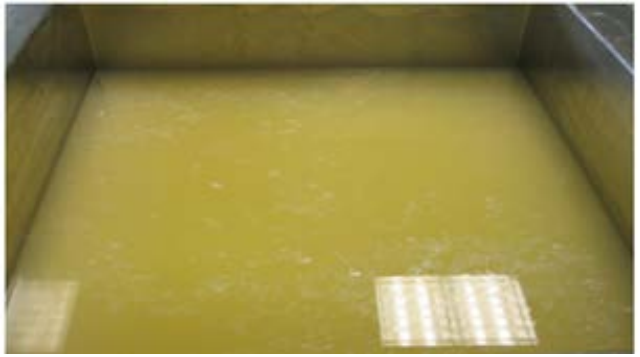


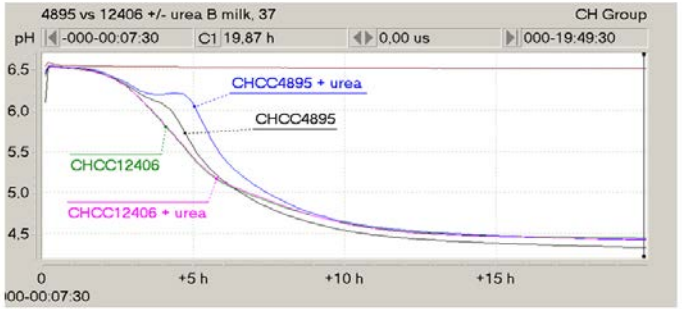
Case #5 : Solving problems with floating curds

Floating curd in Cottage Cheese production due to CO2 production of the starter culture



Use of Urease negative *S. thermophilus* strain as starter culture





Conclusions and future perspectives

Chr. Hansen' state of the art strain discovery platform combines -omics & lab-automation technologies with a proven track record in delivering innovative solutions for the dairy industry

The demand for highly specialized LABs with unique application properties will increase in the future thus requiring customized screening systems and advanced Classical Strain Improvement strategies

Lactic Acid Bacteria can by fine-tuned to make better products by optimizing their metabolism with natural techniques

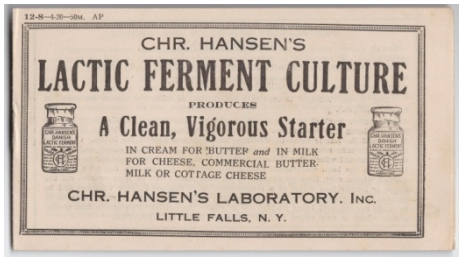
Acknowledgement:


Proceedings

Highly accessed | Open Access

The art of strain improvement of industrial lactic acid bacteria without the use of recombinant DNA technology

Patrick MF Derkx*, Thomas Janzen, Kim I Sørensen, Jeffrey E Christensen, Birgitte Stuer-Lauridsen and Eric Johansen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