Advances in Culture Technology

Yogurt & Fermented Products
Society of Dairy Technology, 28th March, 2012

Hector Scott, Chr. Hansen Ltd.

We are

- A global supplier of bioscience based ingredients to the food, health, pharmaceutical and agricultural industries
- We produce cultures and dairy enzymes, probiotics and natural colors
- Our leading market positions stem from innovative products and production processes, long-term customer relationships and intellectual property

innovation in progress

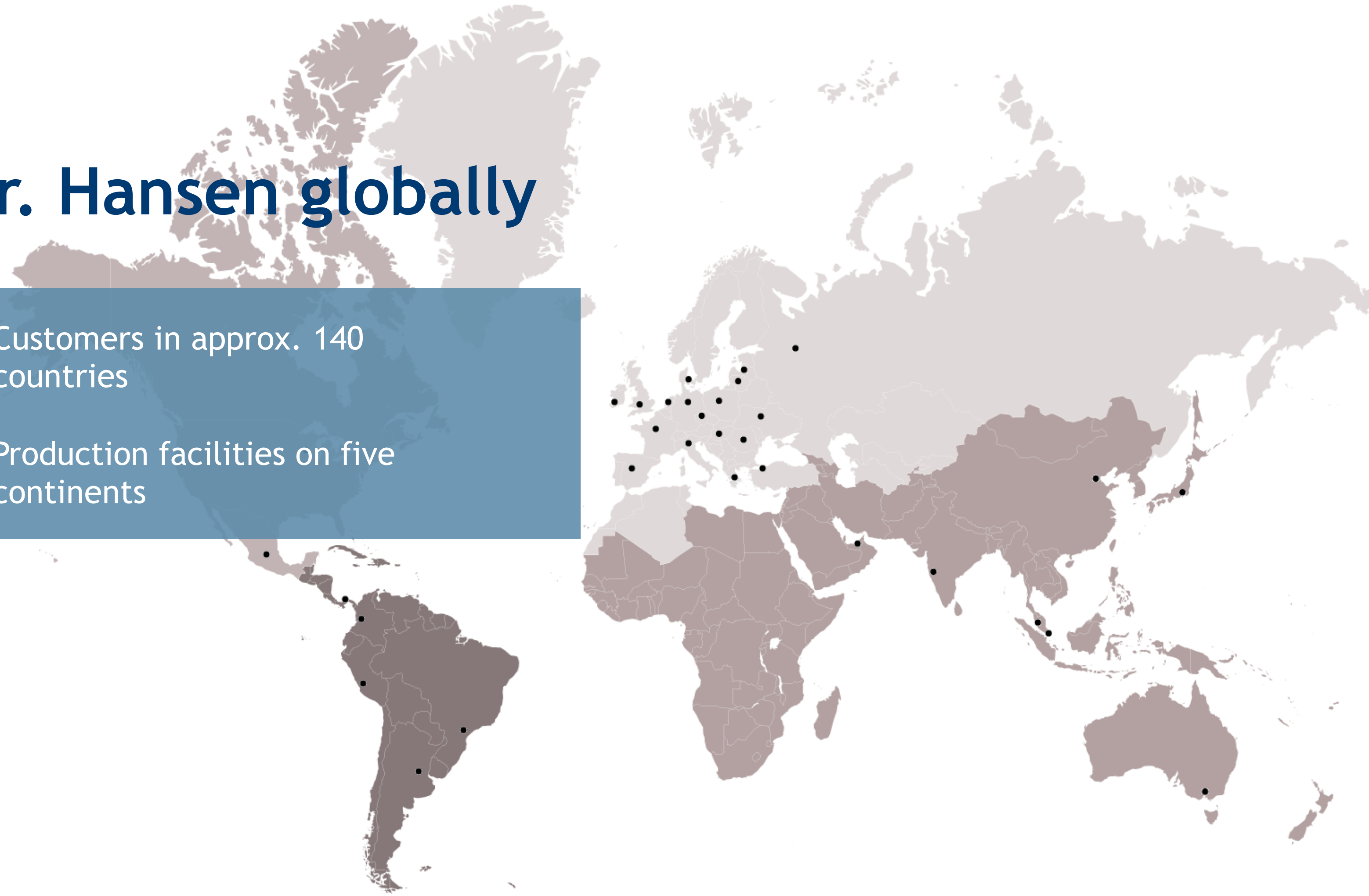
CHR HANSEN

Chr. Hansen in brief

Chr. Hansen HQ
Hørsholm, Denmark

- Founded in 1874 in Copenhagen by Danish pharmacist Christian D.A. Hansen
- Listed on NASDAQ OMX Copenhagen
- 2010/11 turnover EUR 636 million
- Organic growth ambitions of 8-10% annually
- Approx. 2,300 employees

Chr. Hansen globally



- Customers in approx. 140 countries
- Production facilities on five continents

- Subsidiaries and representative offices in over 30 countries

CHR. HANSEN

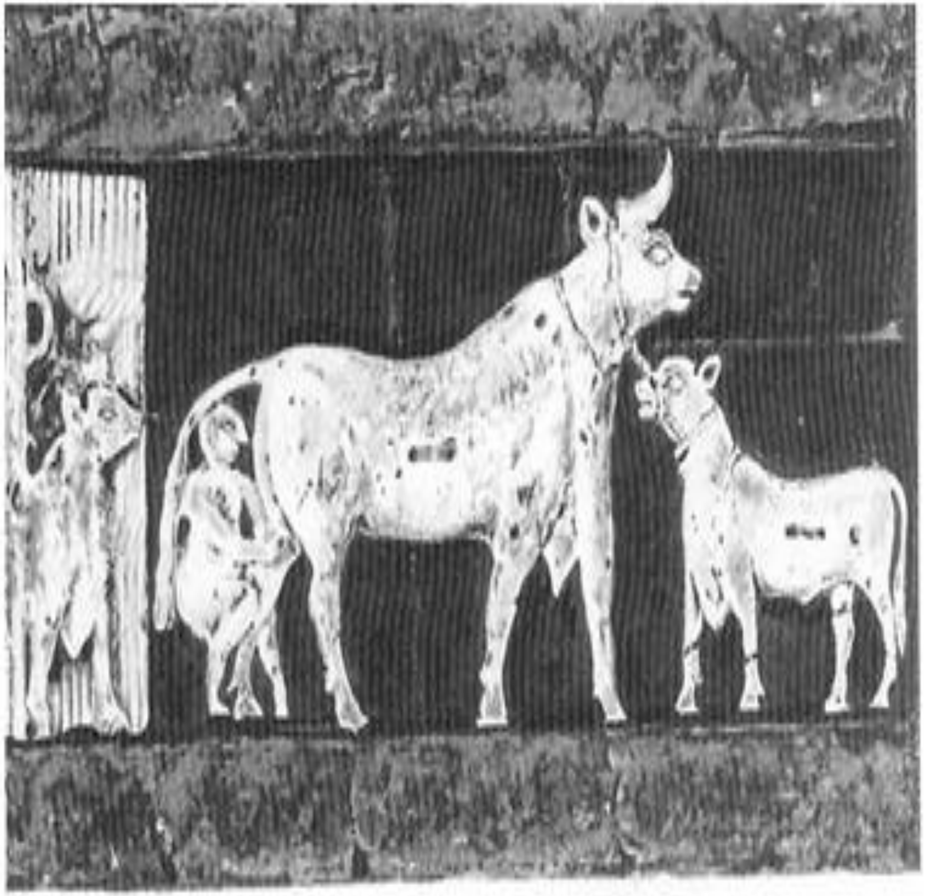
Overview of presentation

- Part 1 – History and early development of starter cultures
- Part 2 – Starter Culture Development in 2012
- Part 3 – Starter cultures and practical features in application

Part 1 – History and early development of Starter Cultures

Early origins of Dairy Fermentation

- Dairy fermentation is thought to have originated in the Middle East as a means of preserving the nutritive value of milk
- The 'Agricultural Revolution' which was the start of domestication of plants and animals (farming and human consumption of animal milks)
- Collection of animal milk + natural environment would have encouraged the acid-coagulation of milk, which on agitation, would have split into curds and whey (early form of cheese, or consumed fresh as a 'yogurt')
- Early fermentation of milk was 'wild' and performed by bacteria naturally present in the milk/animals/equipment



Art from Ur, Mesopotamia depicting early dairying

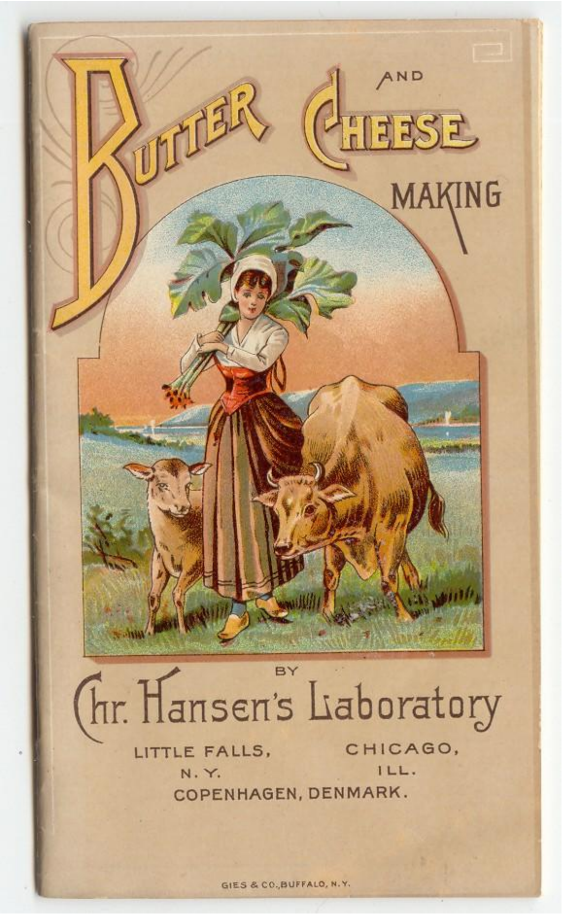
Origins of Commercial Starters

- Dairy production such as yogurt and cheesemaking remained a small scale enterprise for several thousand years

- Industrial Revolution of late 19th century created an urban pop. with a demand for more dairy products

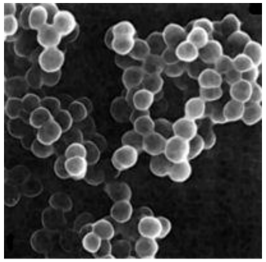
- This led to the start of larger scale commercial dairies for cheese and yogurt manufacture
    - Still using wild or natural fermentations = variable production quality

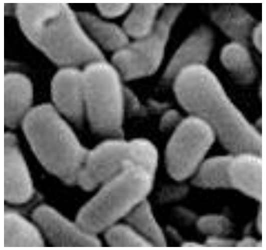
- Advent of pasteurisation in 1880's onwards limited the effectiveness of natural fermentations and thus a requirement for commercial starter cultures was created



Present Day - Commercial Culture Development

- Since the early days of commercial starter cultures, the science and technology has developed to the extent that culture development is considered from many interconnected aspects (for yogurt cultures):
  - Flavour
    - Lactic/acetaldehyde/diacetyl flavour notes etc.
  - Texture
    - Use of polysaccharide producing bacterial strains
  - Performance in a production environment
    - Effect of type/conc. of protein/fat/sugars
    - Interaction of varying bacterial strains
    - Effect of processing parameters (temp./shear pressure etc.)





Starter cultures are composed of Lactic Acid Bacteria (LAB)

What is a starter culture?

A starter culture is a culture of harmless, active bacteria grown in milk, which impart desirable flavour and texture characteristics in fermented milk products.

(Kosikowski & Mistry, 1997).

Genus, species and strains

- Starter cultures are described in terms of Genus and species
- e.g. *Lactococcus lactis* and *Streptococcus thermophilus*

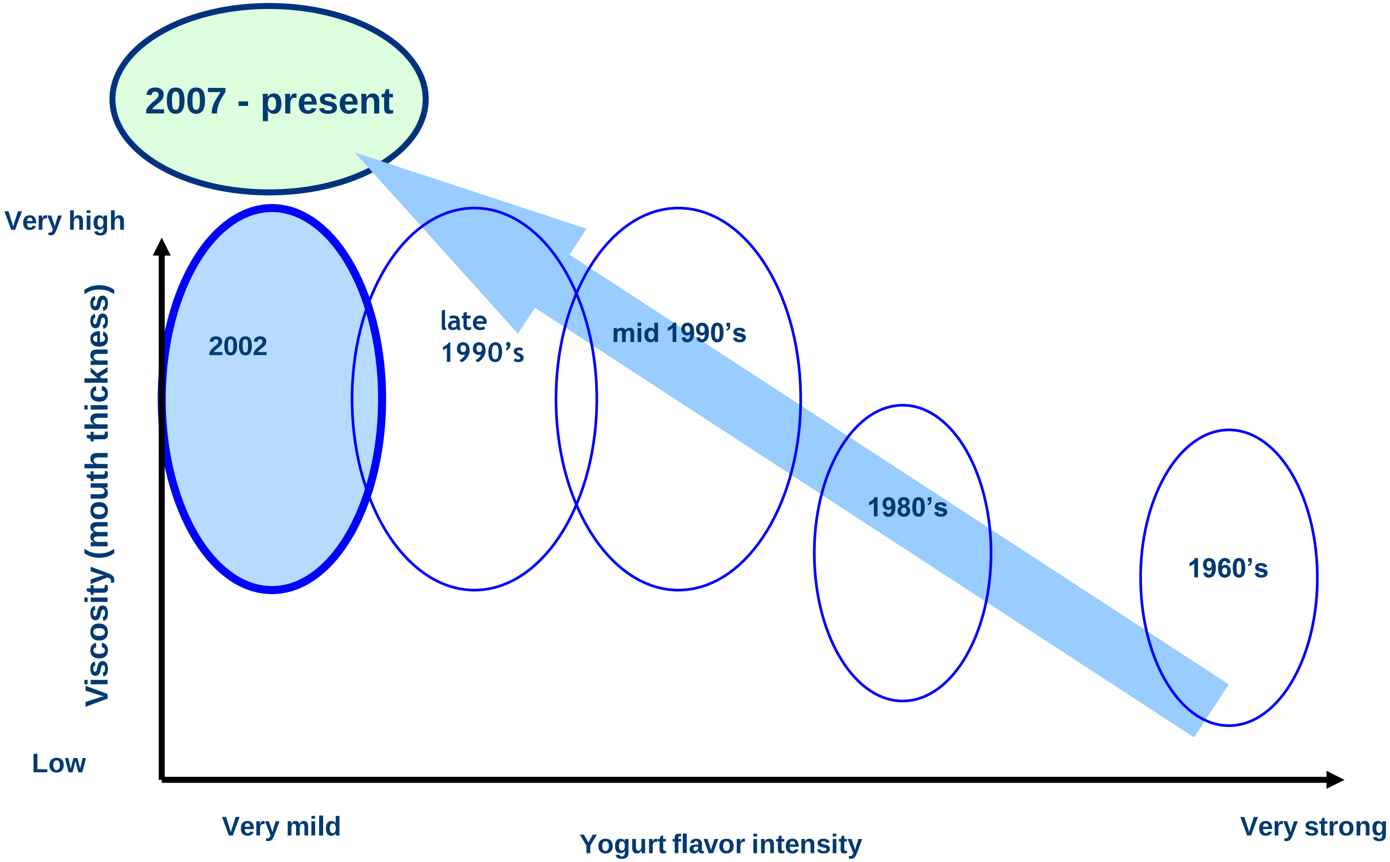
- However, there can be hundreds of different strains. Therefore, you can have 2 strains of *Lactococcus lactis which* are not identical
  - They can differ in various ways, e.g.
    - Acid tolerance
    - Flavour profile
    - Texture development

- **‘Genus” is used to describe a group of Lactic acid bacteria eg Streptococcus**
- **‘Species’ is used to describe the type of bacteria within a Genus eg thermophilus**
- **“Strain’ is used to describe individual bacteria within a species TH3**
- **Example Streptococcus thermophilus TH3**

- **Cultures are formulated by blending individual strains to deliver a desired flavour and texture profile**

Evolution of yogurt market requirements

Trend is moving towards cultures with high mouth thickness and mild flavor



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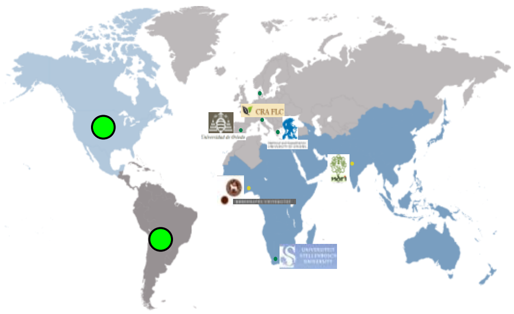
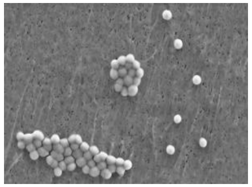
Part 2 - Starter Culture Development

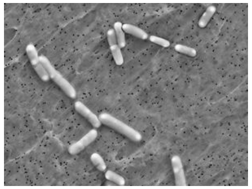
Automated diversity screening to identify leads

Strain Acquisition

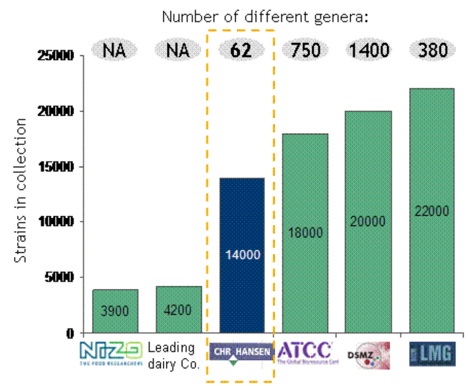


- Worldwide Strain Acquisition
- In compliance with Rio convention
- Partnering with Universities and Institutes:
  e.g. KU-life/Danida



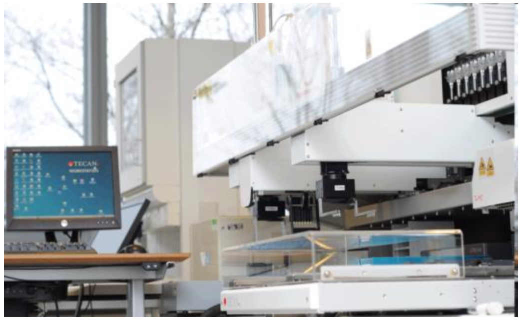


Strain Collection



- CH strain collection >14.000 deposits
- Narrow & deep genera diversity
- Strain purification & deposition (Lean)
- High Throughput single strain screening collection of **>2500** strains in 96 well MTP

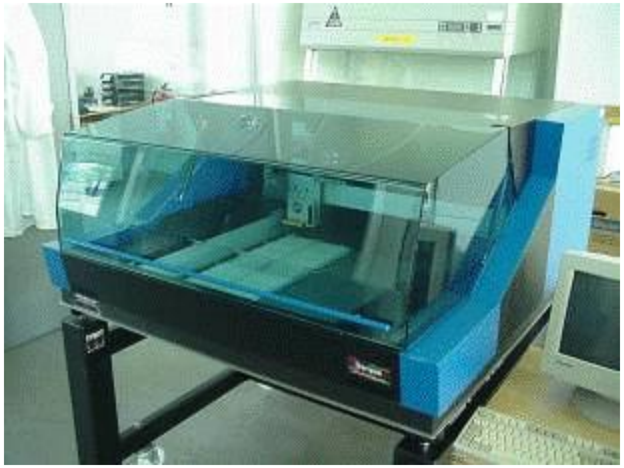
Strain Screening



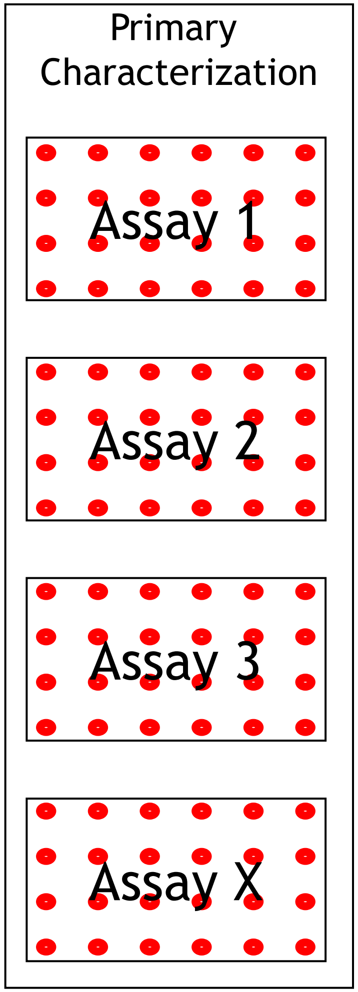
- Automated biological and biochemical characterization using high throughput setup with robots, e.g.
  - Growth assays
  - Acidification speed
  - Antibiotic resistance
  - Metabolite fingerprint
  - Bacteriophage profiling
- Application through characterization

Primary characterization

- High-throughput equipment
  - A colony picker which screens for thousands of colonies per hour
  - A micro arrayer for analysis of gene expression
  - A liquid handling robot (not pictured) for automated enzymatic assays



Primary characterization

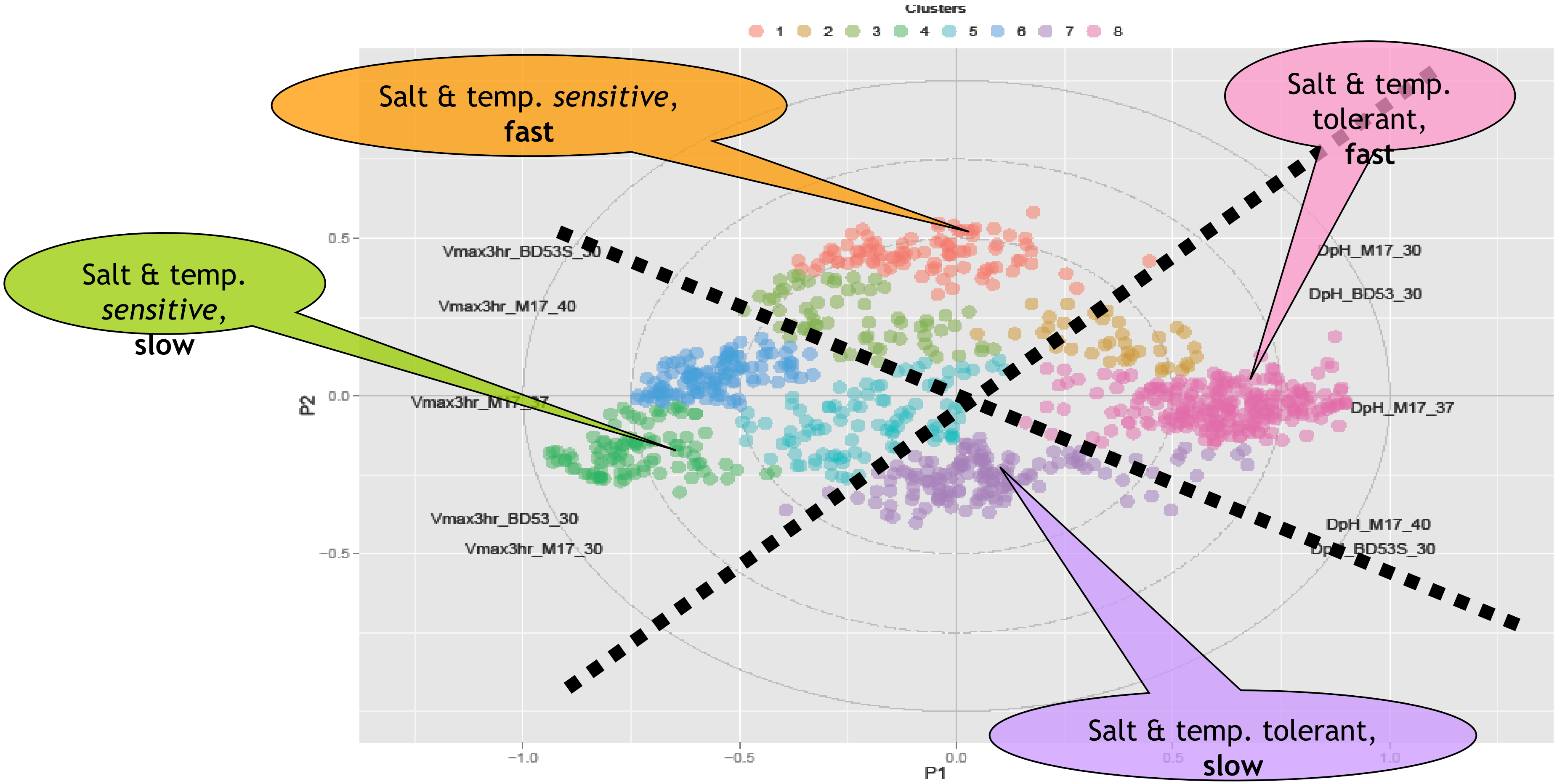


- **Species identification**
  - 16S rRNA sequencing
- **Growth**
  - Media (lab.media (M17/MRS) & ferm.media)
  - Temperature (30 °C; 37 °C; .. °C)
- **Acidification speed**
  - Milk
  - Salt Tolerance
- **Antibiotic resistance screen**
- **Metabolite fingerprinting**
  - Volatile Organic Compounds etc.
- **Phage-HTS profiling**

Primary characterisation - the results

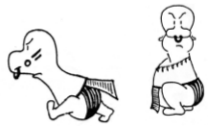
E.G. : Acknowledging *Lactococcus* diversity

Observable clusters among >900 *Lactococcus* strains (work from robotic colony picker and characterisation)



Development of yoghurt cultures

-the ideal world



New strains:

Screening of collections

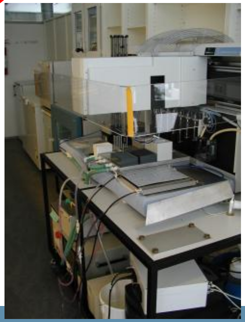
Mutations

Screening of blends using robotic methods

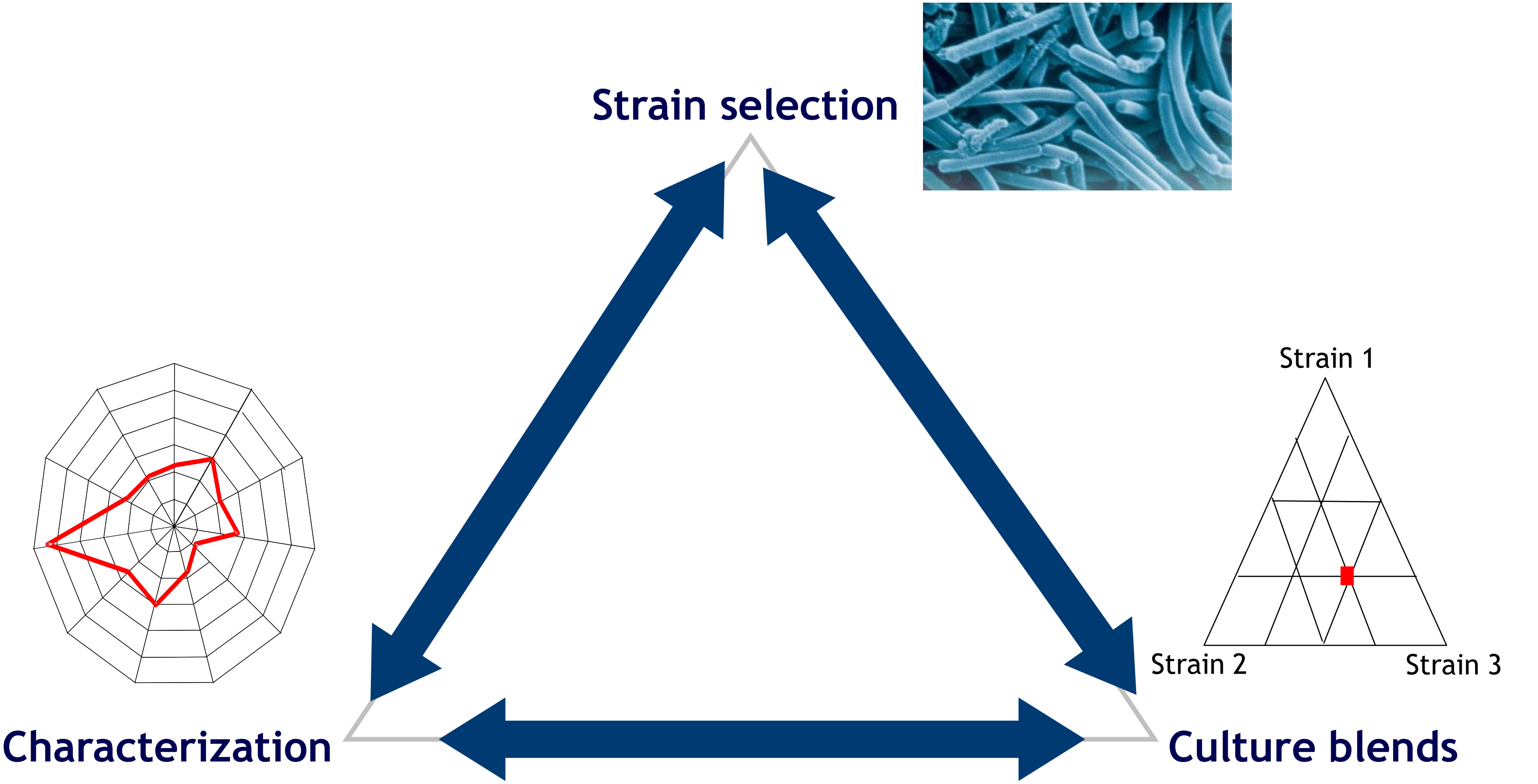
Test of blends in 200 ml scale

Test of blends in 3 l scale simulating full scale post treatment

Characterisation of the final cultures - commercially ready culture



Culture development based on combining strains which have well defined characteristics



Part 3 - Starter Cultures and their features

Fermented Milks

- Covers a broad range of products

- Crème Fraiche
- Sour Cream
- Fromage Frais

- Cultures traditionally using: ‘O’, ‘LD’ and more recently ‘ST’ components
- XT-series/ blended-ready to use convenient cultures for buttermilk and quarg

- CHN- cultures/ mixed cultures for fromage frais/creme fraiche/ sour cream

- Acid gel formation: pH of the white base is reduced to <4.6 (isoelectric point of milk)

- Casein is destabilised and forms a gel

Yoghurts

- Covers a range of formulations
- Stirred or set
- Fat content – 0.1 – 10%
- Greek yoghurt

- Yo-Flex Cultures – ST and LB only

- Nutrish
  - ABT: LA5 + BB12 + ST
  - ABY: LA5 + BB12 + ST + LB

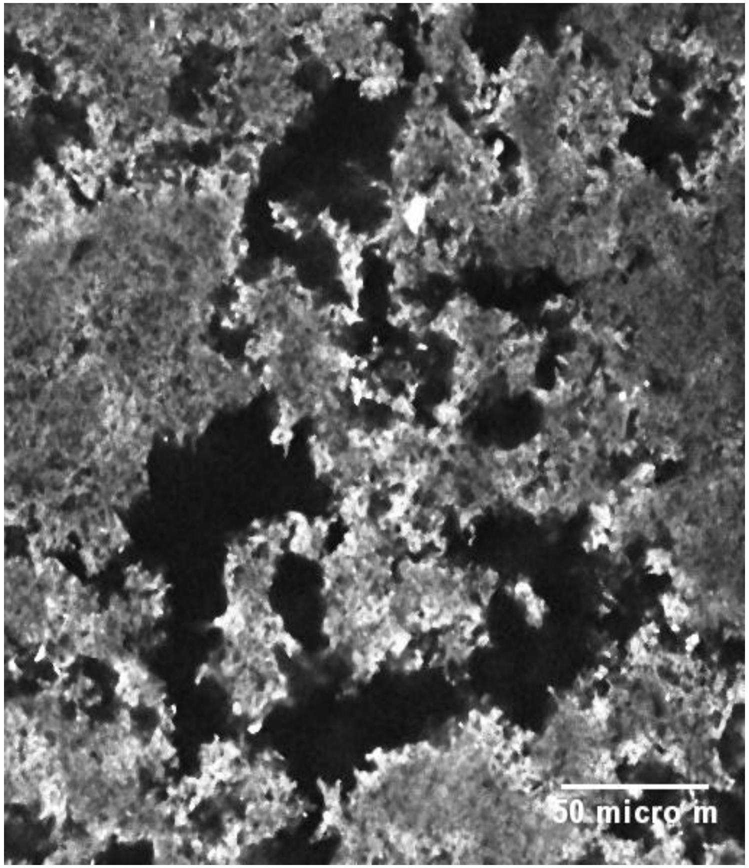
Functions of starter culture

The functions of starter cultures can summarised as:

- **Reduce the pH of milk**
  - Fermentation of lactose to lactic acid
- **Prevents the growth of spoilage micro-organisms** in the milk (preservation)
- **Improved flavour**
  - Production of diacetyl, acetaldehyde, lactate
- **Improved texture**
  - Production of polysaccharide

Acidification and acid-coagulation of milk

- Lactose is the principal sugar in milk (disaccharide composed of glucose and galactose)
- Lactose is metabolised by the starter culture, producing lactic acid, which reduces the pH of the milk, causing the milk protein to coagulate (isoelectric point of milk = pH 4.6)
- At pH below 5.0 a protein network is formed with void spaces containing serum and fat globules
- Protein network i.e. texture can be affected by
  - Fat content
  - Protein level and type
  - EPS-producing cultures



A yogurt protein network with serum pores

Texture Development

- Characteristic yoghurt texture results primarily from:
  - Denatured serum protein complexing with the casein micelles during pasteurisation at elevated temps.
  - Casein precipitation at pH 4.6 (iso-electric point)
- Viscosity can be increased using cultures which produce polysaccharides

Flavour Development

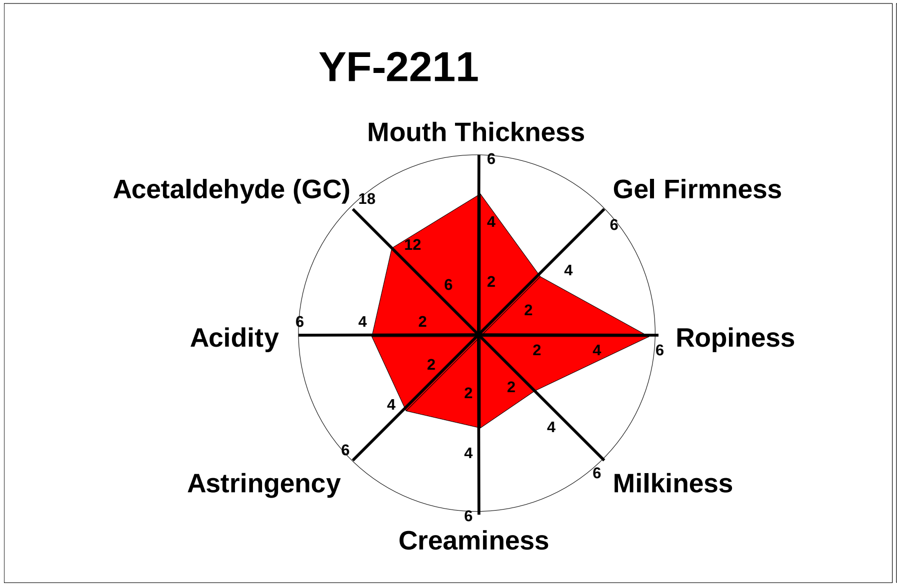
- Acetaldehyde is important to flavour development in yoghurt
- Acetaldehyde is thought to be a by-product of lactose fermentation in thermophilic cultures and proteolysis in mesophilic cultures
- Produced in small quantities (20μg/ml)

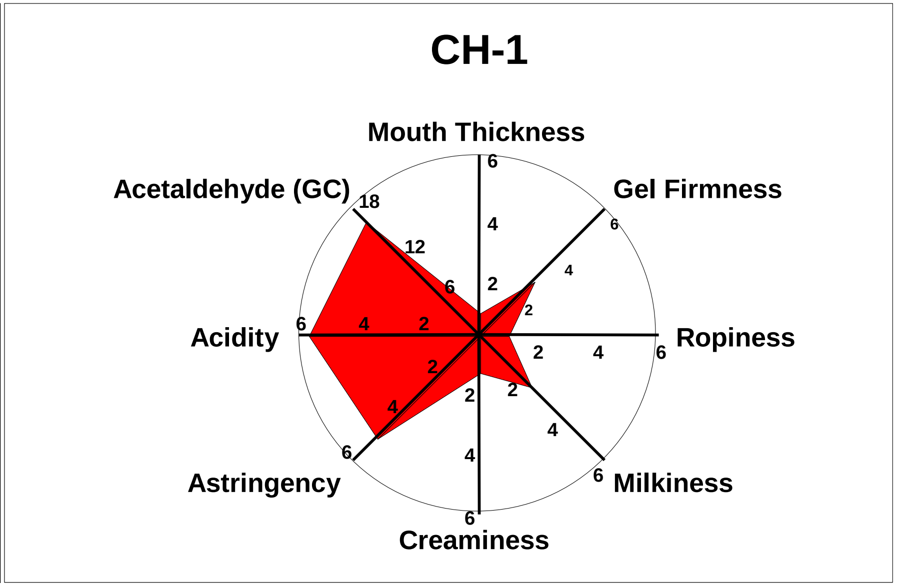
  Lactose $\rightarrow\rightarrow\rightarrow$ Acetaldehyde

However, aside from production of flavourful compounds, the texture of a yogurt will also affect flavour perception

Impact of texture on flavor perception

- Two yogurts with identical pH but differing in sensory evaluation of acidity attributed to a difference in mouth thickness
- YF2211 contains an EPS producing strain, whereas CH1 does not





Diffusion of aroma compounds, flavor release, odor perception are affected by food matrix, protein, fat and thickeners

Acidification and texture formation

- Inclusion of exopolysaccharide (EPS) producing strain(s) can also affects flavour perception
- EPS binds moisture and will reduce the perception of flavour compounds on the tongue and olfactory system – thus producing a 'milder' yogurt

EPS (-) culture:
Slightly stirred

EPS (+) culture:
Slightly stirred

Polysaccharide Production and Viscosity

- EPS produced by starter cultures are formed from saccharide monomers sourced directly from the milk, e.g. glucose/galactose, or from adapted monomers such as rhamnose, furanose etc.

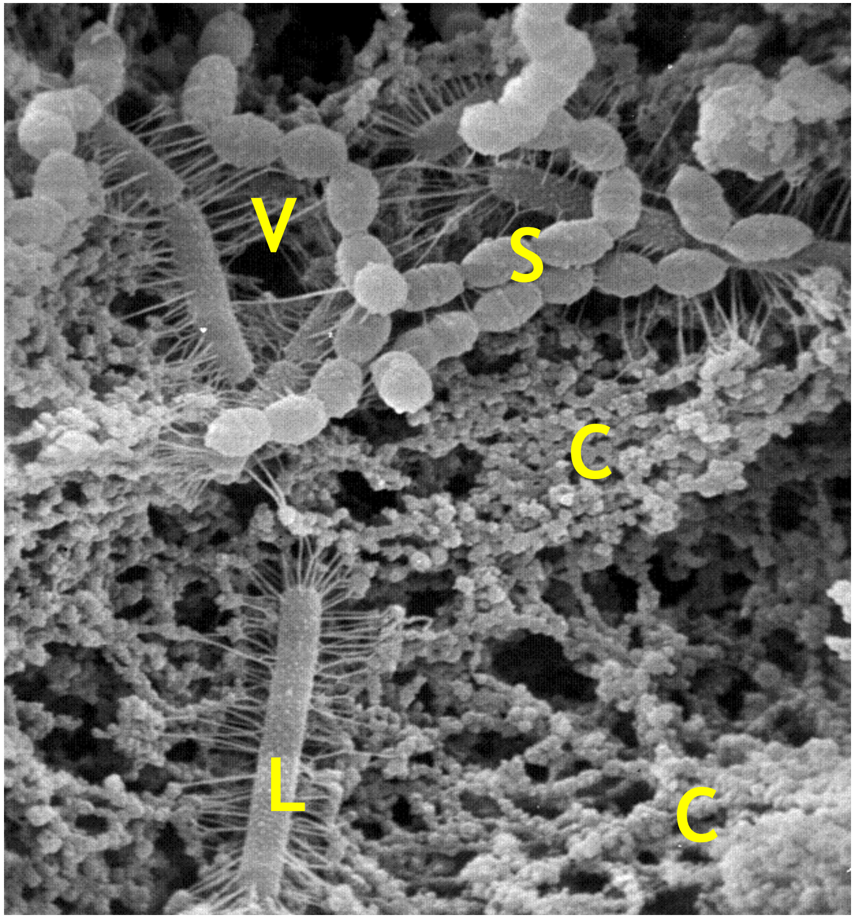
- EPS are often glucans or dextrans

- Manufacturing conditions can influence EPS characterisitics, e.g. Culture X produces a short textured EPS when incubated at >40°C, but a longer textured, ropy yogurt at incubation temps. <40°C

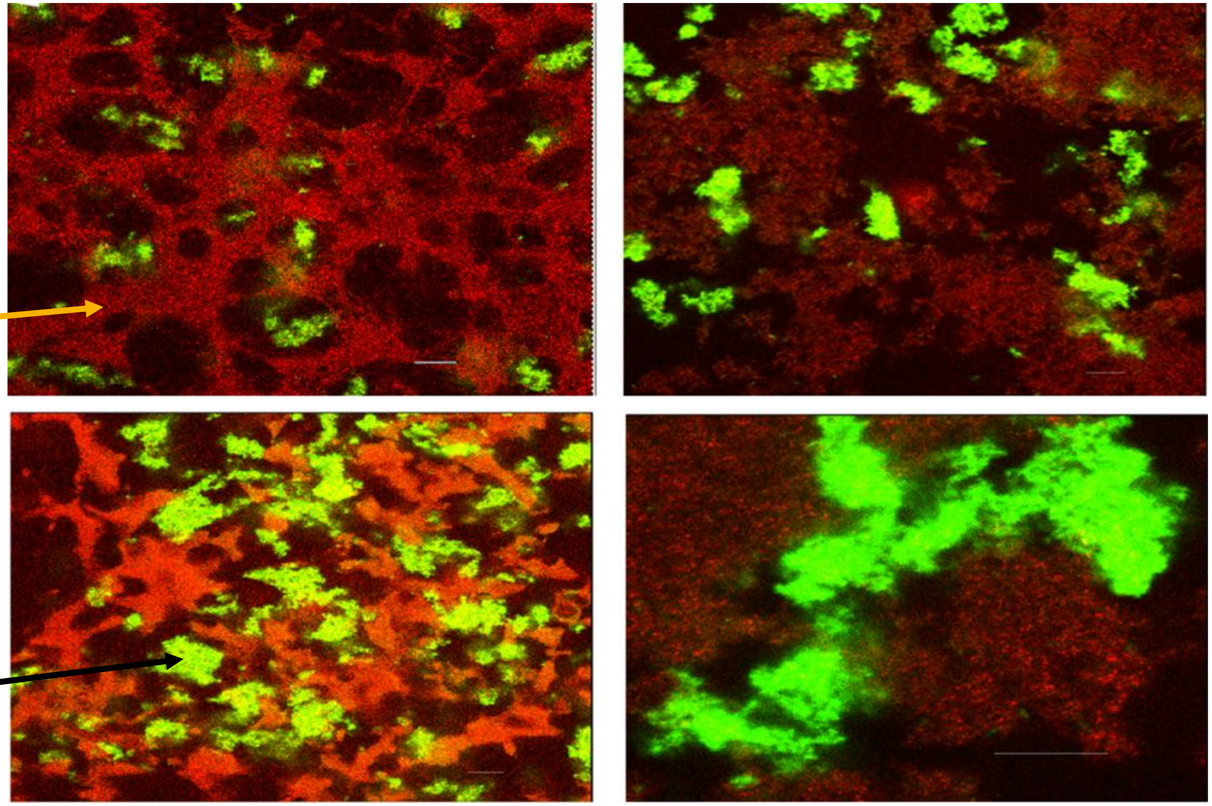
- Chain length of EPS will affect texture, e.g. long or short texture

EPS Production and Viscosity

- Certain LAB contribute extra thickness/viscosity to the final yoghurt
- Can be either Lb or ST
- Production of EPS (exo-polysaccharide), which gives the yoghurt a thick and creamy mouth feel
- Key:
  - L – lactobacillus rod
  - C – casein micelle network
  - V – EPS strands
  - S - Streptococcus



Distribution of EPS in yogurt



Source: Hassan et al. (2002)

Confocal scanning laser microscopy images; courtesy of Dr. Ashraf Hassan

Base formulation and EPS formation

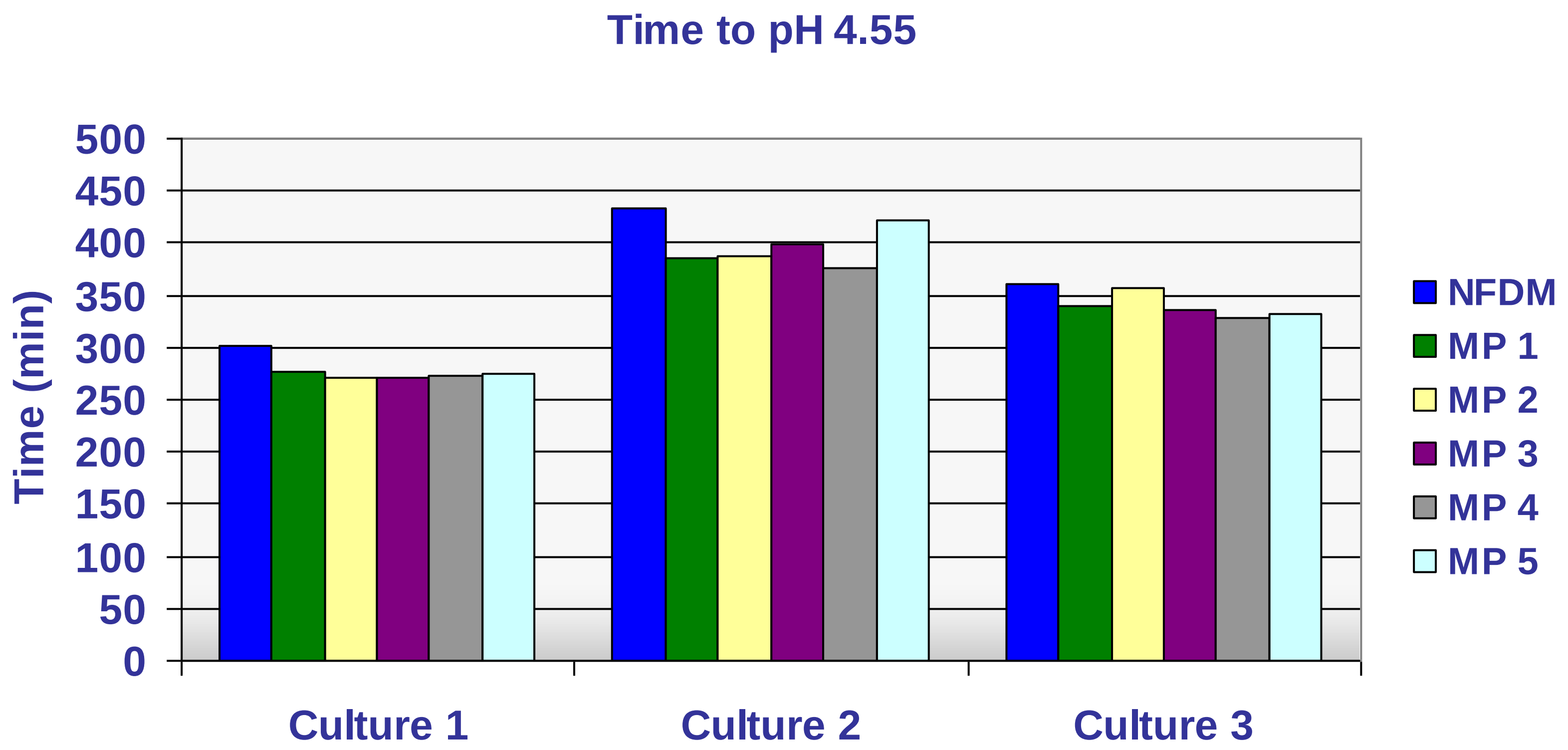
- Base formulation can also trigger changes in EPS formation pathways

  - The combination of lactose + glucose or lactose + fructose, respectively influenced enzyme activity and increased the yield of EPS in S*. thermophilus* LY03 with the former (Degeest and De Vuyst 2000.)

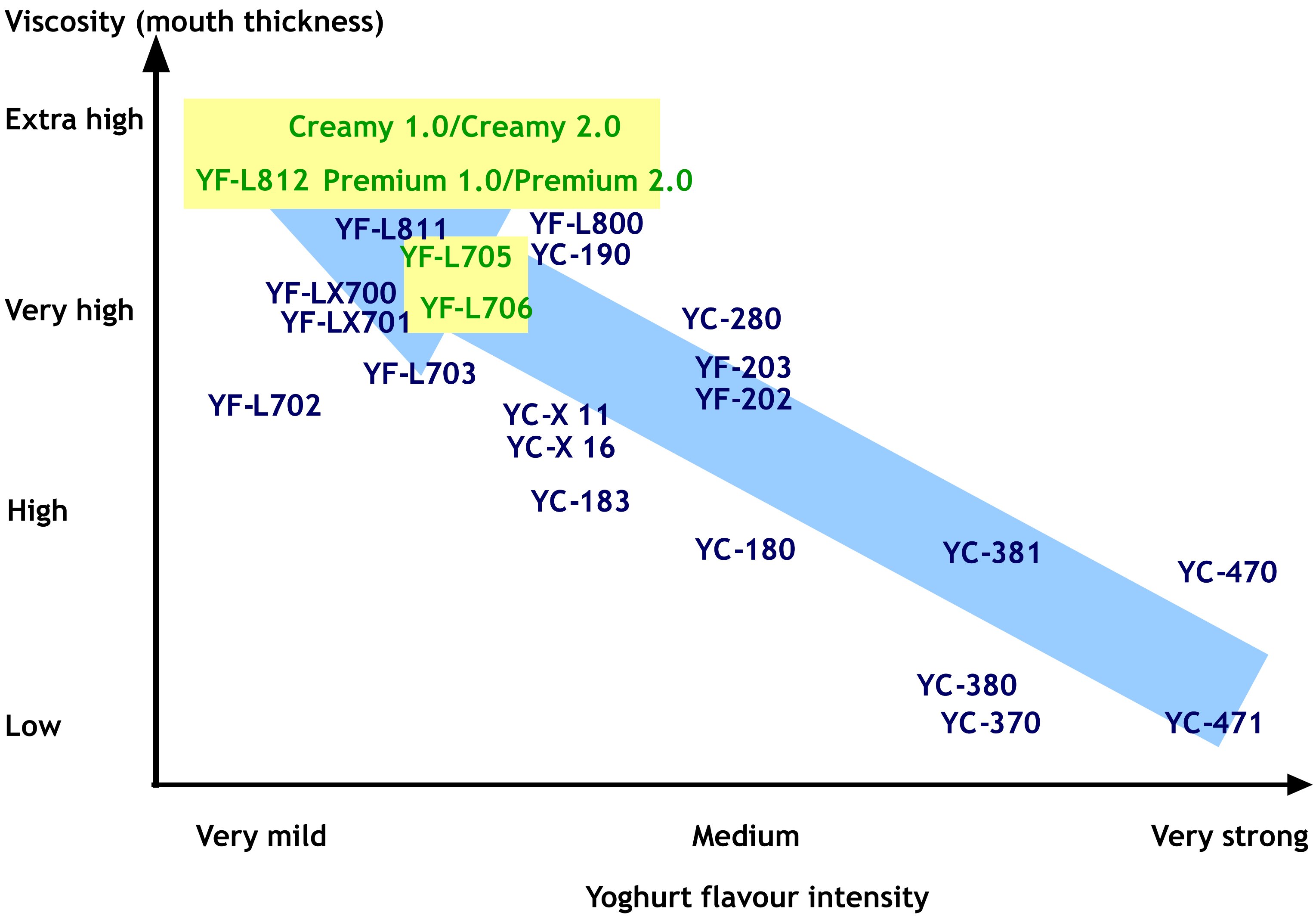
  - Production of EPS in *L. bulgaricus* NCFB2772 increased from 25 mg/L to 80 mg/L when the carbohydrate source switched from fructose to a mixture of glucose and fructose (Grobben et al.,1996).

  - Protein addition will also affect texture, e.g. gel stiffness (short texture) was enhanced by addition of Whey Protein Conc. (WPC) compared to use of Skimmed milk powder (SMP)

Effect of different Milk Protein blends on fermentation time



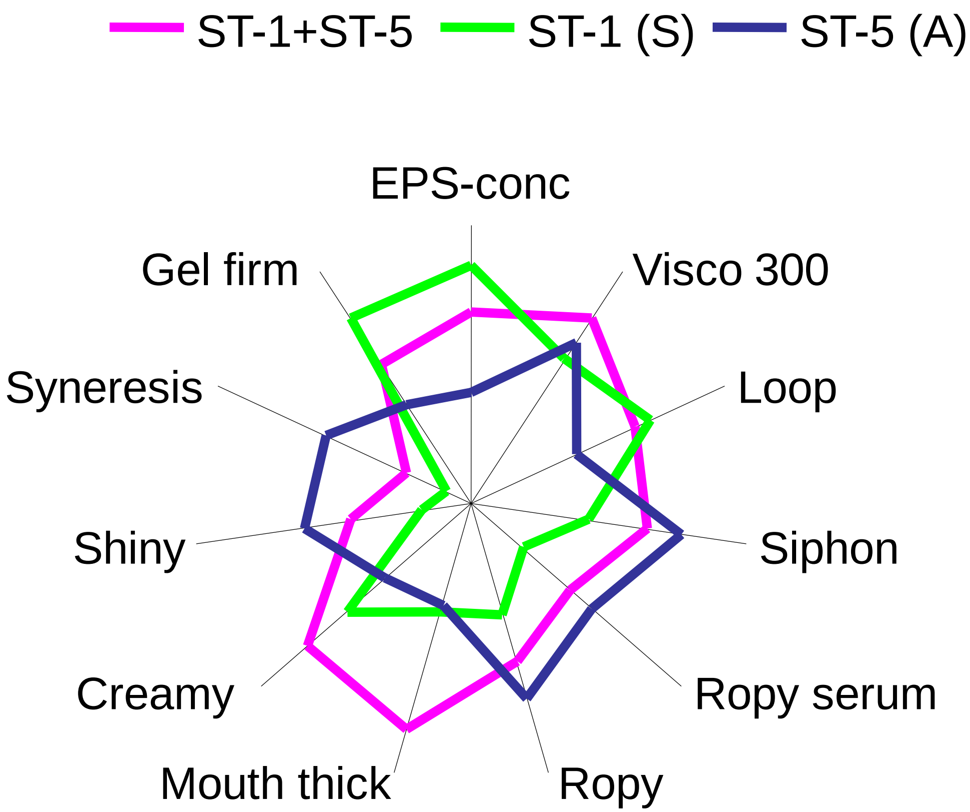
Texture & Flavor Performance - Cultures for Stirred Yogurt



Overall when formulating a culture…….

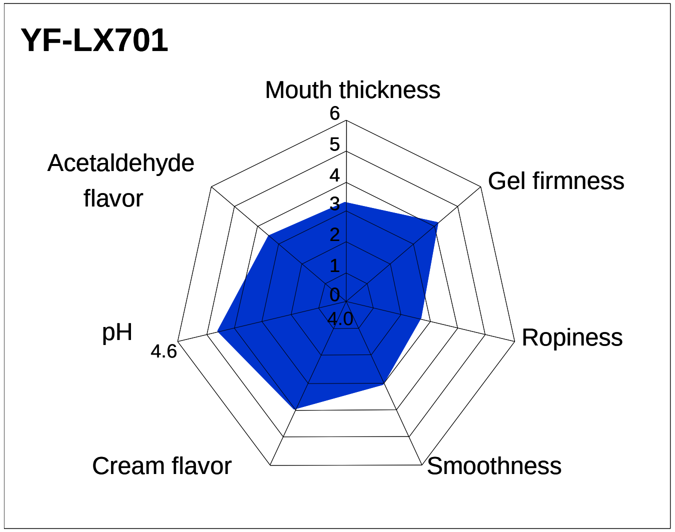
- Main factors that will affect culture performance:
    - Strain interaction, e.g. a combination of 2 different strains will work differently to each strain used in isolation
    - Effect of polysaccharide production/acidification of different strains in a given base formulation (protein selection/sugar selection) – does the selected combination work well in commercial applications?
    - Process robustness (ability to work well across a range of temperatures and process parameters, including high shear)
    - Formulating a range of yogurt cultures that deliver a broad choice in terms of flavour and texture options

Interactions of ST in mixed cultures

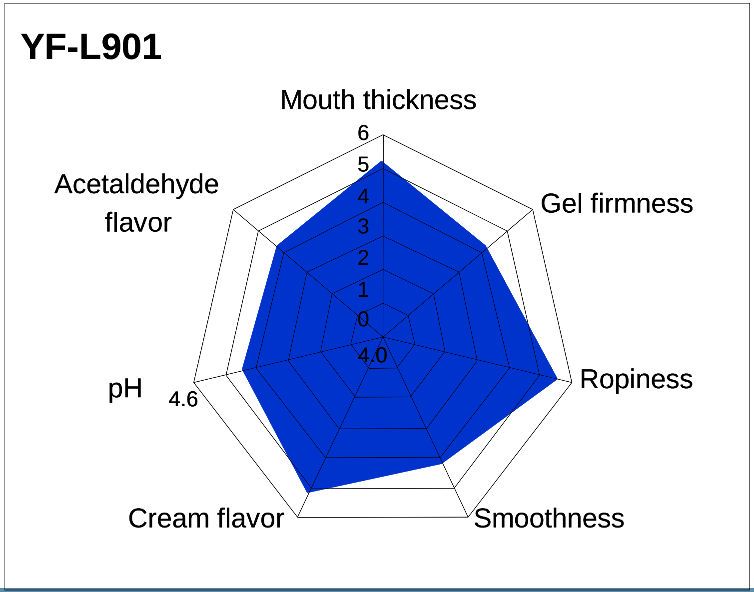


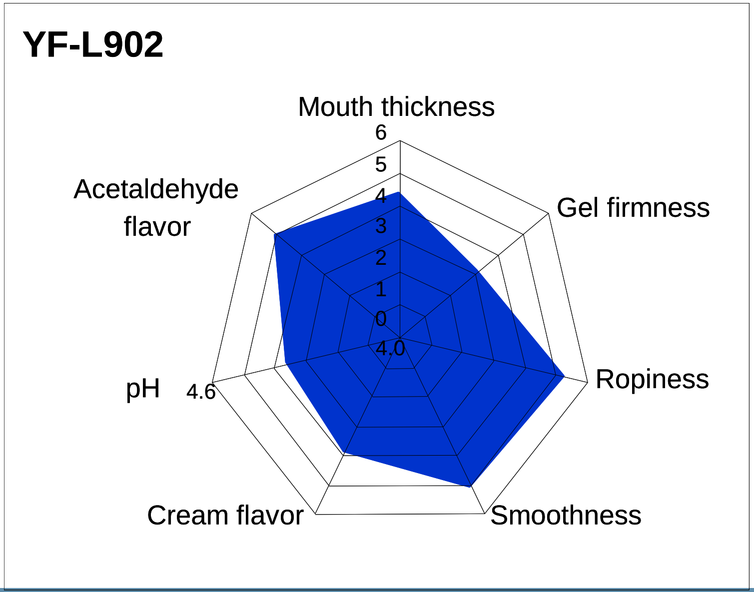
Improved texture when mixing two types of texturing ST-strains

Sensory profiles of low-fat stirred yogurt



Milk base:
1% milk fat, 2% NFDM
No sugar added





Culture development

Compounding – continual feed back loop in terms of culture development (an ongoing/continuous process)