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
Chr. Hansen in brief

- 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
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

- 2007 - present: Very high viscosity (mouth thickness) and very mild yogurt flavor intensity.
- 2002: Very high viscosity and very mild flavor.
- Late 1990's: High viscosity and mild flavor.
- Mid 1990's: High viscosity and mild flavor.
- 1980's: Medium viscosity and mild flavor.
- 1960's: Low viscosity and strong flavor.
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 $\xrightarrow{}$ 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 affect flavour perception.

- EPS binds moisture and will reduce the perception of flavour compounds on the tongue and olfactory system, thus producing a ‘milder’ yogurt.
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 characteristics, 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

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

Set

- Protein network (red)
- EPS (green)

Stirred

- Moderate EPS
- High EPS (ropy)

Source: Hassan et al. (2002)
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 \textit{S. thermophilus} LY03 with the former \citep{Degeest2000}.

  - Production of EPS in \textit{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 \citep{Grobben1996}.

  - 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

Time to pH 4.55

- Culture 1
- Culture 2
- Culture 3

NFDM
MP 1
MP 2
MP 3
MP 4
MP 5
Texture & Flavor Performance - Cultures for Stirred Yogurt

Yoghurt flavour intensity

Yoghurt viscosity (mouth thickness)

Extra high

Very high

High

Low

Very mild

Medium

Very strong

Creamy 1.0/Creamy 2.0

YF-L812 Premium 1.0/Premium 2.0

YC-LX700 YF-LX701 YF-L706

YF-L702 YF-L703 YC-X 11 YC-X 16

YC-183

YC-180 YC-381 YC-470

YC-380 YC-370 YC-471

YC-L812 YF-L800 YC-190

YC-280 YF-203 YF-202

YC-470

YC-471
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 feedback loop in terms of culture development (an ongoing/continuous process)

1. Strain selection
   - Feedback: corrective measures

2. Culture blends
   - Strain 1
   - Strain 2
   - Strain 3

3. Culture characterisation
   - Acidification profile
   - Texture
   - Flavor