17th Biennial Cheese Industry Conference

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Proceedings

Utah State University
17th Biennial Cheese Industry Conference
August 9, 2006
Sun Valley, Idaho

Sponsored by:

Western Dairy Center
Utah State University
Cheese Reporter
Cargill
Danisco
Glanbia Nutritionals
Schering Systems
CHR Hansen
Seventeenth Biennial Cheese Conference - 2006

August 9, 2006
Sun Valley, Idaho

Wednesday, August 9, 2006
Dollar Mountain Lodge

7:30  Registration and Continental Breakfast
8:00  Welcome - Marie Walsh, Utah State University
8:05  Current State of the Dairy Culture Industry – Jeff Kondo, DMI
8:30  Meeting Customer Requirements – Marc Bates, Bates Consulting
9:15  Probiotic Cultures for Cheese – Mary Ellen Sanders, Dairy and Food Culture Technologies
10:00 Break
10:30 Surface Ripening Cultures as Adjuncts – Steve Funk, Cargill
11:15 Dynamics of Microbial Systems – Craig Oberg, Weber State University
Noon Lunch
1:00  Use of Permeabilised and Lytic Strains for Enhanced Flavor Effects in Semi-Hard Cheese – Jonathan Goodwins, Danisco
1:45  Helveticus for Debittering and as Flavor Adjuncts – Jeff Broadbent, Utah State University
2:30 Break
3:00  Cultures and Procedures for Low Fat Cheese – Don McMahon, Utah State University
3:45  Use of Genomics as a Tool for Culture Development and Selection – Bart Weimer, Utah State University
4:30  Adjourn
Current state of the dairy culture industry
Jeff Kondo, DMI

Meeting customer requirements
Marc Bates, Bates Consulting

Probiotic cultures for cheese
Mary Ellen Sanders, Dairy and Food Culture Technologies

Surface ripening cultures as adjuncts
Steve Funk, Cargill

Dynamics of microbial systems
Craig Oberg, Weber State University

Use of permeabilised and lytic strains for enhanced flavor effects in semi-hard cheese
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Helveticus for debittering and as flavor adjuncts
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Cultures and procedures for low fat cheese
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Use of genomics as a tool for culture development and selection
Bart Weimer, Utah State University
Utah State University
17th Biennial Cheese Industry Conference

Current State of the Dairy Culture Industry

Jeff Kondo
DMI
Innovation in the Dairy Culture Industry

17th Biennial Cheese Industry Conference
August 9, 2006

Jeffrey K. Kondo
Director for Product Innovation
Dairy Management, Inc.

Innovation

- Innovation in science and engineering are revolutionizing our 21st century
  - Information intensive, increasingly complex, and rapidly changing
- Rapidly changing technology requires a multidisciplinary approach
  - Information technology
  - Genomics - genome sequence and microarrays
  - Mathematics and physics
  - Engineering and nanotechnology
- New technology creates new value propositions

Genetic Technology: A Disruptive Innovation

- Disruptive innovation
  - Creation of new and conflicting value propositions where social, scientific, and commercial patterns are affected
  - Technology advances faster than our regulatory systems
  - Need for science-based discussions and policies
  - Global markets are affected
- Dairy cultures: an unrealized potential
- Nutrigenomics: the future?
A Brief History of Innovation

- Culture systems
  - Defined strain starter cultures
  - Direct to the vat set
  - External and internal pH control systems
- Culture strains
  - Improved phage resistance (BAR and plasmid technology)
  - Probiotics
  - Extended shelf-life cultures
- Culture technologies
  - Gene transfer systems, cloning, genomics, microarrays
  - DOE Joint Genome Institute (2002) LAB Genome Consortium
- Other products
  - Enzymes for yield, texture, whitening, and flavor
  - Prebiotics
  - Fermentates for shelf-life
  - Functional Foods

New Culture Systems

- Back to "direct to the vat" inoculum
  - Now comparable to pH controlled bulk starter systems
  - Use of Streptococcus thermophilus for fast acid production
  - Accumulation of galactose may lead to browning issues
  - Innovative solutions for galactose
- Fermentation and biomass
  - Use of genome sequences and microarrays

Enzymes

- Phospholipases for yield
- Hexose oxidases for whitening due to galactose transformations
- Flavor enzymes: potential but difficult to control
  - Lipases and proteases
Cultures and Health

- Probiotics
  - Milk as the preferred delivery system
  - Structure/function claims vs. health claims: need better clinical data – and it's coming
  - Functional foods: the time may be right
    - Danone’s “Activa” product
  - Bioactive peptides
    - Blood pressure – Lactobacillus

Flavor & Texture

- Flavor
  - New culture adjuncts for flavor development and debittering, e.g. L. helveticus CNRZ32
  - Enzyme modified cheese flavors
  - Enzymes
- Texture
  - Exopolysaccharides

"The Trend"

- Health and Nutrition
  - Functional Foods - Kelloggs
    - Kashi Vive Probiotic Digestive Cereal launched in August
    - Rice Krispies multi-grain with prebiotics
  - Nutrigenomics

Thank you for your attention!

"The End"
Utah State University
17th Biennial Cheese Industry Conference

Meeting Customer Requirements

Marc Bates
Bates Consulting
Meeting Customer Requirements

17th Biennial Cheese Industry Conference
August 9, 2006
Sun Valley, Idaho

Meet today, Share tomorrow
Based on experience of a farmer at WGI Dairy

August 9, 2006
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Demand vs. Needs

- Demand = quantity purchased at a given price.
- Needs/requirements = those things that the customer expects from you and your product irrespective of the price.
- Customer expects a consistent product with consistent service at minimum

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What Is Your Vision?

- From the Book "Raving Fans" by Blanchard and Shelton
  - Principle #1 Have a vision for your product.

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How Do You View Your Product?

- Cheese: Cougar Gold
  - Gift
  - Education
    - Student Employee
    - Customer
  - Relationship
    - Alumni and friends
    - Current Students and Parents

My Vision for Cougar Gold

- A Unique, Specialty Cheese
  - Not mass produced: University produced only
  - Tie-in to educational/research programs
  - Consistent quality—always aged for ≥ 1 year
  - Unique packaging—vacuum sealed tin
  - Direct contact with the Customer
    - Store, 1-800, internet
  - Self-Sustainable

What is Your Customer’s Vision?

- From the Book “Raving Fans” by Blanchard and Shelton
  - Principle #2: Know your customer and their expectations of you and your product.
How do you know what the customer wants?

- Listen to them
  - Complain/complaints
  - Ask them, but remember to listen!
  - Suggestion box
  - Toll free number
  - info@yourcompany.com
  - Observe them where they use your product
  - If you hear the word "Fine" or there is silence, Beware!

High and consistent quality cheese
- On-time delivery
  - Never earlier or later but just right!
- A Thank you from the gift recipient
  - Gift buyers expect you to find out why they didn't get one!
- Relationship with organization through the current employees
  - Made by, Order taken by and Packed by...

My Customer's Expectations
- High and consistent quality cheese
- On-time delivery
  - Never earlier or later but just right!
- A Thank you from the gift recipient
  - Gift buyers expect you to find out why they didn't get one!
- Relationship with organization through the current employees
  - Made by, Order taken by and Packed by...
Examples of Mismatch Between My Vision and Customer Vision
- Smoky Cheddar Flavor level
- Grabber cookies
- Pumpkin Ice Cream
- Mini Can

Continuity and Improvement
- From the Book "Raving Fans" by Blanchard and Shelton
  - Principle #3 Continuous Improvement
    - The Rule of One percent
  - What is the opposite of growing?
  - Is growth improvement?

Continuity and Improvement
- From my experience
  - Evaluate past performance to identify opportunities for improvement
    - What was the biggest problem the source?
    - What concerns or concerns did we hear most frequently?
    - OPS example
  - On growth. In and out of Book, growth alone won't sustain the organization. The organization has to improve and if it does growth will happen
WD-40 Example

- WD-40
  - Water Displacement on the 40th try.
  - Developed in 1953 for rust prevention
  - Considered a "Johnny One-Note" enterprise as of management change in 1997.
  - Selling a million cans per week in 1997.

WD-40 Example

- Gary Ridge became CEO in 1997
  - His Vision
    - "Don't mess around with my WD-40"
    - Decided for the "sneaky, small and dirt business"

WD-40 Example

- Customer's Vision
  - Tried spray groups with customers as a listening tool
  - Forced customers to use 200X concentration for WD-40
  - Contemplated providing one product in different formats
    - Big Stack for wider spectrums
    - No-Mess WD-40 Pen for precision apparatus
  - Comments told them that "you put your name on it and we will try it. But don't you let us down!"
WD-40 Example

- Continuous Improvement
  - The most common complaint about WD-40 is...
  - Enter the WD-40 Smart Straw
  - Can with a permanently attached straw.

WD-40 Example

- CEO hopes that the new Smart Straw replaces all traditional cans because the customer is paying a 30% premium for the convenience provided by the attached straw.

Branded: American Attitudes toward "The Brand."

- F.A.S.T Food
  - F is for Finance, Fairness and Taste
  - A is for Affordability
  - S is for Safety and Security
  - T is for Truth in Advertising

"In store displays," "word of mouth," and "peer recommendation" were better indicators of the quality or value than traditional references, price or media ads.

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Acknowledgements

- Thanks to:
  - Program Committee for the opportunity
  - WSU Creamery for real life experiences
Utah State University
17th Biennial Cheese Industry Conference

Probiotic Cultures for Cheese

Mary Ellen Sanders
Dairy and Food Culture Technologies
Probiotic Cultures for Cheese

Mary Ellen Sanders, Ph.D.
www.mesanders.com
www.usprobiotics.org
17th Biennial Cheese Industry Conference
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Probiotics

Courtesy of G. Gibson

Live microorganisms which when administered in adequate amounts confer a beneficial health benefit on the host


At least 9 published definitions – all differ

"Probiotics remains a major growth market. The European sector is set to more than triple in value over the next few years, according to Frost & Sullivan, to reach $137.9 million (€118.5m) in 2010."

www.beveragedaily.com
Probiotic yogurt: Activia by Dannon

"Activia® is a great-tasting lowfat yogurt from Dannon® that contains Bifidus Regularis™, a natural probiotic culture that can help regulate your digestive system by helping reduce long intestinal transit time."

www.activia.com/about.asp

PROBIOTICS
Are change in your diet?

Concludes that yogurts are better sources of probiotics than supplements

Methods don't distinguish between starter LAB and supplemental probiotic bacteria

Consumer Reports July 2005

Microbes in fermented dairy products serve different purposes
LAB ≠ probiotic cheese ≠ probiotic food

Transform milk into good tasting products Impart health benefits
Are dairy products good delivery vehicles for probiotics?

- Dairy products:
  - Yogurt - most common carrier for probiotics in US
  - Cheeses - few, but growing
  - Others - kefir
- Good fit in mind of consumer - "live, active cultures" and healthy image fit with dairy products
- Many probiotic products not formulated optimally
  - Unknown levels of probiotics in dairy products
  - Little communication to consumer about probiotics
  - Strains not optimally chosen

Probiotics and Dairy

Stability | Functionality
---|---
In Product Many dairy foods are short shelf life, refrigerated products, which encourages probiotic survival in product
Gastrointestinal Transit Buffer stomach acid
Physiological Activity Dairy products deliver functional nutrition along with probiotic bacteria
Compliance Food vs. pills
The role of delivery vehicle in the functionality of probiotics has not been established

Functional genomics research
Controlled, comparative human studies

Needed to establish this link

Considerations for probiotic preparation and delivery

- Bacterial Growth
  - Growth phase
  - Media composition
  - Temp
  - Sublethal treatments
- Concentration
  - Freezing, Drying
  - Temp, Moisture
- Matrix/Food
  - Direct inoculation or fermentation (added bioactives)
  - Contribution of other bioactive ingredients on functionality
  - Impact of other LAB
  - Impact on stability
- Food processing parameters (shear, pressure, temp)

In vivo function
- How do preceding factors influence delivery to active site in vivo?

As these parameters change, when do efficacy evaluations need to be re-confirmed?

Quality Control
- CFU/g or other viability assessment
- Injured cells?
- Differentiate from other LAB present

Cheeses containing probiotics

- Not many commercial products yet
- Ingredient supply companies and researchers are developing approaches to this area
"The cheese with a plus"

L. rhamnosus GG-containing Emmental-style cheese

www.valio.com

- Offered by Finland's Valio through its Belgian subsidiary
- Gelfilus is a low fat cheese (15% fat on total mass) of the Emmental type, enriched with Lactobacillus GG (LGG).
- "With Gelfilus cheese you do not need as high a dose of LGG per serving as, for example, in capsules.
- "Cheese... is an excellent carrier of all probiotic bacteria, particularly LGG."

Cheese Slice from Germany

- Contains L. acidophilus
- Omega 3
- Low salt content
- Probiotic cultures: "contribute to the balance of the intestinal flora"
- Replacement of dairy fat with vegetable oil - "a natural substance to lower cholesterol"

DSM Food Specialties

Offers probiotics for cheese
Cheddar, gouda, stracchino

- LAFTI probiotic strains
  - Lactobacillus acidophilus LAFTI® L10
  - Bifidobacterium lactis LAFTI® B94
  - Lactobacillus paracasei LAFTI® L26
- Cheese as a delivery system for these probiotics
  - Higher pH than yogurts
  - Better survival rates
- Excellent survivability until the end of the commercial life of the product
Research findings

- Cheddar cheese (Daigle et al. 1999, STELA, Quebec)
  - B. infantis
  - Good survival 4°C for 84 d, >3 x 10^6 cfu/g
  - No significant difference was observed between cheeses

- Camembert cheese (Coeuret et al. 2004, INRA, France)
  - L. plantarum UCN4 3037
  - >10^7 cfu/g after 75 days aging
  - No adverse effects on quality
  - Studies in human microbiota-associated rat model documented that strains eaten in cheese survived intestinal transit

- White-brined cheese (Yilmaztekin et al. 2004, Inonu University, Malatya, Turkey)
  - B. bifidum BB-02, L. acidophilus IA-5
  - 2.5 or 5% inoculation rate
  - 1% cheese 1% water soluble nitrogen, 1% non-protein nitrogen, 1 protease-polymer nitrogen and 1 lysozyme values
  - Probiotic numbers declined 2-3 logs over 90 days storage

- Pugliese cheeses (Corbo et al. 2001, Universita degli Studi di Foggia, Italy)
  - B. bifidum BB-02 and/or B. longum BB-02
  - 1 growth and survival of starter mesophilic lactic acid and S. thermophilus during ripening
  - 16^7 cfu/g at start, 1-2 log drop after 56 d ripening
  - 1 amino acid, 1 lactate, 1 pH 4.6-soluble N/protein N ratio with more pronounced amino-, imino-, and dipeptidase activities
  - No difference in sensory evaluation profiles

- Argentinian Fresco cheese (Vinderola et al. 2000, Universidad Nacional del Litoral, Santa Fe, Argentina)
  - Different combinations of B. bifidum, B. longum, L. acidophilus, L. casei tested
  - Good survival (< 1 log drop)

Survival of probiotic strains in cheddar cheese after 32 wks

Cheddar cheese with *L. paracasei*

www.teagasc.ie/research/reports/dairyproduction/4266/eopr-4266.htm

UCC, Ireland

- *L. paracasei/NFB 338* pilot and commercial plant production
- Used RAPD PCR to differentiate probiotic from non-starter lactobacilli
- *L. paracasei* maintained high levels > 10⁸ cfu/g
- Spray dried skim milk powders containing probiotics produced

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Health effects of probiotics delivered through dairy products

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Medline Search

<table>
<thead>
<tr>
<th>Publication years</th>
<th>Total number of citations for search term &quot;probiotic&quot;</th>
<th>Limited to clinical trials</th>
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<td>Prior to 1990</td>
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<td>1990 - 1992</td>
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<td>0</td>
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<td>2002 - 2004</td>
<td>1193</td>
<td>115</td>
</tr>
<tr>
<td>2005 - June 2006</td>
<td>872</td>
<td>96</td>
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</table>
Published targets for probiotics

- Colds
- Absences from work, daycare
- Diarrhea (rotavirus, travelers', AAD, C. difficile)
- Inflammatory bowel disease
- Irritable bowel syndrome
- H. pylori/stomach infection
- Vaginal infections
- Growth for under nourished young children
- Delivery of cloned components active in gut
  - Anti-inflammatory delivery
  - Vaccine delivery
  - Yeast delivery
  - Tannin receptors
- Alteration of intestinal microbiota
- Colon tumors (primary evidence in animals)
- Harmful intestinal microbe activities
- Lactase digestion
- Allergy development and symptoms
- Immune enhancement
- Dental caries
- Blood pressure
- Blood lipid profiles

Disease vs. health endpoints
Drug vs. food

Effect of probiotic bacteria 5x10⁷/d
B. longum, B. bifidum, L. gasseri on episodes of colds in healthy adults

<table>
<thead>
<tr>
<th>Duration (days)</th>
<th>Probiotic + Vitamin/mineral supplement</th>
<th>Vitamin/mineral supplement</th>
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<tbody>
<tr>
<td>7.0±0.5</td>
<td>8.9±1.0 (p=0.045)</td>
<td></td>
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<tr>
<td>13.2±2.1</td>
<td>19.1±2.7 (p=0.011)</td>
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<tr>
<td>0.24±0.1</td>
<td>1.0±0.3 (p=0.017)</td>
<td></td>
</tr>
<tr>
<td>79.3±17.4</td>
<td>102.5±12.2 (p=0.056)</td>
<td></td>
</tr>
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</table>

Study duration ~3 months
N=479

Effect of probiotic formula on infections in child care centers
Weizman et al. Pediatrics 115:5-9

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>BB-12</th>
<th>L. reuter</th>
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</thead>
<tbody>
<tr>
<td>Fever (d)</td>
<td>0.83</td>
<td>0.86*</td>
<td>0.17*</td>
</tr>
<tr>
<td>Fever (episodes)</td>
<td>0.41</td>
<td>0.27*</td>
<td>0.11*</td>
</tr>
<tr>
<td>Diarrhea (d)</td>
<td>0.59</td>
<td>0.37*</td>
<td>0.15*</td>
</tr>
<tr>
<td>Diarrhea (episodes)</td>
<td>0.31</td>
<td>0.13*</td>
<td>0.02*</td>
</tr>
<tr>
<td>Clinic visits</td>
<td>0.55</td>
<td>0.51</td>
<td>0.23*</td>
</tr>
<tr>
<td>Absences from CCC</td>
<td>0.43</td>
<td>0.41</td>
<td>0.14*</td>
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<tr>
<td>Antibiotic use</td>
<td>0.19</td>
<td>0.21</td>
<td>0.06*</td>
</tr>
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</table>

N=58 control
N=71 BB-12
N=65 L. reuter

No differences in days with or episodes of respiratory illness
Probiotics in undernourished children in India

- Hypothesis: failure to thrive in many stunted children is due to damage to the gut epithelium
- 50 ml fermented curd with and without L. acidophilus 5x10^9 
  cfu/day for 6 months
- 100 children, 2-5 yr
- ↑ weight (1.3 vs. 0.81 kg)
- ↑ height (3.2 vs. 1.7 cm)
- ↓ cases of diarrhea (21 vs. 35 cases)
- ↓ fever (30 vs. 44 cases)
- ↔ duration of diarrhea or cough/cold incidence


The impact of fermented food (6 mo) on weight and height in malnourished infants

Dose: 5x10^9/d L. acidophilus

- ↑ incidence and duration of fever
- ↓ incidence of diarrhea


Workplace absences

N=181 healthy adults
10% L. reuteri ATCC 55730 powder
RDIPC
Study duration: 80 d

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>L. reuteri</th>
</tr>
</thead>
<tbody>
<tr>
<td>% subjects</td>
<td>26%</td>
<td>11%</td>
</tr>
<tr>
<td>reporting sick</td>
<td></td>
<td></td>
</tr>
<tr>
<td>days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median # sick</td>
<td>3 days</td>
<td>3 days</td>
</tr>
<tr>
<td>days per absence</td>
<td></td>
<td></td>
</tr>
<tr>
<td># sick days/total</td>
<td>0.9%</td>
<td>0.4%</td>
</tr>
<tr>
<td>study days (%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Antibiotic Associated Diarrhea
Meta-analysis

Forest diagram
- 7 placebo-controlled studies
  - L. rhamnosus GG (3 studies)
  - Saccharomyces boulardii (3 studies)
  - Lactobacillus spp. (1 study)
- 881 patients

Overall, probiotic supplementation was a protective factor with respect to the incidence of diarrhea measured as a binomial (yes/no) variable.

Meta-Analysis of Lactobacillus as therapy for acute infectious diarrhea in children

"Lactobacillus is safe and effective as a treatment for children with acute infectious diarrhea"


Key Issues in the Development of Probiotic Dairy Products
Strain is important: Same species, different function

Pulsed-field gel electrophoresis of chromosomal DNA from probiotic *L. crispatus* strains

Useful for:
- strain tracking
- strain comparisons
- patent enforcement
- quality control

R: Reference
1: DPTC 013
2: DPTC 014
3: DPTC 015
4: DPTC 016
5: DPTC 009
6: DPTC 017
7: ATCC33199

Yeung, et al., unpublished

Effects Are Strain-Specific

- Not all strains of the same species can be expected to have the same effects
- Clinical data must be viewed as applicable only to the strain or strain combinations tested
  - Other strains of the same species MAY have the same effect.
  - It cannot be assumed, for example, that because one *L. rhamnosus* has a certain effect, that all do.
- No one strain should be expected to have all effects
  - Strain blend approach
  - Targeted strain approach
- Future research will determine what phenotypic traits are important for certain effects
What Levels of Probiotics Need to be Delivered in Products

- Many studies track acute endpoints
  - What benefits are achieved, if any, at lower, long term doses is not known
- Required levels for therapeutic endpoints may be higher than prophylactic endpoints
  - A few studies document effects at 10^9/d
- Synergism has not been studied
  - What levels of probiotics are necessary when combined with other foods functional in the GI tract?
  - What about multiple dietary sources of probiotic bacteria?
  - What about strain interactions as part of blends?

Levels used should be based on levels found to be efficacious in human studies.

What does the "live active culture" seal tell us about probiotics?

- Nothing.

Trademark of the National Yogurt Association
- Minimum level of cultures – 10^7/g at end of shelf life
- Doesn't specifically apply to probiotic content
- Starter cultures usually ~10^8-9/g
- Probiotic bacteria target ~10^6/g, and levels at end of shelf life are not published

Labels

- Truthful and not misleading
- Tell the consumer what your product has in it:
  - Genus, species, strain
  - Levels until the end of shelf life
- Tell your consumer what it can do for them:
  - What do efficacy studies show?
Probiotic products
US and beyond

Some of these US products communicate about "benefits" on their websites, but few have any published studies on the specific product and most do not disclose the specific strains used or the levels of viable strains through the end of shelf life.

DanActive® Cultured Dairy Drink
"Helps Naturally Strengthen Your Body’s Defense System"

- 10 billion per serving of L. casei + yogurt cultures
- Now available in ‘light,’ lower carb format and multiple flavors
- Available in:
  - Whole Foods Markets nationwide
  - Select HEB stores in Texas
  - Select Giant Eagle stores in Pennsylvania, Ohio and Maryland
  - Schnucks stores in the Midwest
  - Select stores in the Denver area

Nancy’s Yogurt
Eugene OR

Nancy’s yogurt is “fully cultured with casei, rhamnosus, acidophilus, bifidum, thermophilus, and bulgaricus”
Kefir by Lifeway

- Microbes:
  - Lactobacillus lactis
  - Lactobacillus cremoris
  - Lactobacillus diacetylactis
  - Leuconostoc cremoris
  - Lactobacillus plantarum
  - Lactobacillus casei
  - Saccharomyces cerevisiae
  - "Basics Plus" dietary supplement line also contains concentrated extracts of colostrum targeted toward enhancing performance of the immune system.

  "It is a good remedy for digestive problems, and is particularly good in reestablishing necessary intestinal microflora, which may have been destroyed by antibiotic or other medical treatment." www.lifeway.net

Canada

Dairyland Plus Value Added Milks

Calcium Extra
Lactose Free
Microfiltered Milk
Carb Aware milk (low-carb)
Omega-3 milk
AB Milk (Acidophilus, Bifidus)

"AB culture is naturally found in the digestive system. AB culture is lost when antibiotics are taken. Thus, taking Dairyland AB milk helps put the 'good' bacteria back in the digestive system."

www.dairyland-ca.com

Probiotic juice product – Canada

"the first and only North American fruit juice containing live and active probiotics"

- www.lassonde.com/va_lassonde/en/adult/1_0/1_1.asp?groupe=5
Vitamin plus probiotic

- Lactobacillus gasseri PA 16/8
- Bifidobacterium bifidum MF 20/5
- Bifidobacterium longum SP 07/3
- Launched July 2006 in US

YogActive Cereal
Contains 15% yogurt pearls with active Lactobacillus acidophilus bacteria.

"Many years of research were required to build a state-of-the-art manufacturing system that allows the yogurt bacteria to remain active in a dry state."
http://www.yogactive.com/indexE.html

BioGaia – L. reuteri and more

- Innovative delivery vehicles - LifeTop™
- LifeTop™ is designed to protect sensitive ingredients during distribution and storage and to release them in a simple and efficient way at the moment of consumption
- Probiotics, but also vitamins, minerals, colors or flavor
Align™
Procter & Gamble

• 10⁹/capsule B. infantis 35264
• Studied on IBS patients
• "Align is a natural supplement that helps promote a healthy, balanced digestive system when taken daily." www.aligngi.com

Infant Formula
Nestle

• S. thermophillus TH4 and Bifidobacterium lactis Bb12
• ~1.5 x 10⁷/ml reconstituted formula (~10⁸/gm powder)
• Europe and Asia (not US)
• Other brands of formula with probiotics in Europe

Probiotic yeast: Florastor
www.florastor.com

• Each capsule of Florastor contains: Saccharomyces boulardii 250mg equivalent to 5 billion live cells.
• From website:
• "The best way to put an end to your intestinal problems"
• Florastor is often used to help with:
  - Antibiotic-Associated Diarrhea (AAD)
  - Acute/chronic diarrhea
  - Traveler's diarrhea
  - Clostridium difficile associated diarrhea
  - Bloating and gas associated with bowel problems
Probiotic blend:
VSL#3

From www.vsl3.com

- 450 billion live bacteria (8 species) per packet
- Medical food probiotic recommended for the dietary management of ulcerative colitis (UC) or an ileal pouch
- Clinically proven to be effective in the dietary management of intestinal disorders.
- Decreases diarrhea, frequency, and urgency

Animal applications

- The use of probiotics and prebiotics in animal agriculture and companion animal industry is huge
- Pathogen shedding, colonization
- Growth promotion, especially now that antibiotics in animal feed are declining
- Digestive health
- Malodors (fecal, breath)
Utah State University
17th Biennial Cheese Industry Conference

Surface Ripening Cultures as Adjuncts

Steve Funk
Cargill
Traditionally yeasts and mold in cheese are seen as undesirable contaminants due to poor sanitation. However, in many parts of the world selected yeasts and molds are used to produce desirable flavors, aromas, and other attributes in cheeses.

Yeast and molds require oxygen to reproduce and potentially exhibit their detrimental traits. By putting them in oxygen limited environments such as Cheddar, Parmesan, Romano, and other cheeses that are ripened in a more enclosed environment, the yeasts and molds can release favorable cheese ripening enzymes and by-products.
• They release these enzymes and by-products through lysis (the actual breaking apart of the cell) and the enzymes help to breakdown protein and fat in the curd matrix to enhance flavor development. This development can either accelerate or accentuate desired cheese flavors and also help to prevent the development of off-flavors such as bitterness for example.

• The level of the organisms used is too low to contribute any detectable yeast or mold in the finished cheese attributed to those selectively added to the cheese vat. This way the cheesemaker can get the desired positive contributions from these organisms without risking contamination.

Organoleptic quality genesis

- Technological factors
- Micro-organisms
- Ripened cheese
- Raw material
- Taste
- Texture
- Sight
- Flavor
- Organolectic qualities
The flavor of ripened cheeses

- A large number of volatile compounds
- Origin: lactose, lipids and proteins breakdown
- Actors: ripening microorganisms

Cheese is a biochemically-active dynamic product that undergoes many changes during its ripening period. Cheese flavor development is one of the consequences of these chemical changes occurring over this ripening period. Flavor compounds are produced through the principal biochemical degradation pathways: glycolysis, lipolysis and proteolysis. Depending on variety, technology, microflora and ripening conditions, flavor compounds are produced to give unique sensory characteristics to each cheese variety.

Proteins breakdown

<table>
<thead>
<tr>
<th>Dairy proteins</th>
<th>Peptides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymes acting on Proteins</td>
<td>Enzymes acting on Peptides</td>
</tr>
<tr>
<td>Same Microflora</td>
<td>Same Microflora</td>
</tr>
<tr>
<td>Peptides</td>
<td></td>
</tr>
<tr>
<td>Amino acids</td>
<td></td>
</tr>
</tbody>
</table>
Amino acids breakdown

Alcohols from proteolysis
Primary and secondary alcohols, along with ketones, are considered to be the most important compounds in the aroma of soft and mold-ripened cheeses.

Sulfur compounds
In ripened cheese, it has been shown that the sulfur flavors are comprised of a structurally diverse class of molecules which provide a wide range of characteristic aromatic notes (e.g. "cheesy," and "garlic") in a particular cheese as evident from the analysis of Cheddar, Limburger, Camembert, Blue and other mold-ripened varieties.
Lipids breakdown

Triglycerides

<table>
<thead>
<tr>
<th>Fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-esters</td>
</tr>
<tr>
<td>Saturated Fatty acids</td>
</tr>
<tr>
<td>Free Fatty acids</td>
</tr>
<tr>
<td>Secondary Alcohols</td>
</tr>
<tr>
<td>Methylketones</td>
</tr>
<tr>
<td>Lactones</td>
</tr>
<tr>
<td>4- or 5-Hydroxyacids</td>
</tr>
<tr>
<td>α or β Hydroxyacids</td>
</tr>
<tr>
<td>Secondary Alcohols</td>
</tr>
<tr>
<td>Lactones</td>
</tr>
<tr>
<td>Ketones</td>
</tr>
</tbody>
</table>

Fatty acids

Fatty acids are important for the aroma of cheese. Not only are they aromatic compounds by themselves, but they also are methylketones, alcohols, lactones and esters precursors.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Goat cheese</th>
<th>Human</th>
<th>Mammalian</th>
<th>Musky</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Ethyl-4-methyl-2-pentanol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Ethyl-4-methyl-2-pentanol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Methyl-2-pentanol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Methyl-2-pentanol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4,4-Dimethyl-2-pentanol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4,4-Dimethyl-2-pentanol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Flavor of goat cheese

- 4-Ethyl-2-octanoic acid
  Quantity in cheese: 5.8 ppm
  Detection threshold: 1.0 ppm
- 4-Methyl-2-octanoic acid
  Quantity in cheese: 0.7 ppm
  Detection threshold: 0.05 ppm
Fatty acids oxidative breakdown

The homologous series of odd-chain alkan-2-ones, from C3 to C15, constitutes one of the most important aroma fractions of surface, mold-ripened cheeses, where the major component is nonan-2-one, and Blue cheeses where the major one is heptan-2-one.

Secondary alcohols, along with ketones, are considered to be the most important compounds in the aroma of soft and mold-ripened cheeses.

<table>
<thead>
<tr>
<th>Saturated Fatty acids</th>
<th>5-Enolactone</th>
<th>5-Acetolactone</th>
<th>Chemolitho</th>
<th>Methylketones</th>
<th>Secondary alcohols</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakdown of C18 fatty acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Linoleic and linolenic acids are precursors of eight carbon aroma compounds, particularly oct-1-en-3-ol, oct-2-en-1-ol, octa-1,5-dien-3-ol, and octa-1,5-dien-1-ol. The principal enzymes supposed to be implied in this alcohol synthesis are a lipoygenase and a hydroperoxide lyase found in molds.

<table>
<thead>
<tr>
<th>Linoleic acid (C18:2)</th>
<th>Linolenic acid (C18:3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipoygenase</td>
<td>Hydroperoxide lyase</td>
</tr>
<tr>
<td>Hydroperoxide</td>
<td>Octa-1-en-3-ol</td>
</tr>
<tr>
<td>Other C8 compounds</td>
<td></td>
</tr>
</tbody>
</table>
**C8 volatile compounds flavor**

Oct-1-en-3-ol is well known for its raw mushroom odor. Its low perception threshold, 0.1 ppm, gives a characteristic touch to mold-ripened cheese aroma. This compound, together with its ketone equivalent the threshold of which is very low are without a doubt key compounds in the global aromatic note of Camembert cheese. Because they are produced by *Penicillium camemberti* metabolism, they only appear late in the cheese ripening process.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molar</th>
<th>Threshold (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct-1-en-3-ol</td>
<td>Methyl</td>
<td>0.01 ppm</td>
</tr>
<tr>
<td>Oct-1-en-3-one</td>
<td>Methyl</td>
<td>0.0005 ppm</td>
</tr>
<tr>
<td>Oct-1-en-1-ol</td>
<td>Ethyl</td>
<td>0.01 ppm</td>
</tr>
<tr>
<td>Oct-1,3-dien-4-ol</td>
<td>Ethyl</td>
<td>0.01 ppm</td>
</tr>
<tr>
<td>Oct-1,3-dien-6-ol</td>
<td>Ethyl</td>
<td>0.001 ppm</td>
</tr>
</tbody>
</table>

**Hydroxyacids breakdown**

- Lactones production
  - Lactone precursors are hydroxylated fatty acids. The closing of the ring occurs by the action of pH and/or microorganisms. The action of microorganisms on the lactone production in cheese has never been clearly elucidated. Hydroxyacids, which are direct lactone precursors, can be present as triacylglycerols in milk.

**Esters formation**

Esterification reactions happen between alcohols derived from lactose (ethanol) fermentation or derived from amino acid catabolism and short- to medium-chain fatty acids. A wide variety of enzymes are implied in esterification reactions as carboxylesterases, having a very wide range of substrates and arylesterases present in most of the micro-organisms contributing to cheese ripening.
Ester flavor notes
Most of the esters encountered in cheese are described as having fruity, floral notes. The most cited aromatic notes to qualify these compounds are pineapple, banana, apricot, pear, floral, rose, honey, and wine. Some of these esters have a very low perception threshold. Thus isvaleric acid is detected in water at a concentration of 2 µg/L.

<table>
<thead>
<tr>
<th>Ester</th>
<th>Flavour</th>
<th>Concentration (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl valerate</td>
<td>Strong</td>
<td>8 µg/l</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>Strong</td>
<td>11 µg/l</td>
</tr>
<tr>
<td>Isobutyl acetate</td>
<td>Neutral</td>
<td></td>
</tr>
<tr>
<td>2-isopropyl hexanol</td>
<td>Neutral</td>
<td></td>
</tr>
<tr>
<td>Butyl acetate</td>
<td>High</td>
<td></td>
</tr>
</tbody>
</table>

Contents

- Flavor compounds and genesis pathway
- Studying techniques
  - Function of main ripening microflora

Methodology

- Sensory analysis
  - Flavor description and quantification by a sensory panel
- Chemical analysis
  - Volatile compounds identification and quantification
Sensory analysis

The first step for studying cheese flavor should be the sensory analysis of the cheeses using descriptive tests, such as quantitative descriptive analysis (QDA). This evaluation allows the establishment of the flavor profiles of the samples by measuring the sensory flavor descriptors which might become pertinent targets for the subsequent instrumental analyses.

Descriptive sensory analysis

Perception analysis
Association of each perception stimulus with a descriptor
Measurement of the perception intensity

No Intense
Mushroom flavor

Chemical analysis

Methodology used for studying volatile flavor compounds includes various steps whose ultimate aim is the identification of the compounds which are really relevant to the flavor. In fact, it is now well recognized that it is not necessary to identify the total volatile content of foods in order to understand the flavor. Not only the extraction and concentration procedures used prior to analysis by gas chromatography-mass spectrometry may lead to the formation of artifacts, but total volatile content in most cases is very difficult to relate to the flavor profile determined by a panel in sensory evaluation. While this determination can provide useful information in some circumstances, it appears much more efficient to concentrate the identification efforts on those compounds that have a significant contribution to the flavor.
Flavor compounds and genesis pathway

Studying techniques

✓ Function of main ripening microflora

Flavoring properties of our ripening micro-organisms

- Pure culture on cheese curd medium
  - 21 days at 12°C.

- Sensory profile by culture sniffing

- Chemical profile by extraction, identification and quantification of key flavoring volatile compounds

Listing of the flavor descriptors used by the selected and trained panel.
Yeast

Flavoring micro-organisms blend

- Sensory Profile
- Aromatic Profile
Micrococci

- Sensory Profile
- Aromatic Profile

Associations of strains in cheeses

Key flavoring compounds
- 103 flavoring compounds
- 27 key flavoring compounds
Many cheese culture suppliers with international expertise have identified and selected these desirable yeasts and molds. They have knowledge of which ones will contribute the traits you are interested in. They have used these internationally for many years and have introduced and expanded the use of these in the U.S. in the last decade.
Utah State University
17th Biennial Cheese Industry Conference

Dynamics of Microbial Systems

Craig Oberg
Weber State University
Introduction

Microbial Ecology - interactions of microorganisms with each other and their environment.

- Tendency is to get smaller and more focused (specific applications)
- Get bigger and make generalizations

Why will there always be a need for microbiologists in the dairy industry?

Microbial Misconceptions

What we teach students in the laboratory that is not entirely true.

1. Microorganisms are easy to cultivate.
2. Microorganisms grow fast.
3. Microorganisms grow as single cells.
4. Microorganisms get along with each other.
5. Microorganisms have a unique identity.
6. Microorganisms produce easily recognizable colonies.
7. Microorganisms lead quiet, solitary lives.
8. Most microorganisms can be grown in the laboratory.
How Microorganisms Really Live

1. Content to do things slowly (metabolism)
2. Stationary Phase - ultimate "couch potato"
3. Resist efforts to get rid of them.
4. Live together in communes.
5. Often antagonistic toward their neighbors.
6. Usually looking for a better home and an easier way to make a living.
7. Make a living anyway they can.
8. Communicate with each other and sometimes are very dependant on each other.

What is really out there?

- Detect (utilizing PCR) 100 to 1000 X the number of bacterial types that can be cultivated.
- Most of these MO do not have the genetic capability to impact the dairy industry but this illustrates the microbial potential.
- Potential dairy problems: Spoilage and emerging pathogens, new spoilage MO, even new fermentation microorganisms or enzyme systems.
16S rDNA Analysis of Anaerobe Isolates

Group 1 - Day 1
Group 2 - Day 12
Group 3 - Day 18

Bacteroides
Deinococcus

Microenvironments

- Growth of microorganisms depends on resources (nutrients) and on growth conditions.
- Differences in the type and quantity of different resources and physiological conditions of a habitat define the "niche" for a MO.
  - Prime niche and secondary niches.
- Because MO are so small their habitats are small.
  - For a 3-mm rod-shaped bacterium, a distance of 3 mm is equivalent to 2 km for a human.
- Different niches can exist across a very small spatial dimension.

Microcolonies of LAB in Cheese

Strategies for Growth

- Where do NSLABs come from?
- Why do they grow to such high numbers in aging cheese?
- What are their nutrient sources?
- How do they out compete starter cultures?
- Is aging cheese really a "hostile" environment?
r and K Selection
How is growth in the natural environment related to growth in a flask?
r-strategists: MO that respond to added nutrients with rapid growth rates (high rates of growth for short periods of time) (dormant state)
K-strategists: characterized by a high affinity for nutrients that are present in low concentration (metabolize slowly and have long generation times) (cell maintenance state)
Direct counts ~2 logs higher than viable counts. Dormant cells may become viable but nonculturable.

r or K Selection Strategies
• Extended periods of exponential growth in nature are rare.
• Slow growth rate reflect:
  – Nutrients are frequently in low supply.
  – Distribution of nutrients in the habitat is not uniform
  – MO must deal with the competitive effects of other MO.
• Most MO grow in nature at less than 1% of the maximal growth rate in the laboratory.

Bacterial species are described as having r or K selection strategies if:

r strategists
Evolution favors either a high rate of reproduction

K strategists
Evolution favors optimal uses of resources

• Continuum
• Most species are somewhere in between
• Mammals compared to microbes.
• Humans compared to rabbits…
**Development of Microbial Communities**

Population Selection within Communities

- **r strategists**
  - Highest reproduction rate
  - Opportunistic
  - They experience population crashes in resource limited environments
  - Populations are subject to extreme fluctuations
  - Prevail in uncrowded communities

- **K strategists**
  - Optimal utilization (conservation) of resources
  - Physiological adaptation to environmental resources
  - Reproduce more slowly
  - Successful in resource limited environments
  - More stable populations
  - Permanent members of communities

---

**Microbial Ecology of Cheese**

![Graph showing microbial population over ripening time](image)

- **Starter**
- **NSLAB**

**Ripening Time (mo)**

---

**Domestication of Microorganisms**

- Domestic Bacteria vs. Wild Bacteria
- Change the properties of MO - loss or suppression of traits

Natural Selection by extrinsic factors (milk environment) LAB fermented milk before starter cultures were thought of...

MO fill empty niches or try to expand environmental (host) range

Searching for new sources of nutrients and more hospital locations for growth.
How do LAB become domesticated?

- Adapted to unique environment (continual selection for a specific function)
- *S. thermophilus* - genome degradation

**Domestidation**
- Genome sequencing and annotation
- Metabolic pathway characterization
  Genome→gene→protein→compound
  (flavor, texture, defect)

**Genome Plasticity**

- *Lactococcus lactis* is a very specialized MO with a reduced genome (2.5 Mb) and a narrow ecological niche.
- Homologous recombination – *L. lactis* IL1403 contains 6 different IS elements (43 total copies)

**Lateral Gene Transfer**

- Bacteria are very successful at acquiring new genes (traits).
- Readily obtain genetic traits from other MO even unrelated MO especially when we constantly place them in close proximity.
- Bacterial speciation is affected by LCT.
- Four types of pathogenic *E. coli*
  - *E. coli* 0157:H7 genes from *Shigella*
Lateral Gene Transfer
Prokaryotic cells are sexually promiscuous and exchange genes across broad phylogenetic lines.
- Horizontal flow of genetic material occurs by:
  - Conjugation
  - Transduction (10x as many phage as bacteria in an environmental sample)
  - Transformation
  - Transposition
Also mutation and natural selection.
Results:
1. Few genes in total number
2. Usually only temporary benefit to the MO
3. Requires selective pressure

Concerns with Domestication
- Loss of "hybrid vigor" – phage sensitivity
- Pathways run in two directions
- Alternate pathways
- Subtle changes in substrates
- Extrinsic effects
- Lateral Gene Transfer
- Phage infections

Types of Lactic Acid Bacteria and Other Dairy-Related Bacteria
- Lactococcus
- Lactobacillus
- Leuconostoc
- Pedicoccus
- Streptococcus
- Enterococcus
- Carnobacterium
- Lactophlaera
- Genococcus
- Aerococcus
- Aliiococcus
- Delostigranum
- Terragenococcus
- Vagococcus
- Biilfidebacterium
- Sporolactococcus
- Wesserllo
- Propionibacterium
- Brevibacterium
Biofilms

- Biofilms – microcolonies of bacterial cells attached to a surface and encased in adhesive polysaccharides.
- In nature MO generally grow on a solid surface in mixed populations embedded in polymers produced by the MO.
- Communicate (quorum sensing) to prevent cells from filling in channels to bring in nutrients and remove wastes.

Biofilms

- Attachment to a surface is a signal for expression of biofilm-specific genes.
  - Synthesize cell-to-cell signaling molecules
  - Begin polysaccharide formation
  - Chemotactic agents that recruit nearby cells

Purpose: Protect cells from antimicrobial agents:

1. Heat
2. Chemicals
3. Sanitizers
4. UV radiation

Reason - very slow growth rate of the cells

- Drinking water pipes – biofilms are resistant to chlorination.
- Sources of contamination

Biofilm at Octopus Springs
Communities of Microorganisms

- *Listeria* problems
- Bulk tank study (861 tanks in 21 states)
  - *Cotiforms* 95% of the tanks
  - *Salmonella* 2.6%
  - *Listeria* 6.5% (95% clinical isolates)
- Cheese Plant survey - *Listeria* found (recalcitrant) in a variety of environmental locations.
- Numerous studies utilizing EPS producing LAB to enhance cheese properties.

EPS Production by *Streptococcus thermophilus*

Results:
- Moisture Retention - yield increases
- Improved rheology for reduced and low-fat cheeses.

Looking for a New Home

Considerations:
1. Nutrients
2. Extrinsic parameters
3. MO Interactions: Mutualism vs. Antagonism
   - Thermophilic LAB used in Italian and Swiss cheese.
   - Bacteriocins - very hostile to each other
Emerging Food borne Diseases

- Hepatitis A 1997 International travel and commerce
- Salmonella DT 104 1996 Microbial adaptation
- Cyclospora cayetanensis 1996 International travel and commerce
- E. coli O157:H7 1995 International travel and commerce
- Norwalk-like Virus 1994 Economic development
- E. coli 0157:H7 1993 Breakdown of public health measures
- Vibrio cholerae 01, El Tor 1991 International travel and commerce

New pathogens that are food borne and pathogens newly recognized as predominantly food borne in the United States in the last 20 years.

- Campylobacter jejuni
- Campylobacter fetus ssp. fetus
- Cryptosporidium parvum
- Cyclospora cayetanensis
- Enterohemorrhagic E. coli O157:H7 and related E. coli (e.g., O111:NM, O104:H21)
- Listeria monocytogenes
- Norwalk-like Virus
- Vibrio parahaemolyticus (cause of amnesic shellfish poisoning)
- Salmonella Typhimurium DT 104
- Vibrio cholerae 01
- Vibrio vulnificus
- Vibrio parahaemolyticus
- Yersinia enterocolitica

Cheese as an OTC Medication

- Change the mind set of consumers
- Integrate nutritional studies
- Use as a probiotic delivery system
Inhibition of Staphylococcus aureus Isolated from Human Nares by Lactic Acid Bacteria

<table>
<thead>
<tr>
<th>Symptom of LAB</th>
<th>LAB isolation at 0% ORP</th>
<th>LAB isolation at 5% ORP</th>
<th>LAB isolation of 10% ORP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibition</td>
<td>13/13</td>
<td>13/13</td>
<td>10/10</td>
</tr>
<tr>
<td>Inhibition</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
</tr>
<tr>
<td>Lactic</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td>Lactic</td>
<td>2/2</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td>Lactic</td>
<td>1/1</td>
<td>1/1</td>
<td>0/0</td>
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<tr>
<td>Lactic</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
</tr>
<tr>
<td>Lactic</td>
<td>1/12</td>
<td>1/12</td>
<td>1/12</td>
</tr>
</tbody>
</table>

**BACKGROUND**

- *Staphylococcus aureus* is a potentially pathogenic bacterium that can be found in about one third of the human nares of healthy individuals. Although carriers may not experience health decline due to carriage of this organism, the presence of persistent carriers may be associated with recurrent infections. Onset of persistent nasal carriage of *S. aureus* also increases the risk of infection in immune-compromised hosts such as patients after surgery.
- As a result, our studies have found that some of these persistent carriers have been saved by specific probiotics. LAB can enhance both specific and nonspecific immune responses.
  - Activate macrophages
  - Alter cytokine expression
  - Increase level of immunoglobulins
- Direct cells to either pro- or anti-inflammatory responses.

**LAB Interactions with Humans**

- LAB can enhance both specific and nonspecific immune responses.
  - Activate macrophages
  - Alter cytokine expression
  - Increase level of immunoglobulins
- Direct cells to either pro- or anti-inflammatory responses.
Novel Uses - There is a MO for every situation

- Trichloroethylene (TCE) contamination – pump whey into ground water, use whey-degrading MO to generate lactate compounds that react with TCE.

Final Observations and Conclusions

- There is still a need for studies concerning the microbial ecology of cheese and other fermented dairy products.
- Apply general principles of microbial ecology to better understand NSLABs.
  - Nutritional sources and growth strategies
  - Biofilm development and control (novel methods) will be continue to be important.
- Examine other types of LAB for genetic traits that will confer advantages to current dairy LAB.
Utah State University
17th Biennial Cheese Industry Conference

Use of Permeabilised and Lytic Strains for Enhanced Flavor Effects in Semi-Hard Cheese

Jonathan Goodwins
Danisco
A little bit of History...

Techniques we use to follow ripening.

Results with Lytic strains.

Results with permeabilised strains.

Where this puts us today....

"Where are the fat rich - eating Cheddars which were on every grocer's counter thirty years ago?... But the perfect Cheddar, which cuts like marrow and yet has a sweet, nutty flavour, is today almost unknown."

Organic Phosphates in Cheese - published by St Ivel Ltd for the London Dairy Show of 1911
The flavour forest

- Natural balance of ripening enzymes
- No labeling / legal issues.
- Technically feasible with good incorporation of cells into the curd matrix
What is meant by Lytic or Permeabilised?

Lysis - destructive disruption of cells: the destruction of cells by disruption of the bounding membrane, allowing the cell contents to escape.

Permeable: allowing (especially liquids) to pass or diffuse through.

Historical use of Lytic & Permeabilised Cells:

1. W. Storch (DE), Hermann Weigmann (D) and Herbert Williams (USA) created the fundamentals of dairy starter cultures, concluding that bacteria are responsible for acidifying the milk.

2. Eduard von Freudenreich isolated in 1897 a Lactobacillus (Lactobacillus brevis).

3. Sigurd Orla-Jensen published in 1919 a monograph entitled "The Lactic Acid Bacteria" which focused attention on these organisms as a group for the first time.

4. Hermann Weigmann also observed in the course of his work that he could isolate a component called "casease" from bacterial cultures that "favours and accelerates opening when added to fresh cheese."

Methods for attenuating cells in this fashion include:
- Heat shock and spray drying
- Freeze shock and freeze drying
- Use of phage or enzymes (lysozyme)
- Chemical treatments (Triton X etc.)
- Physical treatments: Emulsification, homogenisation, pressure, sonication

3. Frey, Marth, Johnson & Olson 1966 Milchwissenschaft 4(1): 681-685
Morgan employed a strain utilizing Lactococcus lactis subspecies lactis which also produced a bacteriocin active against the cremoris acidilier. Lysozyme, sphero-plast creation, and phage reduction of bitterness (produced by using high coagulant levels) through directed lysis of Lactococcus lactis ML8.

Positive roles for Lysis in cheese ripening:

- **Propionibacteria**
  - Saboya, Goudelanche, Moules, Lerayer & Lortal 2001 Lait v81 p699 - 712
- **Lactobacillus casei**
  - Göfani & Vesovi 2005 European Food Research and Technology v220 p477-482
  - Bachmann, Gruinger, Jaws, Rohm & Weinricher

No positive role found:

- Bifidobacteria
  - El-Shafei 1994 Indian Dairy Sci v 47 p774 - 779
- Leuconostoc
  - El-Shafei 1994 Die Nahrung v38 p699 - 605

---

The name comes from the French word *racier* (to scrape).

Mountain region smear cheese.

Is usually eaten after heating & scraping off when melted with a knife.

9 weeks ripening at 12°C

45% fat minimum
53% dry matter.

---

**PepXP**

X. PTP 699 - 710
null
Sensory analysis facilities

- Tasting area: 10 booths with serving area
- Colour: clear
- Lighting: daylight

The Quantitative Descriptive Analysis method chosen
- Key attributes and a frame of reference identified
- 15-17 assessors are trained during more than 10 sessions on Cheddar cheeses
- Performance of assessors is monitored and cheeses are evaluated in duplicate after 6 months.

Products location in flavour map

Std RD686 RD693 RD799
RD797 RD498

Sweet

RD414 Sulphur
RD691 Savoury
RD796 Overall intensity

Bitter

RD498

Generalization of TQA as a sensory method

Key attributes identified: Cheddar characteristic:
- Texture
- Aroma
- Flavor
- Pasteurization
- Overall intensity
"The flavor profiles of cheeses are complex and variety- and type-specific. This was realized back in the 1950s, when Mulder (1952) and Kosikowski and Mocquot (1958) proposed the "component balance" theory. According to this theory, cheese flavor is the result of the correct balance and concentration of a wide variety of volatile flavor compounds."
1. Permeabilised Strains

- Altering Process conditions, for example heat or freeze shocks are the most easy methods.
- Early studies also used chemical agents such as butanol.
- Food grade permeabilising agents are also now available.

2. Selecting a strain for permeabilisation

- Detectable PepN activity with increasing levels of Sodium Dodecyl Sulphate
  - PepN - cell associated
  - PepN - Free enzyme

- Examples of the maximum PepN activity obtained from isolated cell fractions with increasing levels of SDS. The light line shows the final values for a specific SDS concentration.
Attacks bitter peptides in Crescenza cheese

<table>
<thead>
<tr>
<th>Change</th>
<th>Positive Effect on Permeabilisation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth Temperature °C</td>
<td>40°</td>
</tr>
<tr>
<td>Holding time in Stationary phase</td>
<td>35°</td>
</tr>
<tr>
<td>Final pH during Stationary phase</td>
<td>7.5</td>
</tr>
<tr>
<td>Heat Treatment</td>
<td>X</td>
</tr>
<tr>
<td>Freezing Cycle</td>
<td>slower</td>
</tr>
</tbody>
</table>
De-bittering no problem.

Complexity of aroma profile?

Reproducibility?

Future developments

- The results suggest that a balance of lysed and intact cells is important for control of cheese ripening...
- Combined strains for enhanced effects
- Selection for downstream activities such as amino-transferase
- Screening underway:

Crook, Mintley, Coolbear & Roundhill 1995 International Dairy Journal v5 p451 - 472

Thanks for listening
Helveticus for Debittering and as Flavor Adjuncts

Jeff Broadbent
Utah State University
Using *Lactobacillus helveticus* for Debittering and as Flavor Adjuncts

Jeffery R. Broadbent
Department of Nutrition & Food Sciences
Utah State University, Logan

Cheese Flavor Development Requires Lactic Acid Bacteria

LAB in cheese may include:
- Starter culture (lactococci, lactobacilli, streptococci)
- Starter adjuncts (most are lactobacilli)
- Non-starter LAB (predominantly lactobacilli)

Adjuncts and Cheese Quality:
- Contribute to flavor development, but effect is often “atypical”
- Academic trials suggest some strains can dominate NSLAB populations
- Reduced susceptibility to culture-related quality defects
**Key Contributions by LAB to Cheese Flavor Development**

- Proteolysis
- Amino acid catabolism
- Lipase/esterase activity
- Other activities
  - Citrate catabolism
  - Redox

**Proteolysis and Cheese Flavor**:

- Breakdown of the casein network softens cheese texture, which facilitates the release of flavor compounds during chewing.
- Low molecular-weight peptides can directly influence flavor, but effect is typically negative (i.e., bitterness).
- Some free amino acids have a direct effect on flavor, and amino acid catabolism by LAB is a major source of aroma and flavor compounds.

**Contribution of Peptidase Enzymes to Cheese Proteolysis**

- Hydrolyze peptides into smaller oligopeptides and free amino acids
- Broad differences in specificity and relative activity
- All are intracellular enzymes
Intracellular Aminopeptidase Activity

TCA Soluble Amino Acids in Cheddar Cheese made With or Without a *L. helveticus* Adjunct.

Free amino acid content (nmol/ml):

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>control</th>
<th>Adjunct</th>
<th>control</th>
<th>Adjunct</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>24</td>
<td>70</td>
<td>49</td>
<td>190</td>
</tr>
<tr>
<td>Arginine</td>
<td>23</td>
<td>46</td>
<td>22</td>
<td>120</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>40</td>
<td>45</td>
<td>79</td>
<td>200</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>87</td>
<td>140</td>
<td>240</td>
<td>980</td>
</tr>
<tr>
<td>Glycine</td>
<td>24</td>
<td>70</td>
<td>31</td>
<td>170</td>
</tr>
<tr>
<td>Leucine</td>
<td>55</td>
<td>70</td>
<td>190</td>
<td>520</td>
</tr>
<tr>
<td>Lysine</td>
<td>41</td>
<td>94</td>
<td>110</td>
<td>610</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.4</td>
<td>2.0</td>
<td>23</td>
<td>85</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>30</td>
<td>41</td>
<td>100</td>
<td>250</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>5.7</td>
<td>13</td>
<td>13</td>
<td>150</td>
</tr>
</tbody>
</table>


USU Cheese Trials

- Treatment 1 (Control): *Lactococcus lactis* starter culture alone (DVS 850)
- Treatment 2: DVS 850 plus *Lac- L. lactis* adjunct culture CR213
- Treatment 3: DVS 850 plus *Lactobacillus helveticus* adjunct CNRZ 32
- Treatment 4: DVS 850 plus *Lc. lactis* adjunct CR213 and *Lb. helveticus* CNRZ 32
Ripening Procedure for USU's Old Juniper Cheese:

- 35°F to 55°F
- 1.5°C to 13°C

Sensory Analysis

- Cheeses evaluated in duplicate by Dr. MaryAnne Drake's trained sensory panel (n=10) at NCSU
  - 3 mo
  - 6 mo
  - 9 mo
- Data analyzed for main effects (time, temperature, and treatment) and interactions
Impact of Treatment on Flavor

* Denotes significant flavor changes

**Lb. helveticus** CNRZ 32 Draft Genome

- 2.3 Mbp sequenced (~97.5% coverage)

- Commercial cheese starter/flavor adjunct
- ~80 total contigs assembled into ~50 scaffolds
- ≥ 5 rRNA operons
- ≥ 27 distinct transposase sequences; ≥ 160 complete or partial transposase genes
- ≥ 1 prophage
- "Tiled" microarray made
- Functional genomics on:
  - Proteolytic enzymes
  - Amino acid metabolism

---

**The Power of Genomics:**

**Catalog of Lb. helveticus CNRZ32 Proteolytic Enzymes Before/After Genome Sequencing**

**Proteases:**
- PrtH1, PrtH2, & 12 additional proteases

**Endopeptidases:**
- PepE, PepE2, PepF, PepO, PepO2, PepO3, & 2 glycoprotein endopeptidases

**Aminopeptidases:**
- PepN, PepC, PepC2, PepX, & PCP

**Di-Tripeptidases:**
- PepD, PepD2, PepD3, PepD4, PepE, PepQ, PepQ2, PepR, PepT, PepT2, PepV, & 8 additional dipeptidases

**Oligo- and di-peptide transport:**
- OppA, OppA2, OppB-D, OppF, DtpA, DtpA2, DtpT
**Specificity of *L. helveticus* CNRZ32 Endopeptidases Toward β-CN (193-209) & α_{6s}-CN (f1-9)**

(10°C, pH 5.0-5.2, 4% NaCl)

<table>
<thead>
<tr>
<th>β-CN (193-209):</th>
<th>RIVGPVGFSP</th>
<th>PepO2</th>
<th>PepO3</th>
<th>PepF</th>
</tr>
</thead>
<tbody>
<tr>
<td>α_{6s}-CN(f1-9):</td>
<td>RPKHPIKHQ</td>
<td>PepO2</td>
<td>PepO3</td>
<td>PepE</td>
</tr>
</tbody>
</table>


---

**Formation and Degradation of Bitter Peptides**

Casein \[\rightarrow\] Rennet \[\rightarrow\] Microbial Proteases & Peptidases \[\rightarrow\] Free amino acids \[\rightarrow\] Peptidases

O = Hydrophobic residues

---

**Assays for Endopeptidase Specificity and Relative Activity**

- Expression of CNRZ32 pepF, pepO2, pepO3, and pepE in *L. lactis* LM0230 on pTRKH2 under *P_{pepO3}* control
- No *Lc. lactis* transformants for *pepF* construct
- Hydrolysis of β-CN(1193-209) and α_{6s}-CN(f1-9) with CFE of LM0230 derivatives expressing PepO2, PepO3, and PepE at 10°C, pH 5.0-5.2, 4% NaCl:
  - Against individual peptides
  - In a defined peptide mix (both peptides plus α_{6s}-CN (f1-6), (f1-13), & (f1-4))
  - In Cheddar cheese serum
Peptidase Activity & Bitterness

- Specific activity of some CNRZ 32 endopeptidases toward bitter peptides was significantly higher in cheese serum versus buffer.
- General aminopeptidase (e.g., PepN) and post-prolyl endopeptidase (e.g., CNRZ 32 PepO2 or PepO3) activities are likely key to de-bittering activity associated with *Lb. helveticus* adjunct cultures.

Amino Acid Catabolism and Cheese Flavor

- Phenylalanine → phenylacetate, phenylethanol (floral/rosy)
- Methionine → methanethiol (sulfur)
- Leucine → 3-methyl butanal (nutty/malty)

Met Conversion by LAB

- Methionine
  - Transaminase
  - Cystathionine-(β or γ)-lyase
  - Hydroxy-acid dehydrogenase
  - Methanethiol (sulfur flavor)
VSC Production by LAB (pH 6.8)

VSC Production by *Lb. helveticus* CNRZ32 cbl Derivatives

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Product (µM)</th>
<th>WT</th>
<th>Null</th>
<th>Compl.</th>
<th>OEx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met</td>
<td>MeSH</td>
<td>0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>DM-DS</td>
<td>1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>DM-TS</td>
<td>0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.78&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cyst.</td>
<td>MeSH</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.15&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>DM-S</td>
<td>1.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cys</td>
<td>MeSH</td>
<td>0.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.45&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>H&lt;sub&gt;2&lt;/sub&gt;S</td>
<td>1.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.25&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

pH 6.8, 96 h at 37°C

Auxotrophy and Amino Acid Catabolism by LAB

- Primary mechanism for amino acid breakdown by LAB involves the reversible action of enzymes used in amino acid biosynthesis
- Interplay between LAB in amino acid catabolism may be a reflection of the molecular basis for amino acid auxotrophy among the different bacteria in cheese
- Genome sequence information should enhance our ability to predict -and test- an organisms' contribution to amino acid breakdown in cheese
Arginine Biosynthesis & Catabolism

- Arg is required

Aspartate \( \rightarrow \) Argininosuccinate

\[ \text{Aspartate} \quad \text{4.3.4.5} \rightarrow \text{Argininosuccinate} \]

\[ \text{Citrate cycle} \]

\[ \text{Citrate} \rightarrow \text{Argininosuccinate} \quad \text{4.3.2.1} \]

\[ \text{Fumarase} \]

\[ \text{Orotic acid} \quad \text{Carboxypeptidase} \quad \text{aspartate} \quad \text{4.3.3.4} \]

\[ \text{Carbamoyl-P} \rightarrow \text{Orotidine} \]

\[ \text{Orotidine 5'-phosphate} \quad \text{2.1.3.5 (orotidine-5'-phosphate decarboxylase)} \]

\[ \text{Orotidine} \rightarrow \text{Putrescine} \]

\[ \text{Putrescine decarboxylase} \quad \text{2.3.1.17} \]

\[ \text{Putrescine} \rightarrow \text{ATP} + \text{NH}_3 + \text{CO}_2 \]

\[ \text{Glutamate} \]
**Lb. helveticus CNRZ 32 Genotype vs Phenotype: Asp/Asn Biosynthesis**

- Asp or Asn is required

Phosphoenolpyruvate \( \rightarrow \) Glycolysis

\[ \text{Glu} \] \[ \rightarrow \] \[ \alpha\text{-KG} \] \[ \rightarrow \] \[ \text{Aspartate} \]

- Aspartate \( \rightarrow \) Asparagine
- Asparagine \( \rightarrow \) Glu

\[ \text{Citrate} \rightarrow \text{Oxaloacetate} \]

\[ \text{aspartate} \]

\[ \text{asparagine} \]

\[ \text{glutamic acid} \]

\[ \text{citrate} \rightarrow \text{succinate} \]

\[ \text{Malate} \rightarrow \text{Fumarate} \]

\[ \text{Fumarate} \rightarrow \text{Succinate} \]

**Amino Acid Auxotrophy in CNRZ32: Phenotype vs Genotype**

- Good agreement between genome predictions and phenotype
- Auxotrophy for 13 amino acids directly traced to the presence of pseudogenes (Asn/Asp, Met) or the complete absence of genes (Arg, Pro, Leu, Ile, Val, Phe, Tyr, Trp, Thr, Glu, His)
- Auxotrophy is primarily due to complete gene loss rather than point mutations or minor genetic lesions
- Some "remnant" genes contribute to amino acid catabolism and likely impact cheese flavor

**Conversion of Citrate to Succinate by Lb. helveticus**

- Citrate \( \rightarrow \) Oxaloacetate
- Oxaloacetate \( \rightarrow \) Malate
- Malate \( \rightarrow \) Fumarate
- Fumarate \( \rightarrow \) Succinate

Summary

- Cheese technologists and microbiologists have identified many fundamental mechanisms by which LAB affect cheese flavor.
- This knowledge is often translated into industry practice through adjunct cultures like *Lb. helveticus* CNRZ 32.
- Functional and comparative genomics studies of *Lb. helveticus* and other LAB will continue to advance our understanding of the molecular processes that determine cheese flavor development.

Acknowledgments

Collaborators:
- Drs. J. Steele, S. Rankin, V. Smeianov, & D. Banavara (UW)
- Drs. J. Hughes & D. Welker (USU)
- Dr. MaryAnne Drake, NCSU

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- Wisconsin Center for Dairy Research
- Utah State University CURl program
- Wisconsin University-Industry Research program
Utah State University
17th Biennial Cheese Industry Conference

Cultures and Procedures for
Low Fat Cheese

Donald McMahon
Utah State University
Making Low Fat Cheese

Donald McMahon
Jeffrey Broadbent
Steve Larsen

Overview

• What is low fat cheese.
• Strategies for making low fat cheddar cheese
• Initial cheesemaking trials
• Cheese composition
• Texture analysis
• Melting analysis
• Flavor analysis

US Definition of Low Fat

• Low fat foods can contain no more than 3 g of fat per reference amount
  - or per 50 g if reference amount is < 50 g
• Reference amount for cheese = 30 g
• 3 g per 50 g = 6.0%
• Low fat cheese can contain no more than 6% fat irrespective of the cheese variety.
• Other ingredients not permitted in making cheeses with a Standard of Identity, can be used in making low fat cheeses.
Strategies

- Increasing pasteurization temperature
  - Merid et al., 1994, J. Dairy Sci. 77:1783-1790
- Pre-acidifying milk
  - Merid et al., 1994, J. Dairy Sci. 77:1783-1790
- Adding fat mimetics
- Adding lecithin
- Homogenizing milk
  - Nudan et al., 2000, J. Dairy Sci. 81:2065-2075

Pre-Acidifying Milk

- Lowering pH of milk to pH 6.3 prior to adding starters and rennet.
  - Increases calcium phosphate solubility
  - Some calcium (and phosphate) move out of the casein micelles into the serum phase of milk.
  - More calcium phosphate is expelled from the curd during whey syneresis.
  - Cheese curd has lower calcium level.
  - Cheese protein matrix is more hydrated and holds more moisture.
  - Cheese moisture content is raised.
  - Cheese become softer.

High Pasteurization Temperature

- Pasteurizing milk at 185°F for 15 sec.
  - Increases level of whey protein denaturation
  - Denatured whey proteins form large aggregates that are trapped in the cheese curd.
    - Less whey proteins in the whey
    - Aggregates bind to surface of casein micelles
  - Protein matrix cannot contract as much during cheese making.
  - Less whey is expelled from the curd.
  - Cheese has a higher moisture level.
  - Whey proteins in cheese interfere with cheese flavor development.
  - Cheese become softer.
Homogenization

- Homogenizing of milk
  > Casein micelles bind to the newly created fat droplet surfaces
  > Fat droplets are incorporated into the protein network that is formed as a result of renneting.
  > Contraction of protein network is restricted.
  > Can increase the opaqueness of the cheese.

- Homogenize 50% of milk while pasteurizing
  > Aim:
    - Increase opaqueness
    - Slightly retard curd syneresis
    - Slightly increase cheese moisture content.

Initial Trials

<table>
<thead>
<tr>
<th>Trial 1: Milk Treatment</th>
<th>% Cheese Moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Milled Curd</td>
</tr>
<tr>
<td>High Temperature</td>
<td>47.1%</td>
</tr>
<tr>
<td>Pre-acidified</td>
<td>48.6%</td>
</tr>
<tr>
<td>Homogenized</td>
<td>53.2%</td>
</tr>
</tbody>
</table>

- Washed curd method
  > Produced higher cheese moistures.
  > Provided better control on final pH

- Milled curd method
  > When adjusting cheese make procedure to increase moisture above 50%, final cheese pH would drop below 5.10

Trial 2

<table>
<thead>
<tr>
<th>Trial 2: Milk Treatment</th>
<th>% Cheese Moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Milled Curd</td>
</tr>
<tr>
<td>High Temperature</td>
<td>51.9%</td>
</tr>
<tr>
<td>Pre-acidified</td>
<td>53.7%</td>
</tr>
<tr>
<td>Homogenized</td>
<td>52.1%</td>
</tr>
</tbody>
</table>

- Moisture levels could be increased by dropping cook temperature to 96°F.
  > But pH dropped below pH 5.1
  > Cheeses were too acid.

- About 53% moisture appeared best.
Trial 3

<table>
<thead>
<tr>
<th>Milk Treatment</th>
<th>% Cheese Moisture</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenized and</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-acidified</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partial Drain then add</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cold water to dilute</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lactose in whey</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Completely drain whey</td>
<td>53.3%</td>
<td>5.19</td>
</tr>
<tr>
<td>then add cold water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>to curd to wash out</td>
<td>52.9%</td>
<td>5.13</td>
</tr>
<tr>
<td>lactose</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Wash water could be added before or after draining the whey
  >Similar moisture
  >Similar pH
- Washing after draining preferred
  >Less volume of diluted whey to process and dry
  >Can separate diluted whey from regular whey.
  >More suitable for use on cheese belts.

Trial 4

<table>
<thead>
<tr>
<th>High Temp</th>
<th>Homogenized</th>
<th>Pre-acidified</th>
<th>% Moisture</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>X</td>
<td>54.7%</td>
<td>5.05</td>
</tr>
<tr>
<td>X</td>
<td></td>
<td>X</td>
<td>54.7%</td>
<td>5.05</td>
</tr>
<tr>
<td>X</td>
<td>X</td>
<td>X</td>
<td>57.7%</td>
<td>5.18</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>High Temp</th>
<th>Homogenized</th>
<th>Pre-acidified</th>
<th>% Moisture</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td></td>
<td>X</td>
<td>56.7%</td>
<td>5.17</td>
</tr>
<tr>
<td>X</td>
<td>X</td>
<td>X</td>
<td>56.7%</td>
<td>5.32</td>
</tr>
<tr>
<td>X</td>
<td>X</td>
<td>X</td>
<td>56.3%</td>
<td>5.42</td>
</tr>
</tbody>
</table>

- Combining high temperature pasteurization (185°F
  for 15 sec) with pre-acidification had an additive
  effect.
- Homogenization provided no benefit when combined
  with either high temp. or pre-acid.
- Cheeses above 55% moisture were too soft.

Trial 5 Cheese Composition

<table>
<thead>
<tr>
<th>High Temp</th>
<th>Pre-acidified</th>
<th>% Moisture</th>
<th>pH</th>
<th>% Salt</th>
<th>% Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td></td>
<td>52.5%</td>
<td>5.21</td>
<td>1.88%</td>
<td>4.75%</td>
</tr>
<tr>
<td>X</td>
<td>X</td>
<td>57.0%</td>
<td>5.27</td>
<td>2.03%</td>
<td>4.25%</td>
</tr>
<tr>
<td>X</td>
<td>X</td>
<td>54.2%</td>
<td>5.20</td>
<td>1.83%</td>
<td>5.00%</td>
</tr>
<tr>
<td>X</td>
<td></td>
<td>54.8%</td>
<td>5.27</td>
<td>2.01%</td>
<td>4.32%</td>
</tr>
</tbody>
</table>

Mean values from 3 replicates

- High temperature pasteurization (185°F for 15 sec) increased cheese moisture more than pre-acidification to pH 6.3.
- Curd made from milk subjected to high temperature pasteurization absorbed/dissolved more salt.
- Combining high temperature pasteurization with pre-acidification did not seem to increase moisture any further.
- Cheese moisture could be increased without any milk treatments but cheese was more "rubbery"
High moisture cheeses were softer
High temperature pasteurization increased adhesiveness of the cheese more than pre-acidification.
Cheese made using high heat treatment was more cohesive even though it had the highest moisture content.
> pre-acidification helped make the cheese less cohesive.

Cheese made using high temperature pasteurization melted slightly more than the others, but this may just be a function of its high moisture content.
Only minor differences in overall melting.
Cheese made using pre-acidification had slightly faster initial melting than the control cheese, but virtually the same final melting.

2 means with the same letter(s) are not different (p<0.05)
Means in row followed by different letters are different (p<0.05)
 Attributes not listed (fruity, free fatty acid, salty, sour) were not detected.
Trial 5 Flavor

- Adding adjunct cultures including *L. helveticus* CNRZ32 increased flavor of low fat cheese.
- Compared to full fat cheese made using the same cultures, the low fat cheese:
  > had less milkfat (dairy) flavor; completely missing
  > had more cooked flavor, whey flavor, diacetyl flavor and sulfur flavors
- Cheese made using high heat treatment tended to develop rosey and other non-characteristic cheddar cheese flavors.

Cheesemaking Summary

- Use adjunct cultures
- Pre-acidify milk to pH 6.3
- Reduce cook temperature (98-100°F)
- Drain at pH 6.1
- Use washed curd method after draining and cool curd to 80-82°F
- Salt at pH 5.8

Cheese Summary

<table>
<thead>
<tr>
<th>Moisture</th>
<th>54%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>6%</td>
</tr>
<tr>
<td>Salt</td>
<td>2.0%</td>
</tr>
<tr>
<td>pH</td>
<td>5.20-5.30</td>
</tr>
</tbody>
</table>
Acknowledgments

- Cheese composition, texture, and melting by Shaun Adams
- Funding from
  > DMI,
  > Utah State University Agricultural Experiment Station
- Flavor analysis by MaryAnne Drake.
Utah State University
17th Biennial Cheese Industry Conference

Use of Genomics as a Tool for Culture Development and Selection

Bart Weimer
Utah State University
Use of Genomics as a Tool for Culture Development and Selection

Bart Weimer
Center for Integrated BioSystems
Utah State University

Core Questions...

- Bacteriophage
  - Phage evolution
  - Host/phage interactions
- Cellular metabolism
  - Lysis
  - Stress response
- Microbial ecology
  - Succession
  - Cellular interaction
  - Host/microbe
- Fermentation
  - Optimization
  - Acid production
  - Phage resistance
  - Survival
  - Flavor production
- Cheese flavor
  - Pathway networks
  - Isogenic mutants
  - Identify critical reactions
  - Role of lysis

Important Traits for Fermentation

- Lactic acid production
  - Glycolysis
- Proteolysis
  - Protease
  - Peptidases
  - Amino acid catabolism
- Flavor production
  - Positive
  - Negative
- Stress and growth
  - Salt
  - Temperature
  - Osmotic
  - Redox

The challenge is to integrate metabolism, genome editing, and gene expression to create productive knowledge.
**Biogenesis of Flavor Compounds**

<table>
<thead>
<tr>
<th>Lactose, citrate</th>
<th>Proteins</th>
<th>Lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic acid</td>
<td>Peptides</td>
<td>Lactones</td>
</tr>
<tr>
<td>Dicarboxyls</td>
<td>Amino acids</td>
<td>Lactones</td>
</tr>
<tr>
<td>Aldehydes</td>
<td>Amines</td>
<td>Aldehydes</td>
</tr>
<tr>
<td>Alcohols</td>
<td>Fatty acids</td>
<td>Fatty acids</td>
</tr>
<tr>
<td></td>
<td>Sulfur compounds</td>
<td></td>
</tr>
</tbody>
</table>

Reactions between classes (e.g., 5-methyl-2-furan) (Law, 1994)

**Flavor Improvement Targets**

**Undesirable**
- Aromatic off-flavors
  - Aromatic amino acids
- Bitterness
  - Protease
  - Chymosin
  - Aminopeptidases

**Desirable**
- Sulfur
  - Methanethiol
  - Hydrogen sulfide
- Lipolysis
  - Lipase/esterase
  - Branched chain amino acids
- Citrate
  - Diacetyl

**Precursors to VFAs**

<table>
<thead>
<tr>
<th>Precursor</th>
<th>VFA</th>
<th>Aroma Threshold (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. Amyl.</td>
<td>propionate</td>
<td>40.3</td>
</tr>
<tr>
<td>Mr. Lipolyse</td>
<td>butyrate</td>
<td>0.3 (pH 5.2)</td>
</tr>
<tr>
<td>propionate</td>
<td>40.3</td>
<td></td>
</tr>
<tr>
<td>isobutyrate</td>
<td>5.3 (pH 2.0)</td>
<td></td>
</tr>
<tr>
<td>4-methylpentanoate</td>
<td>0.61 (pH 2.0)</td>
<td></td>
</tr>
<tr>
<td>6-methylheptanoate</td>
<td>0.84 (pH 2.0)</td>
<td></td>
</tr>
<tr>
<td>2-methylbutyrate</td>
<td>3.2 (pH 2.0)</td>
<td></td>
</tr>
<tr>
<td>l-valerate</td>
<td>6.5 (pH 5.2)</td>
<td></td>
</tr>
<tr>
<td>d-valerate</td>
<td>7.3 (pH 5.2)</td>
<td></td>
</tr>
<tr>
<td>l-leucinate</td>
<td>6.5 (pH 5.2)</td>
<td></td>
</tr>
</tbody>
</table>
**Flavor & Fatty Acids**

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Threshold (ppm)</th>
<th>Flavor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid</td>
<td>N.A.</td>
<td>pungent, sweaty</td>
</tr>
<tr>
<td>Propanoic acid</td>
<td>N.A.</td>
<td>acid, sharp</td>
</tr>
<tr>
<td>Butanoic acid</td>
<td>6.2</td>
<td>rancid, sharp, acid</td>
</tr>
<tr>
<td>Pentanoic acid</td>
<td>6.5</td>
<td>nutty, cheese-like</td>
</tr>
<tr>
<td>Hexanoic acid</td>
<td>8.6</td>
<td>acidic, sweaty</td>
</tr>
<tr>
<td>Heptanoic acid</td>
<td>0.28</td>
<td>soapy, fatty acid-like</td>
</tr>
<tr>
<td>Octanoic acid</td>
<td>8.7</td>
<td>goaty, waxy, soapy</td>
</tr>
</tbody>
</table>

**Casein**

Chymosin

High Mol. Wt. Peptides

- Proteinases
- Endopeptidases
- Aminopeptidases

Low Mol. Wt. Peptides

- Aminopeptidases
- Di- & Tripeptidases

**Proteolysis & Beyond**
Lactic Acid Bacteria
Genome Consortium

Lactococcal Genomes...

- *L. lactis* IL1403
  - BCAA & His auxotroph
  - Plasmid-free
  - Total 2,310 ORFs
  - ~3-5x coverage

- *L. cremoris SK11*
  - Contains 5 plasmids
  - Lac & Protosyn (+)
  - 2.4 Mb total size
  - ~30% GC
  - 2,685 total ORFs
  - ~500 unknown
  - ~10x coverage
  - *L. cremoris MG1363
  - *L. cremoris QA5
**Total Genome Comparisons**

<table>
<thead>
<tr>
<th>Size (Mb)</th>
<th>GC (%)</th>
<th>ORFs</th>
<th>Total IS Elements</th>
<th>IS Types</th>
<th>tRNA</th>
<th>rRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.3</td>
<td>35.4</td>
<td>2310</td>
<td>43</td>
<td>6</td>
<td>6</td>
<td>62</td>
</tr>
<tr>
<td>2.4</td>
<td>35.9</td>
<td>2685</td>
<td>&gt;140</td>
<td>4</td>
<td>6</td>
<td>61</td>
</tr>
<tr>
<td>~2.5</td>
<td>37.1</td>
<td>~2500</td>
<td>62</td>
<td>8</td>
<td>6</td>
<td>?</td>
</tr>
</tbody>
</table>

Looks to be relatively similar, but does it hold on a genome-wide basis?

(Klaenhammer et al., '02; JGI web site; Bolotin et al. '99, '02)

**LAB Phylogenetic Cluster**

**New Phylogeny of LAB**
LAB Relationships to Other MOs

Amino Acid Metabolism - Genomes

Comparison of LAB
Comparisons Using a Macroarray

- Whole genome spotted array with a single probe/OK2 in triplicate
- Hybridized 75 strains
  - Lactobacilli, lactococci, St. thermophilus
  - Only lactococci hybridized

Whole Genome Classification

Stress Response in IL1403

<table>
<thead>
<tr>
<th>Induce</th>
<th>Repress</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>30</td>
</tr>
<tr>
<td>24</td>
<td>26</td>
</tr>
<tr>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>
The consistency of DNA macroarray results

Gene Expression in Cheese

Array results don't match laboratory growth conditions

45 days old

Expression of Metabolism in Cheese

<table>
<thead>
<tr>
<th>Class (%)</th>
<th>Curd*</th>
<th>24 h</th>
<th>45 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO</td>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>AA use</td>
<td>3</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>Protein</td>
<td>0.3</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>DNA meth.</td>
<td>1</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Stress</td>
<td>0.5</td>
<td>0.3</td>
<td>0.3</td>
</tr>
</tbody>
</table>

*% of top 25
### Most Expressed Genes in Cheese

<table>
<thead>
<tr>
<th>Curd</th>
<th>24 h</th>
<th>45 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>arcA,B,C,D</td>
<td>arcA,B,C,D</td>
<td>arcA,B,C,D</td>
</tr>
<tr>
<td>pldA,B,C</td>
<td>pldA,B,D</td>
<td>pldA,B,D</td>
</tr>
<tr>
<td>ploH</td>
<td>ploH</td>
<td>ploH</td>
</tr>
<tr>
<td>enoB</td>
<td>enoA,B</td>
<td>enoA,B</td>
</tr>
<tr>
<td>dnaK</td>
<td>fbhA</td>
<td>fbhA</td>
</tr>
<tr>
<td>cysK</td>
<td>pepF,M</td>
<td>pepF,M</td>
</tr>
</tbody>
</table>

### Changes in Expression

<table>
<thead>
<tr>
<th>Protein</th>
<th>Lab.</th>
<th>Curd.</th>
<th>Curd.</th>
<th>45 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>jgd</td>
<td>YEPD</td>
<td>jgd</td>
<td>jgd</td>
<td>jgd</td>
</tr>
<tr>
<td>pyk</td>
<td>pyk</td>
<td>pyk</td>
<td>pyk</td>
<td>pyk</td>
</tr>
<tr>
<td>gapK,A,B</td>
<td>gapK,A,B</td>
<td>gapK,A,B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dnaK</td>
<td>dnaK</td>
<td>dnaK</td>
<td>dnaK</td>
<td>dnaK</td>
</tr>
<tr>
<td>ydh</td>
<td>ydh</td>
<td>ydh</td>
<td>ydh</td>
<td>ydh</td>
</tr>
<tr>
<td>flbA</td>
<td>flbA</td>
<td>flbA</td>
<td>flbA</td>
<td>flbA</td>
</tr>
<tr>
<td>lidh</td>
<td>lidh</td>
<td>lidh</td>
<td>lidh</td>
<td>lidh</td>
</tr>
</tbody>
</table>

### Stress in Lactococcus lactis IL1403

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Motility</th>
<th>30°C</th>
<th>40°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL1403</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>IL1403</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>IL1403</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>IL1403</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>
Proteins & Gene Expression

Expression of opp & opt Operons

Promoter comparison of opp operons in L. lactis ssp. lactis IL1403 and SSL135
Influence of Exogenous Peptide Pool

Influence of Nitrogen Starvation

Distribution of the Proteolysis Network
A Profile of Starters and Adjuncts

VFA Profile of Starters and Adjuncts

- Propionic
- Isobutyric
- Caproic
- Lauric

VFA Profile from Keto Acids

- Acetic
- Propanoic
- Isobutyric
- Butyric
- Isovaleric
- Caproic

Cell Growth & CHO Starvation

- pH 7.2
- pH 5.2
CHO & Energy

Viability

BCAA Degradation
Aminotransferase Comparison

Pyruvate Carboxylase (pyrA)
- Found in all lactococci sequenced
- 1,137 AA with 26 AA differences between the sequences (97.7% homology)
Lactococcal pyrA

Carboxylase Gene Expression

Induction of Thiol Production
Methionine & Enzymes for Thiol Production

Thiol Production - Whole Cells

VSC from Lactobacilli
Methionine Metabolism

Methionine and VSC via Cystathionine Lyase

ATase Expression Profiles with Sulfur
Sugar Metabolism in LAB

Metabolomic Visualization - CIBCyc

Pathway Webbing
Conclusions

- Large scale strain classification was done using the entire genome allowing rational strain selection for specific uses.
- Genomic analyses provide information about physiology and cell cycles that can be used to guide systematic selection of gene candidates for metabolic engineering.
- Cellular systems were found that were assembled into systems for directed control of flavor production.
Acknowledgments

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- Kathy Baldwin
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- U.S. Dept. of Energy
- Joint Genome Institute
- Fidelity Systems Inc.
- LABGC

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Thank You!

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