Dairy food safety (and microbiology) – Current issues

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Martin’s 2014 issues

• *Listeria, Listeria, and more Listeria*
  – And new FDA tools to allow better trace back
• Raw milk cheeses
• Coliforms
• Yeast and molds: developing capabilities at Cornell
Foodborne Illness Source Attribution Estimates for *Salmonella*, *Escherichia coli O157* (*E. coli O157*), *Listeria monocytogenes* (*Lm*), and *Campylobacter* using Outbreak Surveillance Data

Report

*Interagency Food Safety Analytics Collaboration (IF SAC) Project*

February 2015

This report was written by members of the Interagency Food Safety Analytics Collaboration (IF SAC) and includes contributions from others in the Centers for Disease Control and Prevention (CDC), the U.S. Food and Drug Administration (FDA), and the U.S. Department of Agriculture’s Food Safety and Inspection Service (USDA/FSIS).
There are distinct differences in the distributions of attribution point estimates across the 4 pathogens, as evidenced by the number of food categories responsible for roughly 75% of illnesses. Only 2 food categories accounted for the majority of estimated illnesses caused by *Campylobacter*, *E. coli* O157, and *Lm*, but 7 food categories accounted for a similar percentage of *Salmonella* illnesses. Seventy four percent of *Campylobacter* illnesses were attributed to Dairy (66%) and Chicken (8%), 82% of *E. coli* O157 illnesses were attributed to Beef (46%) and Vegetable Row Crops (36%), and 81% of *Lm* illnesses were attributed to Fruits (50%) and Dairy (31%). In contrast, *Salmonella* was more broadly attributed, as 77% of model-estimated illnesses were attributed to; Seeded vegetables (18%), Eggs (12%), Fruits (12%), Chicken (10%), Sprouts (8%), Beef (9%), and Pork (8%).
<table>
<thead>
<tr>
<th>Food category</th>
<th>Salmonella (N=597)</th>
<th>E. coli O157 (N=170)</th>
<th>Campy (N=161)</th>
<th>Lm (N=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef</td>
<td></td>
<td></td>
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<tr>
<td>Pork</td>
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<td></td>
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<tr>
<td>Chicken</td>
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</tr>
<tr>
<td>Turkey</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Other meat &amp; poultry</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Game</td>
<td></td>
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</tr>
<tr>
<td>Dairy</td>
<td></td>
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<tr>
<td>Eggs</td>
<td></td>
<td></td>
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<tr>
<td>Fish</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Other seafood</td>
<td></td>
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<td></td>
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<tr>
<td>Grains-beans</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Oils-sugars</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruits</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seeded vegetables</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sprouts</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetable row crops</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other produce</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CDC: Listeria Probably Contaminated WI Cheese During Production Process

BY NEWS DESK | APRIL 8, 2014

A Listeria outbreak in the Midwest linked to one death and a miscarriage likely was caused by contamination during the cheese-making process, according to a new report from the U.S. Centers for Disease Control and Prevention.

“Inspection of the cheese-making facility revealed that substantial sanitation deficiencies during the cheese-making process itself, after the milk was pasteurized, likely led to contamination,” the agency’s April 4 Morbidity and Mortality Weekly report stated.
Multistate Outbreak of Listeriosis Linked to Roos Foods Dairy Products

• Feb 2014

• A total of eight persons infected with the outbreak strain of Listeria monocytogenes were reported from two states: California (1) and Maryland (7)
  – 7 ill persons were hospitalized. One death was reported in California. Five of the illnesses (2 mother-newborn pairs and a newborn) were related to pregnancy.

• Whole-genome sequences of the Listeria strains isolated from Roos Foods cheese products were available after the recall and were found to be highly related to sequences of the Listeria strains isolated from the patients.
Oasis Brands, Inc. Cheese Recalls and Investigation of Human Listeriosis Cases

• On August 4, 2014, Oasis Brands, Inc. voluntarily recalled quesito casero (fresh curd) due to possible Listeria monocytogenes contamination after the pathogen was isolated from quesito casero. On October 6, 2014, Oasis Brands, Inc. recalled cuajada en hoja (fresh curd) after U.S. Food and Drug Administration (FDA) isolated Listeria monocytogenes from environmental samples collected from the production facility.

• On October 16, 2014, Oasis Brands, Inc. recalled various Lacteos Santa Martha and one HonduCrema brand cheese and dairy products.
Whole-genome sequences of the *Listeria monocytogenes* strains isolated from recalled quesito casero cheese produced by Oasis Brands, Inc. were found to be highly related to sequences of *Listeria* strains isolated from one person who became ill in September 2013 and four persons who became ill during June through October 2014.

- Illnesses occurred in Georgia (1), New York (1), Tennessee (2), and Texas (1).
- Four persons were hospitalized and one died.
- All ill persons reported consuming Hispanic-style soft cheese. Two persons who were able to answer questions about specific varieties of Hispanic-style soft cheeses reported consuming quesito casero, though neither could remember the brand.
Examples of *L. monocytogenes* ribotypes
DNA sequencing-based subtyping

Isolate 1 AACATGCAGACTGACGATTCGACGTAGGCTAGACGTTGACTG
Isolate 2 AACATGCAGACTGACGATTCGACGTAGGCTAGACGTTGACTG
Isolate 3 AACATGCAGACTGACGATTCGACGTAGGCTAGACGTTGACTG
Isolate 4 AACATGCA\textcolor{red}{TA}CTGACGATTCGACG\textcolor{red}{A}GGCTAGACGTTGACTG
Cost per Genome

- $100M
- $10M
- $1M
- $100K
- $10K
- $1K

Moore's Law

NIH National Human Genome Research Institute

genome.gov/sequencingcosts
Tip-dated maximum clade credibility tree based on SNP data for 47 Montevideo isolates
How to address *L. monocytogenes*

- Control strategies need to focus on preventing post kill step re-contamination of products (at plants as well as at retail)
  - Sanitary equipment design
  - Appropriately designed and implemented SSOPs (sanitation standard operating procedures)
  - Environmental testing
Innovation Center for US Dairy recommendations

- **Minimum**: PEM samples are collected at least weekly and include samples at eye level, below and above. A minimum of 30 swabs are taken per 50,000 sq. ft. per week: Raw:7, RTE/HH: 20, Zone 4: 3

- **Best of class**: PEM samples are collected at least weekly and include samples at eye level, below and above. Greater than 55 swabs are taken per 50,000 sq. ft. per week: Raw:14, RTE/HH 35, Zone 4: 6. As facility ages, swabbing increases to reflect increased risks.

Dairy processing and environmental pathogen sampling

• Considerable industry expertise in many larger companies

• Challenges remain:
  – How to develop and implement environmental pathogen control plans in small and medium facilities
  – How to scientifically validate sampling plans
• FSMA???
Project goals

• Develop and implement environmental pathogen control plans in small and medium facilities
  – Focus on *Listeria monocytogenes*, *Listeria* spp., and *Salmonella*

• Develop an approach to scientifically validate sampling plans for a given plant
  – Ultimate goal is to provide processors with data that can be used to demonstrate to regulatory agencies and others that a scientifically validated approach is used to control environmental pathogen sources
Validation sampling for *Listeria*

- Completed after at least six months of routine sampling
- Performed sampling of the “routine sites” (under supervision or by other individuals)
- Utilized sample size calculations to determine number of sample sites in validation
  - number of samples needed to have an 80% power to determine whether the *Listeria* spp. frequency observed during routine sampling was no more than twice the “true” frequency as estimated by the validation sampling
- Sites were selected to represent zone 2 to 4 sites that were deemed likely transfer points or niches
## Prevalence data – routine sampling and validation

<table>
<thead>
<tr>
<th>Plant ID</th>
<th>Prevalence (from routine)</th>
<th>Goal for validation</th>
<th>Prevalence (from validation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5.12% (34/664)</td>
<td>&lt;10.24%</td>
<td>1.33% (2/150)</td>
</tr>
<tr>
<td>E</td>
<td>11.97% (88/735)</td>
<td>&lt;23.95%</td>
<td>10% (6/60)</td>
</tr>
<tr>
<td>F</td>
<td>&lt;0.3% (0/334)</td>
<td>&lt;10%</td>
<td>6% (3/50)</td>
</tr>
<tr>
<td>G</td>
<td>8.33% (19/228)</td>
<td>&lt;16.67%</td>
<td>2.35% (2/85)</td>
</tr>
<tr>
<td>H</td>
<td>22.64% (24/106)</td>
<td>&lt;45.28%</td>
<td>8% (2/50)</td>
</tr>
<tr>
<td>J</td>
<td>0.94% (1/106)</td>
<td>&lt;10%</td>
<td>14% (7/50)</td>
</tr>
</tbody>
</table>
Summary and conclusions

• *L. monocytogenes* prevalence across small and medium artisan cheese processing plants was 1.89%

• *Salmonella* was only isolated from one plant; introduction from farm environment as likely source

• Validation sampling showed similar *L. monocytogenes* prevalence as routine sampling
  – For one plant, validation sampling suggested that routine sampling data underestimated true prevalence

• **Develop or review your environmental sampling plan now**
  – Cornell is available to help with validation of sampling plans
Martin’s 2014 issues

- *Listeria, Listeria, and more Listeria*
  - And new FDA tools to allow better trace back

- Raw milk cheeses

- Coliforms

- Yeast and molds: developing capabilities at Cornell
Joint FDA / Health Canada
2012
This predicted mean risk of invasive listeriosis from consuming a raw-milk soft-ripened cheese serving at random is 112, 96, 157 and 157 times higher than the mean risk for pasteurized-milk cheese for the Elderly, Pregnant women, Immunocompromised and General populations in the U.S., respectively.
<table>
<thead>
<tr>
<th>Alternative</th>
<th>Relative risk compared to Baseline, Pasteurized-milk cheese</th>
<th>Relative risk compared to Baseline, Raw-milk cheese</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Canada</td>
<td>United States</td>
</tr>
<tr>
<td>Pasteurized-milk cheese, Baseline</td>
<td>1 (Reference)</td>
<td>1 (Reference)</td>
</tr>
<tr>
<td>Raw-milk cheese, Baseline</td>
<td>53*</td>
<td>112</td>
</tr>
<tr>
<td>Raw-milk cheese, no 60 day aging condition</td>
<td>36</td>
<td>62</td>
</tr>
<tr>
<td>Raw-milk cheese if a process that leads to a 3 log_{10} reduction of the <em>L. monocytogenes</em> contamination in incoming milk is applied</td>
<td>7.4</td>
<td>11</td>
</tr>
<tr>
<td>Raw-milk cheese if the milk is tested in farm bulk tank, at every milking***</td>
<td>2.2</td>
<td>3.0</td>
</tr>
<tr>
<td>Raw-milk cheese if the cheese lots are tested***</td>
<td>0.080 = 1/12</td>
<td>0.134 = 1/7.4</td>
</tr>
</tbody>
</table>

* Interpretation: “The mean risk of invasive listeriosis from a serving of soft-ripened cheese made from raw milk is 53 times the mean risk of invasive listeriosis from a serving of soft-ripened cheese made from pasteurized milk for servings consumed by individuals from the Elderly population in Canada”.

** Ratios <1: the mean risk of the alternative is smaller than the reference; this example, which is the same as the preceding one, could read: “The mean risk of invasive listeriosis from a serving of soft-ripened cheese made from pasteurized milk is 0.019 times the mean risk of invasive listeriosis from a serving of soft-ripened cheese made from raw milk for servings consumed by individuals from the Elderly population in Canada”.

*** Volume tested: 25 ml for milk, 25 g composite made of 5 g from each of 5 cheeses at random for cheese lot; single *L. monocytogenes* detection probability: 0.75, test frequency: 100% of farms, tankers, dairy silos, cheese lots, respectively. Detected positive units are removed from production.
The FDA began a food sampling pilot aimed at aligning with the goals of FSMA, which mandates a risk-informed and preventive approach to food safety. Through one portion of this pilot, the FDA is seeking information on the rates of microbial contamination in raw milk cheese aged for 60 days. Upon completion, the FDA will have a statistically significant data set to help inform the agency’s decision making process. During this pilot process, the agency has been closely monitoring the product collections and analysis and stakeholder feedback in an effort to gather lessons learned and make course corrections, if necessary, to meet the goals of the pilot.
At this time, the FDA has collected and analyzed approximately 55% of the 1600 raw milk, aged cheese samples requested as part of this pilot. These samples comprise raw milk cheeses coming from Austria, Belgium, Bulgaria, Canada, Cyprus, Denmark, France, Germany, Ireland, Italy, Mexico, Netherlands, Nicaragua, Poland, Portugal, Spain, Swaziland, Switzerland, United Kingdom, and the U.S. We are considering ways to share more interim data with you and hope to provide more information in the next few months. We also, plan to issue a report at the completion of the pilot to share our findings and key lessons learned with industry.
At this time, we want to inform our industry partners about one such course correction that we are making with regard to non-toxigenic Escherichia coli (E. coli) policy in raw milk, aged cheese. One industry partner asked that we re-examine non-toxigenic E. coli policy and subsequent action taken by the agency on imported cheeses during the pilot. Upon re-examining the basis for the non-toxigenic E. coli in cheese compliance policy, FDA determined that adjustments to testing and regulatory action information were necessary. Specifically, the agency has updated instructions to its field laboratories to limit testing for non-toxigenic E. coli in raw milk cheese to five (5) subsamples. **Subsequent regulatory information was also modified to indicate that only lots with non-toxigenic E.coli levels in three or more subsamples of the five examined exceeding 10 MPN/g but less than 100 MPN/g would be considered violative. A single result above 100 MPN/g remains a violation.** Adjustments were made to facilities subject to import alert based on the policy changes. It is important to note that ten (10) subsamples will continue to be collected and analyzed for the Salmonella spp., Listeria monocytogenes and E. coli O157:H7. The regulatory information applied to these microbial findings will remain unchanged.
Cheese Standards

No federal standards for coliforms in cheese

Nontoxigenic *E. coli*

\[ n=5 \]
\[ c=2 \]
\[ m=10 \text{ MPN/g} \]
\[ M=100 \text{ MPN/g} \]
Coliforms

Coliforms=Coliform bacteria

Definition:
- Gram-negative
- Aerobic/Facultative anaerobic
- Rod shaped
- Non-sporulating
- Lactose utilizing
- Gas producing
- Acid producing
- 48h/32-37°C
Introduction

Coliforms=Coliform bacteria

- Very old concept
- Originally developed for water testing
- Originally developed to indicate fecal contamination
Introduction

**Coliforms=Coliform bacteria**

- Thermotolerant fecal coliform bacteria *(growth at 44.5 - 45.5°C)*
- Thermotolerant ubiquitous coliform bacteria *(growth at 44.5 - 45.5°C)*
- Psychrotolerant environmental coliform bacteria
What genera/species are covered by coliform definition?
*Serratia marcescens* has a long history in the church, as well as in microbiology. Numerous historical incidents recount that the blood red pigment produced by *S. marcescens* growing in bread was interpreted as a sign of blood. *S. marcescens* has a fondness for growth on polenta (corn meal mush), bread and communion wafers, where the pigmented, aged colonies have been mistaken for drops of blood (https://answersingenesis.org/biology/microbiology/serratia-marcescens-the-miracle-bacillus/)
Introduction

**Indicator** microorganism

**Indicator** microorganism

**Index** microorganism

**Surrogate** microorganism

**Marker** microorganism
Introduction

**Indicator microorganism**

Marker whose presence relates to the general microbiological condition of the food or environment (i.e., hygienic quality).

Strawn et al., 2013
Introduction

**Index** microorganism
Marker whose presence relates to the possible occurrence of **ecologically similar** pathogen(s).

Strawn et al., 2013
Indicators are never to be considered as Index
Introduction

**Surrogate microorganism**

Is a non-pathogenic microorganism which resembles certain pathogenic microorganism as much as possible and is used to represent behavior of this pathogen in the food production process (example: validation of a thermal treatment).
# Grade “A” Dairy Product - Standards

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Coliform</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Raw Milk:</strong></td>
<td></td>
</tr>
<tr>
<td>Producer</td>
<td>100,000 cfu/mL</td>
</tr>
<tr>
<td>Commingled</td>
<td>300,000 cfu/mL</td>
</tr>
</tbody>
</table>

 Applies to all milk used to make Grade “A” Dairy Products

<table>
<thead>
<tr>
<th>Pasteurized Milk:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk-Shipped</td>
<td>Not to exceed 10 cfu/mL</td>
</tr>
<tr>
<td>Bulk-Shipped</td>
<td>Not to exceed 100 cfu/mL</td>
</tr>
</tbody>
</table>
Grade “A” Dairy Product - Standards

Grade “A” Whey & Buttermilk  
Coliform  
Not to Exceed 10 cfu/mL

Yogurt  
Coliform  
Not to Exceed 10 cfu/mL
Butter

Quality Requirements

SPC, Not to Exceed 100,000 cfu/g
Proteolytic count, Not to Exceed 100 cfu/g
Yeast & mold count, Not to Exceed 20 cfu/g
Coliform count, Not to Exceed 10 cfu/g
Ice Cream

Coliform

Not to Exceed 10 cfu/g
Not to Exceed 20 cfu/g

Flavored (USDA)
(chocolate, nuts and fruits added)
Sampling plan

240 CHEESE SAMPLES

RAW / PASTEURIZED MILK

COW / SHEEP / GOAT

Hard
Semi-Hard
Soft
Blue
Brined
Fresh
Cheese samples positive for Coliforms

Number of samples positive for coliforms: 56/125 (45%)

30/125 (24%) samples above 10 cfu/g (NY state limit)
Distribution of positive results by cheese type

- 21/57 (37%) Raw Milk Cheese
- 9/68 (13%) Pasteurized Milk Cheese
- 18/74 (24%) Cow Milk Cheese
- 8/27 (30%) Goat Milk Cheese
- 4/19 (21%) Sheep Milk Cheese
## Results

<table>
<thead>
<tr>
<th>Cheese Description</th>
<th>Coliforms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pasteurized, Semi-hard, Sheep</td>
<td><em>L. monocytogenes</em></td>
</tr>
<tr>
<td>Raw milk, Semi-Hard, Cow</td>
<td><em>L. monocytogenes, L. seeligeri</em></td>
</tr>
<tr>
<td>Raw milk, Soft, Cow</td>
<td><em>L. seeligeri</em></td>
</tr>
<tr>
<td>Raw, Hard, Sheep</td>
<td><em>L. innocua</em></td>
</tr>
<tr>
<td>Pasteurized, Soft, Goat</td>
<td><em>L. innocua</em></td>
</tr>
<tr>
<td>Raw, Blue, Cow</td>
<td><em>L. innocua</em></td>
</tr>
<tr>
<td>Raw, Hard, Goat</td>
<td><em>stx 1</em></td>
</tr>
<tr>
<td>Raw, Hard, Sheep+Cow</td>
<td><em>stx1, stx2</em></td>
</tr>
</tbody>
</table>
Take home messages – coliform testing in cheeses

➢ Pasteurized milk cheeses
  ▪ Testing for post-pasteurization contamination of value for some products (e.g. cottage cheese, ricotta)
    • There may be better options than coliform testing
  ▪ Testing for coliforms of less value for other products (e.g., pasteurized surface ripened products)
Other possible Indicator Organisms

- **Coliform** vs. **EB** vs. **Total Gram-negative**
  - **Coliform** is required by PMO, long history of use as indicator organism, fast (24h), easy, non-comprehensive
  - **Enterobacteriaceae** is used predominantly in Europe, fast (24h), easy, includes more potential microorganisms of interest
  - **Total Gram-negative** testing, relatively fast (48h – room for improvement?), easy, most comprehensive
Take home messages

- Understand that coliforms are indicator organisms (i.e., for hygiene issues) not index organisms
  - Coliform testing in finished products cannot be used instead of pathogen control programs (e.g., environmental sampling; finished product testing of raw milk cheeses etc.)
Take home messages

- Review use of coliforms in your product
  - Understand specific state, federal, and international regulations for coliforms that are applicable to your product
  - Coliforms in your product → Indicator of PPC?
    - With what? → Pathogens?, Spoilage coliforms?, G-bacteria?

- For many companies, the main value of coliform testing may be to help reduce regulatory risk
Capri Sun

27,145 likes
407,794 shares

Rachel Phillips
WARNING TO ALL PARENTS: my friend Jennifer gave her son a caprisun Saturday and he told her it tasted funny. She took a sip and said it tasted like straight alcohol. Cut it open and it was nothing but mold inside. Evidently, this happens a lot. They say the lack of preservatives leaves them susceptible to fermentation. I will never let Delo drink another. FYI: this pouch expires April 2013 and we don't own a delorian to go "back to the future" so it wasn't expired. 😊 God bless, have a great day! — with Rachelle Lenise Bennett, Melissa Jones Stevens, Kate Watson and 39 others.

February 4, 2013 near Dexter, MO

Karen Dean Keega, Clotiel Young, Soraya Kay and 27,142 others like this.

407,794 shares

Capri Sun: Action Taken

Summary and conclusions

- *Listeria monocytogenes* is not going away
- Raw milk and raw dairy products continue to hurt the reputation of dairy
- May be time to re-assess use of coliform testing
- Cornell is developing expertise in Yeast and Mold tracking
We are here to help
Molecular Analysis and Clinical Significance of *Lactobacillus* spp. Recovered from Clinical Specimens Presumptively Associated with Disease

Raquel M. Martinez, Kristina G. Hulten, Uyen Bui, Jill E. Clarridge III

Department of Laboratory Medicine, University of Washington, Seattle, Washington, USA; Department of Pediatrics, Baylor College of Medicine, Houston, Texas, USA; Veterans Administration Puget Sound Health Care System, Seattle, Washington, USA

*Lactobacillus* spp. are part of the normal human flora and are generally assumed to be nonpathogenic. We determined the genotypic identification of > 100 *Lactobacillus* isolates from clinical specimens in the context of presumed pathogenic potential (e.g., recovered as the single/predominant isolate from a sterile site or at $\geq 10^5$ CFU/ml from urine). This study assessed the clinical significance and the frequency of occurrence of each *Lactobacillus* sp. We identified 16 species of *Lactobacillus* by 16S rRNA gene sequence analysis, 10 of which could not be associated with disease. While *Lactobacillus rhamnosus*, *Lactobacillus gasseri*, and *Lactobacillus paracasei* were associated with infections, *L. gasseri* was also a common colonizing/contaminating species. *Lactobacillus casei*, *Lactobacillus johnsonii*, and *Lactobacillus delbrueckii* were associated with at least one infection. Species commonly used in probiotic products (e.g., *L. rhamnosus* and *L. casei*) were identical, by 16S rRNA gene sequencing, to our isolates associated with disease. Human isolates of *Lactobacillus* spp. have differing site associations and levels of clinical significance.
Molecular Diagnosis of Endocarditis Due to *Lactobacillus casei* subsp. *rhamnosus*

Frédéric Wallet, Rodrigue Dessein, Sylvie Armand, and René J. Courcol

Laboratoire de Bactériologie-Hygiène, Hôpital A. Calmette, CHRU de Lille, Lille, France

We report a case of endocarditis due to *Lactobacillus casei* subsp. *rhamnosus*. The bacterium isolated from blood cultures and from valve tissue specimens was identified using both phenotypical analysis and DNA sequence analysis, which revealed that the rod profiles of the pathogens recovered from blood cultures and valve tissue specimens were the same.

*Clinical Infectious Diseases* 2002; 35:e117–9
Sporeformers – classification

• Microbes and sporeformers can be classified by growth temperatures:
  – Psychrotolerant: Can grow at cold temperatures (around 6 C), but optimum temperature is higher
  – Mesophilic: Optimum growth around 32 C; enumerated by “mesophilic spore count” (MSC)
  – Thermophilic: Optimum growth around 55 C; enumerated by “thermophilic spore count” (TSC)

• Two General Classes of sporeforming bacteria: Bacilli and Clostridia
Class Bacilli:
Notable Member:
- B. anthracis
- B. cereus

Common in Milk:
- Psychrotolerant: 
  - Paenibacillus spp.
  - B. weihenstephanensis
- Mesophiles: 
  - B. licheniformis
  - B. pumilus
  - B. sporothermodurans
  - B. subtilis
- Thermophiles: 
  - Anoxybacillus flavithermus
  - Geobacillus spp.

Class Clostridia:
Notable Members:
- C. botulinum
- C. perfringens
- C. difficile

Common in Milk:
- Mesophiles:
  - C. tyrobutyricum
  - C. beijerinckii
  - C. butyricum
  - C. sporogenes
Examples of microbiological requirements for dairy powder

- **Clostridium perfringens** <10/g
- Sulfur reducing clostridia <10/g
- Thermophilic sporeformers <10/g
- *Cronobacter*: <1/25 g
- *Enterobacter* <10/g
- Aerobic Plate Count
  - <10,000/g (@30°C)
  - < 5,000/g (@55°C)
- Coliform <1/10g
- *Salmonella* <1/25g
- Coagulase Positive Staph <10/g
- Yeast and Mold <30/g
Previous Study: Thermophilic Sporeformers in Whole Milk Powder Processing

Vol 60, No 2 May 2007

Figure 4 Thermophilic spore counts of samples taken every 2 h over an 18-h run period of two standard whole milk powder runs (runs 5 and 6). Each point represents the mean of triplicate counts.

Plant 2 – MSC

Log Bacterial Count

<10 cfu/g >0.2 cfu/g

25%  0%  25%  0%  50%  12.5%  0%  0%  50%

Beginning  Middle  End  Beginning  Middle  End  Beginning  Middle  End

Raw
n=12

WIP
n=48

Finished
n=12
Plant 2 – TSC

Log Bacterial Count

<10 cfu/g >0.2 cfu/g

0% 0% 25%

12.5% 6.25% 0%

0% 25% 0%

Raw  n=12

WIP  n=48

Finished  n=12
Sequencing-based DNA fingerprinting

| Isolate 1 | AACATGCAGACTGACGATTCGACGTAGGCTAGACGTTGACTG |
| Isolate 2 | AACATGCAGACTGACGATTCGATTCGTCGTAGGCTAGACGTTGACTG |
| Isolate 3 | AACATGCAGACTGACGATTCGACGATTCGACGTAAGGCTAGACGTTGACTG |
| Isolate 4 | AACATGCAATACTGACGATTCGACGATTCGACGATTCGACGATTGACTG |
### Initial molecular subtyping data – plant 2

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Species isolated from powder plants

- *Bacillus licheniformis* (AT 1, AT 6, AT 9 and AT 169) n=48
- *Anoxybacillus* (AT 353) n= 14
  - 12 additional *Anoxybacillus* isolates identified using 16S sequencing
- *Bacillus megaterium* (AT 351) n=3
- *Geobacillus* (n=19), *Aeribacillus* (n=3), *Bacillus* spp. (n=2) and *Ureibacillus* (n=1) identified using 16S sequencing
Comparison with subtype frequency in raw milk

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Location of samples positive for *Anoxybacillus* (AT 353)
Anoxybacillus isolated from 1 sample cycle, 2 sample cycles or 3 sample cycles
Location of samples positive for *Bacillus licheniformis* (AT 1)

- Raw Milk Receiving
  - Raw Milk Silo
    - Balance Tank (Whole)
      - Regenerator
    - Balance Tank (Skim)
      - Preheater
      - Preheater
      - HTST (Past. & Regen)
        - Evaporator (Effects 1-4)
          - Evaporator (Effects 5-7)
            - Dryer Feed Tank
              - Preheater
              - Spray Dryer
              - Collection Silo
              - Sifter
              - Bagging