THE BOVINE MILK MICROBIOOME

Mark McGuire
FLOW OF MILK FROM A FARM TO PROCESSOR
HOW TO ASSESS PRESENCE OF BACTERIA?

Culture-**dependent** methods
- Relies on specific culture media conditions for specific bacterial genera/species (mostly pathogenic)
- Only see what you’re looking for

Culture-**independent** methods
- Rely on molecular techniques and specific primers
- Identification of bacterial taxa often related to genetic variation in 16S rRNA gene

Using a combination of both types of methods is very powerful.

Image: Rodolfo Parulan Photography


“Hypervariable”
# COMMON BACTERIA GROWN FROM BOVINE MILK

<table>
<thead>
<tr>
<th>Species</th>
<th>Colony forming units per ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactococcus</td>
<td>$8.2 \times 10^1$ to $1.4 \times 10^4$</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>$1.4 \times 10^1$ to $1.5 \times 10^4$</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>$1.0 \times 10^2$ to $3.2 \times 10^4$</td>
</tr>
<tr>
<td>Leuconostoc</td>
<td>$9.8 \times 10^1$ to $2.5 \times 10^3$</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>$2.6 \times 10^1$ to $1.6 \times 10^3$</td>
</tr>
</tbody>
</table>

Photos from Janet Williams

COMPARISON OF METHODS WITH MILK

- Compared various papers in the literature that used culture dependent (CD) or independent (next generation DNA sequencing; NGS) methods
- Not necessarily directly comparable
  - Assumes similarity across all samples within a species
  - Breadth of assessment limited in some cases

IS THERE A DIFFERENCE BEFORE AND AFTER MILKING?

- 15 cows
- 2 quarters
- Used aseptic method
- V1-V2 16S rRNA

Reynolds, Hunt, Williams and McGuire, unpublished
SOURCES OF BACTERIA IN RAW MILK

- Irish dairy
- Sampled bedding, feces, grass, silage, soil, teat swabs and compared to individual cow and bulk tank milk
- V3-V4 16S rRNA

ARE THE BACTERIA FROM THE TEAT?

Needle aspirate through surgically scrubbed teat wall
- 15 cows
- 2 teats

Reynolds, Hunt, Williams and McGuire, unpublished

University of Idaho
College of Agricultural and Life Sciences
DOES BACTERIAL CULTURE ASSESS THE BIODIVERSITY PRESENT?


- Culture conditions are tailored to a pathogen of interest
- Conditions cannot meet the requirements of all microbes present
  - Nutrients; aerobic vs. anaerobic growth
  - Biological functions of the host
  - \(<10^4\) colony forming units/ml; culture 0.01 ml of milk; chance of growth?

Clearly there is a need for a method to identify bacteria without culture!
BOVINE MILK MICROBIAL COMMUNITY IN MILK WITHOUT GROWTH

- 3 farms
- 10 cows
- V1-V2 16S rRNA

Clinical mastitis without growth vs. healthy within same cow

Two quarters with low somatic cell count (LSCC)

BOVINE MILK MICROBIAL COMMUNITY BY SOMATIC CELL COUNT

- 2 farms
- 177 samples
- V1-V2 16S rRNA

Healthy
1 = < 20,000 cells/ml
2 = 21,000 to 50,000 cells/ml
3 = >50,000 cells/ml

Subclinical Culture positive
4 = >400,000 cells/ml

5 = Mastitis culture negative

BOVINE MILK MICROBIAL COMMUNITY BY SOMATIC CELL COUNT

- 2 farms
- 103 cows by quarter
- V1-V3 16S rRNA

Somatic Cell Count (SCC)
Low = < 200,000 cells/ml
Medium = 200,000 to 400,000 cells/ml
High = >400,000 cells/ml

Brooker and McGuire, unpublished
CLINICAL MASTITIS IN COWS

Reynolds, Hunt, Williams and McGuire, unpublished
IS THERE A DIFFERENCE BY FARM?

- Bulk tank milk from 19 farms over 2 months

# BACTERIA RELATED TO MILK QUALITY

<table>
<thead>
<tr>
<th>Higher Somatic Cell Count</th>
<th>Higher Standard Plate Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corynebacterium</td>
<td>Acinetobacter</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>Enterobacteriaceae</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>Corynebacterium</td>
</tr>
<tr>
<td>Coxiella</td>
<td>Streptococcus</td>
</tr>
<tr>
<td>Arthrobacter</td>
<td></td>
</tr>
<tr>
<td>Lactococcus</td>
<td></td>
</tr>
</tbody>
</table>
VARIATION IN PREDOMINANT BACTERIA IN RAW MILK

- Two dairy production facilities in California
- 899 tanker truckers
- V4 16S rRNA

Kable et al (2016) mBios 7:e00836-16
CORE RAW MILK MICROBIOME FROM TANKER TRUCKS

- Found in all 899 tanker trucks
- Also 17 others between 0.25 and 0.97 %

<table>
<thead>
<tr>
<th>Family or Genus</th>
<th>% Relative abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus</td>
<td>6.51</td>
</tr>
<tr>
<td>Unidentified Clostridiales</td>
<td>6.33</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>5.45</td>
</tr>
<tr>
<td>Unidentified Ruminococcaceae</td>
<td>4.35</td>
</tr>
<tr>
<td>Corynebacterium</td>
<td>3.70</td>
</tr>
<tr>
<td>Turicibacter</td>
<td>2.45</td>
</tr>
<tr>
<td>Unidentified Peptostreptococcaceae</td>
<td>2.22</td>
</tr>
<tr>
<td>Unidentified Lachnospiraceae</td>
<td>2.03</td>
</tr>
<tr>
<td>Clostridium</td>
<td>1.47</td>
</tr>
<tr>
<td>Unidentified Clostridiaceae</td>
<td>1.33</td>
</tr>
<tr>
<td>Acinetobacter</td>
<td>1.19</td>
</tr>
<tr>
<td>Unidentified Planococcaceae</td>
<td>1.09</td>
</tr>
</tbody>
</table>

Kable et al (2016) mBios 7:e00836-16
South Dakota and Minnesota

PRESENCE OF PATHOGENS

- 131 dairy herds
- Bulk tank milk samples collected using National Mastitis Council methods

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Percent of samples</th>
</tr>
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<tbody>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td>9.2</td>
</tr>
<tr>
<td>Shiga toxin-producing <em>Escherichia coli</em></td>
<td>3.8</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>4.6</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>6.1</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>6.1</td>
</tr>
</tbody>
</table>

26.7% of samples contained one or greater pathogens

Pennsylvania

**PRESENCE OF PATHOGENS**

- 248 dairy herds
- Bulk tank milk samples collected using National Mastitis Council methods

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Percent of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td>2</td>
</tr>
<tr>
<td>Shiga toxin-producing <em>Escherichia coli</em></td>
<td>2.4</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>2.8</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>6</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>1.2</td>
</tr>
</tbody>
</table>

13% of samples contained one or greater pathogens

NAHMS Dairy 2002

PRESENCE OF PATHOGENS

• 861 bulk tank milk samples
• 21 states

<table>
<thead>
<tr>
<th>Pathogen (cultured)</th>
<th>Percent of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>2.0</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>2.4</td>
</tr>
<tr>
<td>Coliforms</td>
<td>95.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pathogen (PCR)</th>
<th>Percent of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella enterica</em></td>
<td>11.8</td>
</tr>
</tbody>
</table>

NAHMS Dairy 2007

PRESENCE OF PATHOGENS

• 536 bulk tank milk samples
• 519 in-line milk filters
• Used PCR for *S. enterica* and pathogenic *E. coli*
• Used culture for *L. monocytogenes*

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Percent of operations</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>7.1</td>
</tr>
<tr>
<td><em>Salmonella enterica</em></td>
<td>28.1</td>
</tr>
<tr>
<td>Shiga toxin-producing <em>Escherichia coli</em></td>
<td>15.2 (bulk tank milk)</td>
</tr>
<tr>
<td></td>
<td>51.0 (filter)</td>
</tr>
</tbody>
</table>

PASTEURIZED MILK ORDINANCE (PMO)

- Total bacteria count leaving farm is <100,000 cfu/ml
- Total bacterial count in commingled milk at the processor is <300,000 cfu/ml
- Total somatic cell count is <750,000 cells/ml
PASTEURIZED MILK ORDINANCE

- All raw milk shall be cooled to 10 °C within 4 hours of 1st milking and to 7 °C or less, within 2 hours after completion
- All farm bulk milk tanks have approved temperature-recording device
IS RAW MILK SAFE?

“Usually, the bacteria in milk are harmless, and if this were always true there would be no reason to cool milk, except to delay souring. There is, however, no way for the dairy operator or regulating officer to be absolutely sure that no disease bacteria have entered the milk, even though observance of the other Items of this Ordinance will greatly reduce this likelihood. The likelihood of transmitting disease is much increased when the milk contains large numbers of disease bacteria. Therefore, it is extremely important for milk to be cooled quickly, so that small numbers of bacteria, which may have entered the milk, will not multiply.”

Grade “A” Pasteurized Milk Ordinance 2015 Revision, p. 59
IS RAW MILK SAFE?

- Risk associated with consumption of unpasteurized cow’s milk and cheese
- Data from the National Outbreak Reporting System (US, 2009-2014)
- Disease related to Shiga toxin-producing *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes*, and *Campylobacter* spp. from dairy products
- 760 illnesses per year; 22 hospitalizations per year
- Unpasteurized milk and cheese consumed by 3.2% and 1.6% of the population, respectively, caused 96% of illnesses from contaminated dairy products

IS RAW MILK SAFE?

Unpasteurized dairy products caused 840 times more illness and 45 times more hospitalizations than pasteurized dairy products!

SUMMARY – MILK IS NOT STERILE

Wide variety of bacteria present in milk
  - At low concentrations $<10^5$ cfu/ml
Large variation in bacterial communities among cows, across farms, through processing
  - What are the major factors influencing the variation?

Bacterial community changes with mastitis

Pathogenic bacteria are present in milk
  - Factors driving presence have not been clearly identified.
  - Also impacted throughout the milking system.

Pasteurization is a great tool to minimize risk of foodborne illness from milk!
THANK YOU!

Michelle McGuire
Janet Williams
Katherine Hunt Yahvah
Sarah Brooker
Susan Reynolds
Larry Fox
SRA 2017 Advancing the Science Webinar Series Continues:

Microbiota Informing Next-Generation Risks & Benefits

1. Rodney Dietert (Cornell University), *Protecting the Human Superorganism* (January 24)


4. Anne Mendelson (Culinary Historians of New York), *History of the Continuing Milk Wars* (July 18, 5:30 EDT)

A panel of microbial risk assessors will deliberate evidence of microbiota influences on risk and benefit for fresh unprocessed and pasteurized milk (*October, TBD*) prior to SRA workshop and Round Table Panel Symposium (*December 10-14, Arlington, VA*).