Spore concerns and testing in dairy: Reducing variability and establishing standards

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Overview

• What are they and why do we care
• Raw to finished product contaminants
• Testing methods matter
Spores: Defense strategies for harsh environmental conditions

- Endospores are formed under stressful environmental conditions
- Allow bacteria to persist in environments that would otherwise be lethal
  - **Heat (pasteurization)**
  - Drying (powders)
  - Radiation
  - Sanitizers
- Under favorable conditions, spores return to a metabolically active state
Sporulation and germination – The key to the spore challenge in dairy foods
Dairy relevant sporeforming bacteria

Fluid Milk

- Psychrotolerant Sporeformers
  - *Paenibacillus*
  - *Viridibacillus*
  - *Bacillus weihenstephanensis*
Dairy relevant sporeforming bacteria

- Anaerobic Butyric Acid Bacteria (BAB)
  - *Clostridium tyrobutyricum*
  - *C. butyricum*
  - *C. sporogenes*

- Historical association with high levels of BAB in silage – raw milk used for certain cheeses in Switzerland cannot come from cows fed silage
- Penalties used to regulate BAB in raw milk used for cheesemaking in the Netherlands
Dairy relevant sporeforming bacteria

- Mesophilic and Thermophilic Sporeformers
  - *Bacillus licheniformis*
  - *Geobacillus*
  - *Anoxybacillus*

### Table 1. Typical example of Spore-Formers Specifications-Infant Formula for Export

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Range of limit (CFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesophilic plate count</td>
<td>Max: &lt;5,000 to 10,000/g</td>
</tr>
<tr>
<td>Thermophilic plate count</td>
<td>Max: &lt;5,000/g</td>
</tr>
<tr>
<td>Aerobic spore, mesophilic</td>
<td>&lt;500 to &lt;1,000/g</td>
</tr>
<tr>
<td>Aerobic spore, thermophilic</td>
<td>&lt;500 to &lt;1,000/g</td>
</tr>
<tr>
<td><em>Bacillus Cereus</em></td>
<td>Max: &lt;100/g</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>Max: negative /0.1g</td>
</tr>
<tr>
<td>Sulfite reducing <em>clostridia</em></td>
<td>Max: &lt;10 to &lt;25/g</td>
</tr>
</tbody>
</table>

*Source: USDEC*

### Table 2. Typical example of Spore-Formers-Recombined and UHT for Export

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Limit (CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesophilic plate count</td>
<td>&lt;10,000</td>
</tr>
<tr>
<td>Thermophilic plate count</td>
<td>&lt;5,000 to &lt;10,000</td>
</tr>
<tr>
<td>Aerobic spore, thermophilic</td>
<td>&lt;500 to 2,000</td>
</tr>
<tr>
<td>Thermoresistant spore count</td>
<td>&lt;500</td>
</tr>
</tbody>
</table>

*Source: USDEC*
Sporeforming bacteria- Farm to finished product contaminants
Sporeforming bacteria in dairy products
Sporeforming bacteria in dairy products
Mesophilic spores represent the highest proportion of sporeforming bacteria in raw milk.
Spores are present in low levels in raw milk
Bacillus licheniformis accounts for nearly half of spores in bulk tank raw milk.
Sources of sporeforming bacteria

- Soil
- Feed
- Bedding
Sources of sporeforming bacteria

- Manure
- Drinking water
Sources of sporeforming bacteria

- Air
- Equipment swab
- Filter sock
- Individual cow milk
- Teat swab
- Towel
- Wash water
- Bulk tank milk
Sporeforming bacteria- Farm to finished product contaminants
Sporeforming bacteria in processing environments

**Post-processing contamination**

- Inadequate cleaning and sanitization leading to biofilm formation
- Indicators of PPC in fluid milk include Gram-negative bacteria (e.g., *Pseudomonas*)
- Sporeformers can also be introduced into processed dairy products through biofilm formation
Sporeforming bacteria in processing environments

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.
Testing methods matter

Spore Pasteurization
80°C (176°F)/12m
- PSC
  6°C (42.8°F)/10d incubation
- MSC
  32°C (89.6°F)/48h incubation
- TSC
  55°C (131°F)/48h incubation

Highly Heat Resistant Spore Treatment
100°C (212°F)/30m
- HHR-MSC
  32°C/48h incubation
- HHR-TSC
  55°C/48h incubation

Specially Heat Resistant Spore Treatment
106°C (222.8°F)/30m
- STSC
  55°C/48h incubation

Butyric Acid Bacteria (BAB)
75°C (167°F)/15m
- BAB Most Probable Number (MPN)
  37°C (98.6°F)/7d incubation
Spore populations in raw milk vs. dairy powders

Table 3. Spore genera and species isolated from samples from 4 different dairy powder processing plants and bulk tank raw milk from 33 farms

<table>
<thead>
<tr>
<th>Genus and species</th>
<th>SW</th>
<th>NDM</th>
<th>AW</th>
<th>WPC-80</th>
<th>BT raw milk</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aeribacillus pallidus</em></td>
<td>0.6</td>
<td>2.1</td>
<td>0.0</td>
<td>4.3</td>
<td>1.3</td>
<td>1.7</td>
</tr>
<tr>
<td><em>Anoxybacillus</em> spp.</td>
<td>38.0</td>
<td>9.4</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>10.6</td>
</tr>
<tr>
<td><em>Bacillus cereus</em> group</td>
<td>17.2</td>
<td>1.3</td>
<td>0.0</td>
<td>13.3</td>
<td>4.7</td>
<td>7.6</td>
</tr>
<tr>
<td><em>Bacillus clausii</em></td>
<td>0.3</td>
<td>2.1</td>
<td>5.9</td>
<td>0.5</td>
<td>5.2</td>
<td>2.8</td>
</tr>
<tr>
<td><em>Bacillus licheniformis</em></td>
<td>6.3</td>
<td>44.9</td>
<td>73.5</td>
<td>31.9</td>
<td>47.4</td>
<td>36.5</td>
</tr>
<tr>
<td><em>Bacillus pumilus</em></td>
<td>0.0</td>
<td>6.4</td>
<td>0.5</td>
<td>0.5</td>
<td>9.1</td>
<td>5.2</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> s.l.</td>
<td>2.4</td>
<td>1.1</td>
<td>2.7</td>
<td>4.9</td>
<td>4.9</td>
<td>3.0</td>
</tr>
<tr>
<td><em>Bacillus thermoamylolovorans</em></td>
<td>0.0</td>
<td>2.1</td>
<td>2.9</td>
<td>16.0</td>
<td>6.4</td>
<td>5.1</td>
</tr>
<tr>
<td><em>Geobacillus</em> spp.</td>
<td>27.1</td>
<td>16.0</td>
<td>18.1</td>
<td>0.2</td>
<td>0.2</td>
<td>12.1</td>
</tr>
<tr>
<td><em>Lysinibacillus</em> spp.</td>
<td>0.0</td>
<td>1.3</td>
<td>0.0</td>
<td>1.1</td>
<td>3.2</td>
<td>1.7</td>
</tr>
<tr>
<td><em>Paenibacillus</em> spp.</td>
<td>0.9</td>
<td>3.5</td>
<td>0.1</td>
<td>0.5</td>
<td>4.2</td>
<td>2.8</td>
</tr>
<tr>
<td><em>Ureabacillus</em> spp.</td>
<td>0.3</td>
<td>1.1</td>
<td>1.6</td>
<td>0.3</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Other*2</td>
<td>0.9</td>
<td>1.1</td>
<td>5.9</td>
<td>0.0</td>
<td>2.4</td>
<td>1.5</td>
</tr>
<tr>
<td>Other <em>Bacillus</em> spp.*</td>
<td>6.0</td>
<td>7.5</td>
<td>8.8</td>
<td>9.6</td>
<td>10.8</td>
<td>8.7</td>
</tr>
<tr>
<td>Total isolates, n</td>
<td>332</td>
<td>374</td>
<td>34</td>
<td>188</td>
<td>595</td>
<td>1,523</td>
</tr>
</tbody>
</table>

1SW = sweet whey; NDM = nonfat dry milk; AW = acid whey; WPC-80 = whey protein concentrate 80; BT = bulk tank.
2Other includes *Brevibacillus* spp., *Oceanobacillus* spp., *Sohbacillus* spp., *Sporosarcina* spp., and *Terrabacillus* spp.
3Other *Bacillus* spp. includes species *aerophilus*, *coagulans*, *firmus*, *gibsonii*, *hornbeckiae*, *megaterium*, *murulis*, *nealsoni*, *neutrophilus*/*sojae*, *safen-sis*, *smithii*, and other *Bacillus* spp.
Spore testing challenges

• Low level contamination, stochastic variation
• Population diversity
• Germination and recovery variability on different media
• “Countability”
  • Swarmers, spreaders and microaerophiles, oh my!

*What is the “right” method?*
*Must consider implementation*
Spore enumeration
Milk powder A, SP TSC on BHI, Pour Plate

Milk powder A, SP TSC on BHI, Spread plate

Milk powder A, SP TSC on BHI, Pour Plate with overlay
Plating type is a source of variation
Summary and moving forward

• Spores are ubiquitous and must be controlled at the farm and processing level

• Sample type (raw milk, powder, etc), Media type, plating type, heat treatment and incubation parameters all play an important role in spore method variation
  • Populations in different dairy products can vary widely contributing to variation seen between spore testing methods

• Identify a set of methods and parameters that provide standards for spore testing
Acknowledgments

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- NDC
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