USING METAGENOMICS FOR INDUSTRIAL HYGIENE RISK ASSESSMENT
Exploration of Molecular Tools for Evidence-based Hygiene Verification in Low Moisture Food Manufacturing Environments

- **WGS**
  - Reactive
    - Incident management
    - Root Cause analysis
    - Corrective Action verification

- **WGS**
  - Preventive
    - GMP Verification

- **16S Metagenomics**
  - Preventive
    - Cleaning validation
    - Hygienic Engineering verification
Assessment Strategy

Understand the Microbiome
- Using 16S Metagenomics: What does the normal microbiome of low moisture food factory environment look like?

Develop Reporting Tools
- ...that will be understood intuitively by the relevant personnel.

Examples
- Provide an example of the use of Metagenomics in process hygiene and equipment design assessment
Exploring the Microbiome of Various Relevant Factory Environments

- Firmicutes and Proteobacteria are > 70% of the flora
- Proteobacteria include known efficient biofilm formers (e.g., *Pseudomonas, Moraxella, Acinetobacter*...) 
- *Actinobacteria* and *Bacteroidetes* are the next highest in abundance
- The other members have a less defined interpretation from a hygiene perspective
Drill Down is Always Possible

Proteobacteria

Firmicutes
### Determining the Base Line

**PCA: Analysis of indicator/target positive and negative swabs**

<table>
<thead>
<tr>
<th>+ I (n=93)</th>
<th>− I (n=22)</th>
<th>+ T (n=8)</th>
<th>− T (n=107)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proteobacteria</strong></td>
<td><strong>Bacilli</strong></td>
<td><strong>Proteobacteria</strong></td>
<td><strong>Bacilli</strong></td>
</tr>
<tr>
<td><strong>Gamma-Proteobacteria</strong></td>
<td><strong>Bacteriodales</strong></td>
<td><strong>Gamma-Proteobacteria</strong></td>
<td><strong>Lactobacillus</strong></td>
</tr>
<tr>
<td><strong>Moraxellaceae</strong></td>
<td><em>Bacillus coagulans</em></td>
<td><strong>Pseudomonades</strong></td>
<td><strong>Fervidobacteraceae</strong>**</td>
</tr>
<tr>
<td><strong>Pseudomonades</strong></td>
<td></td>
<td><strong>Moraxellaceae</strong></td>
<td><strong>Thermotogae</strong>**</td>
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<tr>
<td><strong>Enhydrobacter</strong></td>
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<td><strong>Enhydrobacter</strong></td>
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<tr>
<td><strong>Acinetobacter</strong></td>
<td></td>
<td><strong>Rhodobacteriales</strong></td>
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</tr>
<tr>
<td></td>
<td></td>
<td><strong>Acinetobacter</strong></td>
<td>**<strong>Thermophilic bacteria</strong></td>
</tr>
</tbody>
</table>

**Are we detecting mixed species biofilms?**

**EB was not a significant differentiator**
Simplifying the Taxonomy

Credit: FDA
Definition of Risk: Low Moisture Food Manufacturing
“conditions for high relative abundance...”

The presence of high levels of an indicator microorganism is a good indication of conditions that may support the presence and potential for growth of a target microorganism. However, testing for indicator alone is not sufficient, since even low levels do not guarantee absence of the target microorganism.

I propose that for LMF plants “The presence of a high relative abundance of Proteobacteria is a good indication of conditions that may support the presence and potential for growth of target microorganism (i.e. biofilms).

“Risk” is defined as a relative abundance of Proteobacteria > Firmicutes.
Determining Risk in Cleaning Methods
High Taxonomic Level Scouting for at Risk Environments

Relative abundance (% DNA)

- **Dry**: Firmicutes > Proteobacteria
- **Wet**: Proteobacteria > Firmicutes
Wet Cleaning

Pre clean WWE
F: 40.3 - P: 41.2 = 1.0

Pre clean WWE-env
F: 17.3 - P: 72 = 4.2

Post clean WWE
F: 19.5 - P: 69.7 = 3.6

Post clean WWE-env
F: 11.2 - P: 82.1 = 7.3
Dry Cleaning

i 0/17 / t 0/50

Pre clean DCE
F:40.4 - P:22.1 = 0.5

Post clean DCE
F:30.6 - P:44.9 = 1.5

Pre clean DCE-env
F:59.5 - P:20.6 = 0.3

Post clean DCE-env
F:46.7 - P:33.5 = 0.7
If water is the driver of risk, why do Metagenomics?
Understanding the Microbiome of a complex line with difficult access
The trend is clear .... The risk is proximal
(Hidden) high moisture conditions or mostly dry low moisture conditions can be recognized by analyzing the microbiome....
Preliminary Conclusions

16S Metagenomics is useful in assessing the relative risk with a specific unit operation’s hygiene design and management.

Risk is defined as presence of an environment conducive to microbial biofilms.

This tool can be used in hygienic engineering and cleaning validation and verification.

This tool is likely not practical yet for routine use in environmental monitoring.