Molecular-based Surveillance in Food Manufacturing Facilities Using Next Generation Sequencing Techniques and Software

Cian O’ Mahony, Chief Science Officer, Creme Global
Seamus Fanning, UCD
IAFP Europe 2016
BACKGROUND
Background

Phylogenetic Profiles of In-House Microflora in Drains at a Food Production Facility: Comparison and Biocontrol Implications of *Listeria*-Positive and -Negative Bacterial Populations

Edward Fox, Katie Solomon, John Moore, Patrick Wall and Séamus Fanning

Applied and Environmental Microbiology, 2014, 80(11):3369
Fox et al.

Study

- Examined interaction between Listeria species and other microorganisms
- Data gathered from drains in a meat production facility
- Four drains examined – two Listeria positive, two Listeria negative
- Microbiome of drains examined, and overall diversity analysed
Microbiome Analysis

Negative

Positive

Classes

Families

- Lachnospiraceae
- Pseudomonadaceae
- Rikenellaceae
- Enterobacteriaceae
- Halomonadaceae
- Bacillaceae
- Ruminococcaceae
- Prevotellaceae
- Flavobacteriaceae
- Comamonadaceae
- Unclassified and Others

- Gammaproteobacteria
- Clostridia
- Bacteroidia
- Bacilli
- Alphaproteobacteria
- Deltaproteobacteria
- Betaproteobacteria
- Actinobacteria
- Fusobacteria
- Unclassified and others
●: *Listeria*-negative drains, overall diversity; ○: *Listeria*-negative drains, classified OTUs only; ■: *Listeria*-positive drains, overall diversity; □: *Listeria*-positive drains, classified OTUs only.
Results

Microbiome

- 21 families found only in Listeria positive drains
- Janthinobacterium, Prevotella, and Pseudomonas all associated with higher abundance in Listeria-negative drains

Co-Culturing

- Presence of J. Lividum reduced the attachment and biofilm growth of L. monocytogenes
- E. Gallinarum caused significant increase in L. monocytogenes attachment and biofilm formation
Suggested Control Strategy

1. Identify drains colonised by *Listeria*, and drains free of *Listeria*

2. Determine microbiome and profile the local microbial communities

3. Determine potential protagonists and antagonists

4. Isolate key in-house protagonists and antagonists

5. Examine the effect of these strains on *Listeria* attachment and biofilm formation

6. Seed drains with non-pathogenic ‘in-house’ bacterial species that show inhibition of *Listeria* colonisation
Questions

- Can we quantify the relationship between the microbiome of a manufacturing facility and specific bacteria of interest?
- Can we quantify/predict what would be an effective biocontrol strategy?
- Can we examine specific bacteria using WGS in parallel with the microbiome?
- What traits can be identified using WGS and monitored over time and space?
THE S.A.F.E. PROJECT
The SAFE Project

- Sequencing Alliance for Food Environments
- Three year project in collaboration with:
  - University College Dublin Centre for Food Safety (UCD-CFS)
  - Five postdoctoral researchers
  - Creme Global
  - Six industry partners
Project Goals

1. Develop a pipeline of data using genome-based technology (WGS & WMS)
2. Develop mathematical models of safety and quality
3. Link gene-based information to phenotype for tracking/tracing of bacteria of interest
4. Use phenotypic data to highlight potential solution strategies to food safety and quality
5. Provide software tools to improve food safety & quality decision making steps
Tasks

Task 1
- Sampling plan design

Task 2
- Whole Genome Sequencing
- Microbiome Characterisation

Task 3
- Bioinformatic analysis

Task 4
- Production solution scenarios

Task 5
- Modelling and Software
Industry Partners

KERRY

Dairygold

glanbia

MeadJohnson Nutrition

Nutrition Supplies

Dawn Farms
Sampling Plan

Molecular Surveillance

- Multiple Times
- Sequenced Isolates

Multiple Zones

- Microbiome
Sampling Plan

Monitored over two years in six facilities

Molecular Surveillance

- Multiple Times
- Multiple Zones

- Sequenced Isolates
- Microbiome
Sampling Plan

Monitored over **two** years in **six** facilities

- Molecular Surveillance
  - Multiple Times
  - Multiple Zones
    - Sequenced Isolates
    - Microbiome
Sampling Data

WGS
- Indicators of quality and safety
- Identifiable Traits
- Changes over time and space

Metagenomics
- Microbiome of Facility
- Complete bacterial ecology
- Changes over time and space
Sampling Data

WGS
- Indicators of quality and safety
  - Identifiable Traits
  - Changes over time and space

Metagenomics
- Microbiome of Facility
  - Complete bacterial ecology
  - Changes over time and space

Analysed separately and in parallel
# Whole Genome Sequencing

## Bacteria of interest to be sequenced

<table>
<thead>
<tr>
<th>Safety</th>
<th>Quality</th>
</tr>
</thead>
</table>

## Dedicated Strain Database

<table>
<thead>
<tr>
<th>Track and trace back</th>
<th>Associated metadata</th>
<th>Link to phenotypes</th>
<th>Thermal-resistance profiles</th>
</tr>
</thead>
</table>

## Control Strategies

| Assessment of in-house strategies | Explore bio-control strategies |
Final Output

WGS

Sampling Plan

Metagenomics

Cloud Computing
SOFTWARE PLATFORM
SAFE

EMAIL

PASSWORD

LOGIN
## Samples

<table>
<thead>
<tr>
<th>Year</th>
<th>Month</th>
<th>Area</th>
<th>Sample Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017</td>
<td>Feb</td>
<td>Dry room</td>
<td>12345</td>
</tr>
<tr>
<td>2017</td>
<td>Feb</td>
<td>Wet room</td>
<td>22345</td>
</tr>
<tr>
<td>2017</td>
<td>Mar</td>
<td>Wet room</td>
<td>32345</td>
</tr>
</tbody>
</table>
## WGS Analysis

<table>
<thead>
<tr>
<th>Isolate ID</th>
<th>Date</th>
<th>Location</th>
<th>AM Resistance</th>
<th>Virulence</th>
<th>Thermal Resistance</th>
<th>Biofilm Formation</th>
<th>Biocide</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-00001</td>
<td>Feb-17</td>
<td>Dry Zone, High Risk</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
</tr>
<tr>
<td>10-00002</td>
<td>Feb-17</td>
<td>Wet Zone, Low Risk</td>
<td>Red</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
</tr>
<tr>
<td>10-00008</td>
<td>Feb-17</td>
<td>Dry Zone, High Risk</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
</tr>
<tr>
<td>10-00009</td>
<td>Feb-17</td>
<td>Wet Zone, Low Risk</td>
<td>Green</td>
<td>Green</td>
<td>Orange</td>
<td>Green</td>
<td>Green</td>
</tr>
<tr>
<td>10-00017</td>
<td>Feb-17</td>
<td>Dry Zone, High Risk</td>
<td>Orange</td>
<td>Red</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
</tr>
<tr>
<td>11-00005</td>
<td>Jun-17</td>
<td>Wet Zone, Low Risk</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
</tr>
<tr>
<td>11-00537</td>
<td>Jun-17</td>
<td>Wet Zone, Low Risk</td>
<td>Green</td>
<td>Green</td>
<td>Orange</td>
<td>Green</td>
<td>Green</td>
</tr>
<tr>
<td>11-00538</td>
<td>Jun-17</td>
<td>Wet Zone, Low Risk</td>
<td>Green</td>
<td>Green</td>
<td>Orange</td>
<td>Green</td>
<td>Green</td>
</tr>
<tr>
<td>11-01558</td>
<td>Jun-17</td>
<td>Wet Zone, Low Risk</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
</tr>
<tr>
<td>11-01559</td>
<td>Jun-17</td>
<td>Wet Zone, Low Risk</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
</tr>
</tbody>
</table>
Sample 123

- Gammaproteobacteria
- Clostridia
- Bacteroidia
- Bacilli
- Alphaproteobacteria
- Deltaproteobacteria
- Betaproteobacteria
- Actinobacteria
- Fusobacteria
- Unclassified and Others

- Lachnospiraceae
- Pseudomonadaceae
- Rikenellaceae
- Enterobacteriaceae
- Halomonadaceae
- Bacillaceae
- Ruminococcaceae
- Prevotellaceae
- Flavobacteriaceae
- Comamonadaceae
- Unclassified and Others
Thank You

cian.omahony@cremeglobal.com
@CianOnData
Expert Models for Decision Makers™

Thank You

cian.omahony@cremeglobal.com
@CianOnData
Expert Models for Decision Makers™

Thank You

cian.omahony@cremeglobal.com
@CianOnData