IFSH and Creme Global Present

WEBINAR
High-Throughput Sequencing and Predictive Microbiology: Mapping the microbiome to manage risk during production

Wednesday, February 26*, 2020 at 10:30 am CST
Introduction

A couple of announcements
IFSH: A short video
IFSH HTS Initiative
Laboratory & bioinformatics
Collaborative projects in IFSH

Dr. Behzad Imanian

The field of predictive microbiology: An overview
Prof. Seamus Fanning

Microbiological analysis from the SAFE project
Dr. Scott Nguyen

Developing predictive models: Our approach
Noel Rogers

The service Implementation
Brendan Ring

Questions/Answers
High-Throughput Sequencing: A Tutorial and Hands-on Workshop
Analytical & Bioinformatic Tools and Programs

May 19, 2020
Moffett Campus
6502 S Archer Rd, Bedford Park, IL

Register: https://www.eventbrite.com/e/86076462015
The 5th IFSH High-Throughput Sequencing Symposium
Food Safety & HTS: Latest News and Views

May 20 – 21, 2020
Chicago Marriott Southwest Hotel
Burr Ridge, Illinois

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Questions/Answers
High-Throughput Sequencing or HTS
IFSH High-Throughput Sequencing Laboratory & Bioinformatics Facilities
IFSH HTS Laboratory Services

- Nucleotide extraction, purification and quantification
  - DNA
  - RNA
  - Pure or mixed samples
- Library preparation
- WGS (pure samples)
  - Bacterial, viral, mitochondrial and chloroplast genomes
- Metagenomics (mixed/environmental samples)
  - 16s rRNA or shotgun
  - Transcriptomics (pure or mixed samples)
Bioinformatics at IFSH

- High-Performance Computers (HPCs)
  - Fast
  - Secure
  - Reliable

- High-capacity data back up system
IFSH HTS Bioinformatics Services

- Sequence quality inspection and filtration (QC)
- Sequence assembly (WGS, RNA-seq or transcriptomics)
- Sequence assembly assessments (QC)
- Sequence assembly comparisons
- BLAST search and results
- Sequence annotation
- SNP analysis (CFSAN SNP Pipeline or kSNP analysis)
- Phylogenetic analysis and phylogeny reconstruction, tree visualization
HTS at IFSH

- IFSH HTS Industry Advisory Committee
  - 20 members

- IFSH HTS Industry-Governmental Agencies Council
  - Representatives from FDA, CDC, USDA FSIS and IFSH HTS Industry Advisory Committee
The Next Generation Sequencing Revolution: Impacts on Manufacturing Safety

February 2020
Presenters

Prof Shéa Fanning

Dr Scott Nguyen

Noel Rogers

Brendan Ring
Agenda

The field of predictive microbiology: An overview

Microbiological analysis from the SAFE project

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The service Implementation
The field of predictive microbiology: An overview

Prof Shéa Fanning
FDA Food Safety Modernization Act

In 2011, Congress enacted the FSMA as the largest expansion of FDA’s food safety authority since the 1930s.

Rule established “Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventive Controls for Human Food” Document 80 FR 55907

21 CFR 117.3

Key Provisions of FSMA:
- Increased frequency of inspections at food facilities
- Tighter record-keeping requirements
- Extend oversight to certain farms
- Mandate product recalls if a firm fails to institute them voluntarily
- Food processing, manufacturing, shipping, and other regulated facilities must conduct an analysis of the most likely safety hazards and to design and implement risk-based controls to prevent them
- The establishment of science-based "performance standards" for the most significant food contaminants
- Improve the nation's foodborne illness surveillance systems
- Increased scrutiny of food imports, which account for a growing share of U.S. consumption
Who’s Looking?

Supply chain → Processing → Distribution → Consumption → Health impact

- Customs and USDA random sampling
- Regulator random and targeted WGS swabathons
- Regulator random sampling using WGS
- Social media, influencer blogs, etc.
- CDC using WGS to identify strain
GenomeTrakr

Retrospective NGS study on a 2012 *Salmonella* outbreak from spicy tuna sushi rolls showed high levels of resolution over PFGE. Outbreak isolates were linked to a processing facility 8 km away.

This study convinced FDA-CFSAN to invest in whole genome sequencing (WGS) technologies and start GenomeTrakr.

GenomeTrakr has been collecting WGS data in 2012 from foodborne pathogens.

GenomeTrakr + FSMA powers allowed the FDA to close a *Listeria* contaminated cheese facility identified by NGS data in 2014.
FDA Swab-A-Thons: What To Expect In The Search For Pathogens

By Sam Lewis, editor, Food Online

What To Expect When the FDA Visits Your Facility For a Swabathon

Steve Decker / August 10, 2017
Who’s Looking?

www.IWasPoisoned.com
Outbreak!

Understanding the microbiome could have prevented this type of scenario.

French dairy expands baby milk recall to include 83 countries

By News Desk on January 16, 2018

Fallout from a Salmonella outbreak traced to baby milk products from Lactalis is raining down on the French dairy company and retailers that continued selling the contaminated recalled products.

Friday Lactalis officials expanded the recall to include 83 countries and more than 12 million boxes of infant milk products. It is the second expansion since the company’s initial recall in December 2017 when 30 countries were involved.

Three dozen infants in France have been confirmed sick and other children in Spain and Greece are possible cases. Lactalis does sell products in the United States, but as yet none of the infants in the U.S. or Canada has been implicated.
Technology Background

Dr Scott Nguyen
Next Generation...

Traditional Technique

Move towards NGS techniques
Next Generation...

Traditional Technique

**Slow:**
Pathogen present already

Move towards NGS techniques

**Fast:**
Detects all (non-culturables), data can be used to model risks
Culturable vs. Non-cultured

< 10% of bacteria is culturable

> 90% are not cultured

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NGS - All bacteria

NGS: 100% detectable

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Next Generation Sequencing (NGS)

NGS has emerged as the preferred technology for various regulatory agencies.

Costs of next generation sequencing has dropped, outpacing Moore’s Law.

Thus more regulatory agencies have dropped traditional identification methods such as PFGE in favour of NGS due to speed, reliability, and affordability.
WGS vs 16S rRNA

WGS
To strain level

Single Isolate
To strain level

Shotgun Metagenome

16S rRNA Gene
Amplicon Microbiome
**WGS vs 16S rRNA**

- **WGS**
  - To strain level

- **Single Isolate**
  - To strain level

- **Shotgun Metagenome**

- **16S rRNA Gene Amplicon Microbiome**
  - Family: Listeriaceae
  - Genus: Listeria
  - Species: Listeria monocytogenes EDGE
WGS vs 16S rRNA

WGS
To strain level

Single Isolate
To strain level

Shotgun
Metagenome

16S rRNA Gene
Amplicon Microbiome

Family
Listeriaceae

Genus
Listeria

Species
Listeria monocytogenes EDGE

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Outbreak Identifier
Microbiome as Microbial Census

If we view the microbiome as a snapshot of the community, how do we distinguish which members are permanent residents and which members are merely visiting?

How can population structure give us insights into how the community evolves and assess risks for pathogen establishment?

For example, if we see a lot of “empty homes” in the neighborhood, we can make predictions and be prepared for pathogen “squatters”.

CRM Global
Microbiome as Microbial Census

Likewise, if we know what is present in the community, we can assess the likelihood of pathogens establishing through data modelling and machine learning.

Case study: Tan et al. showed in a recent study that harmless bacteria (*Pseudomonadaceae*) in apple-packing facilities can shelter *Listeria* and protect *Listeria* from repeated sanitation:

https://www.sciencedaily.com/releases/2019/08/190821101416.htm

Microbiological analysis from the SAFE project
SAFE
Sequencing Alliance for Food Environments

Food safety & quality risk assessment using Next Generation Sequencing (NGS)
Data Volume

- Sampling Rounds: 6
- Sampling Points: 98
- Samples Analyzed: 515
- Distinct genera detected: 2,085
Data Volume
SAFE Sampling Runs

Run 1

Run 2

Run 3

Run 4

Run 5

Run 6

2016 2017 2018
The 16S rRNA gene is present in all microbes.

Hypervariable regions (specific to family/genus) are flanked by highly conserved sequences (for all) which allows for genus level identification (V3-V4 focus).

Captures all microbes present (not something traditional microbiology would be able to assess)
16S rRNA Gene Sequencing

The output from the sequencing are screened and processed for quality control.

Sequences are binned based on percent identity (~97%). Representative sequence from each bin is aligned to a reference database for taxonomic placement. (OTUs)

The counts will vary based on sample quality so we focus on relative abundance rather than absolute counts.

16S RNA gene sequencing **will not** capture strain level information.
Developing predictive models: Our approach
Noel Rogers
Microbiome as Bacterial Census

By capturing the culturable and non-culturable bacteria population, we can assess the 'neighborhood' and make predictions.

These predictions are on a case-by-case situation. For certain environments, low diversity is preferable while in other situations, higher diversity may inhibit pathogenic bacteria.
Climate Related Trend

Trend related to unusually hot summer.

Spike for particular bacteria at many locations.
Seasonal Trend

Trends in the entire microbiome, single sample site.

Trends in the microbiome composition over time for a single sampling point. Each colour represents a distinct genus.
Spatial Trend

Bacteria differences by location

Map of a processing facility showing average abundances at a number of sampling points of a bacteria typically found on human skin.
Uniqueness of Microbiomes

We can take a ‘personalised’ approach to plant safety by understanding the bacteria profiles unique to that environment.
Insights from Clustering

Coloured by sample date
Anomaly!

Clustering of samples for a single plant. Samples are coloured based on the date they were taken.
Insights from Clustering

Coloured by sample **type**

Anomaly!

Clustering of samples for a single plant. Samples are coloured based on the type of sample (swab, raw ingredient, finished product, etc.)
Predictive Modelling

Machine Learning
Predictive Modelling
Motivating Idea

Different bacteria will thrive in different conditions

Tendency for co-culturing between certain species

(Fox et al. 2014)
Predictive Modelling

Predictions

Present

Yes

No

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Predictive Modelling

Predictions

Present Model Prediction

Yes
No
Yes
No
The service Implementation

Brendan Ring
Predictive Microbiology

Next generation genomic techniques monitoring factory wide microbiome. Machine Learning is used to predict the likelihood of safety or quality concerns.

**Objective**
To predict the likelihood of occurrence of a safety or quality issue in a manufacturing environment.

**How**
By using the latest genomic techniques, machine learning and fundamental microbiological growth information to determine the conditions compatible for pathogen or spoiler presence or growth.

**Unique Offering**
Combining the strengths of characterizing the microbiome, genomics, IIOT and machine learning enables the prediction of the likelihood of occurrence of a safety or quality issue. This gives a far greater degree of accuracy and detail than possible from using current traditional microbiological techniques while also benefiting from the insights of domain experts and world-leading academics.
Process

1. Assessment and Agreement
   Initial site screening and service contract

2. Setting the Frame
   Swab-plan tailored to your site, staff training, organisation of kits and shipment

3. Review of Available Metadata Recording Device
   Conduct analysis of existing infrastructure to capture temperature, humidity, etc. and install loggers and or IIOT sensors as required

4. Collect Swabs and Metadata
   Take swabs and record metadata

5. Logistics
   Kit is collected and shipped

6. Extracting and Sequencing
   Anonymous DNA extraction and sequencing in 3rd party lab

7. Data Processing and Analysis
   Converting raw sequence data into meaningful insights

8. Your Results
   Results available for viewing via weekly email
## Viewing the Results

### Current Week Sample Results

<table>
<thead>
<tr>
<th></th>
<th>Location 1</th>
<th>Location 2</th>
<th>Location 3</th>
<th>Location 4</th>
<th>Location 5</th>
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</thead>
<tbody>
<tr>
<td>Bacteria 1</td>
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<td>Bacteria 4</td>
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<tr>
<td>Bacteria 5</td>
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</tr>
</tbody>
</table>
## Metadata

### Once off
- Process step
- Zone (high, medium, low, undefined)
- Surface type (tile, concrete, metal, paint, other)

### Ongoing

#### Swabbing
- Swab ID
- Swab location, time & date
- Person doing the swabbing

#### Survey
- Cleaning log
- Sanitizer
- CFU

#### Automated Data Capture
- Temperature, RH, staff presence, particles in the air, air flow, air pressure differential, surface water, ...

#### Other
- Weather from local data collection centre

### Retrospective
- Incident Information
Conclusion
SAFE

Supply chain → Processing → Distribution → Consumption → Health impact

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Predictive Microbiology

Enabling a preventative approach to managing risk in your environment
SAFE

Supply chain → Processing → Distribution → Consumption → Health impact

Efficient root cause analysis
- Microbiome changes
- Operational practices

Implement targeted change
- Zoning
- Sanitation replacement
- Raw material checks
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