Application of ‘Omics’ Technologies in Food Safety

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Outline

• Overview
• *E. coli* O104:H4 – applied genomics
• *E. coli* O157 – comparative genomics
• *E. coli* O157 – applied transcriptomics
• Conclusions
Overview

In the last two decades a number of technologies have emerged to profoundly advance the efficiency of biological investigation:

- Genomics - complete genome sequence has accelerated genetic knowledge generation
- Transcriptomics – complete complement of expressed mRNA
- Proteomics – complete complement of expressed proteins
- Metabolomics – complete complement of metabolites
- Phenomics – high-throughput phenotype arrays
- Metagenomics – the genomic complement of mixed genomes (e.g. From complex environments such as poultry carcass)
German outbreak of *E. coli* O104:H4

Started in May, 2011

- High rate of HUS (women, adults)
- Initially: Shiga toxin producing *E. coli*
- *E. coli* O104:H4 isolated from patients

4,075 cases, 50 deaths (as of 21st July, 2011)

- 908 cases of HUS
  - 34 deaths related to HUS (kidney failure)
  - 16 deaths from other problems (heart failure, seizures etc)

<table>
<thead>
<tr>
<th><em>E. coli</em></th>
<th>Year</th>
<th>Outbreak</th>
<th>No. cases</th>
<th>% HUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>O157:H7</td>
<td>1993</td>
<td>USA (hamburger – Jack in the Box)</td>
<td>583</td>
<td>7</td>
</tr>
<tr>
<td>O157:H7</td>
<td>1997</td>
<td>Japan (sprouts)</td>
<td>5,000 - 12,680</td>
<td>0 – 3</td>
</tr>
<tr>
<td>O157:H7</td>
<td>2006</td>
<td>USA (spinach)</td>
<td>204</td>
<td>15</td>
</tr>
<tr>
<td>O104:H4</td>
<td>2011</td>
<td>Europe (fenugreek sprouts)</td>
<td>4,075</td>
<td>22</td>
</tr>
</tbody>
</table>
Outbreak

16 countries in Europe and North America

Rising Toll
Sixteen countries have reported cases of E. coli infections to the World Health Organization.

<table>
<thead>
<tr>
<th>Country</th>
<th>Cases</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>GERMANY</td>
<td>2,988</td>
<td>30</td>
</tr>
<tr>
<td>UNITED KINGDOM</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>SPAIN</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>FRANCE</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>SWITZERLAND</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>POLAND</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>CZECH REPUBLIC</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>AUSTRIA</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>SWEDEN</td>
<td>47</td>
<td>1</td>
</tr>
<tr>
<td>NORWAY</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
| Source: World Health Organization
E. coli O104:H4 outbreak

- Investigations into source of outbreak
  - Difficult to pin point source
  - Spanish cucumbers
    - E. coli detected, but not O104:H4

- Eventually thought to be sprouts
  - Links to one sprout farm
  - Massive trace back investigation
  - Second outbreak in France
    - Also linked to sprouts

- Seeds used for sprouting from Egypt
  - Complex distribution

http://www.landscapingrevolution.com/ingrid-garden/sprouts/fenugreek-closeup.jpg
Fenugreek seed distribution

Fenugreek seeds exported from Egypt
Quantity: 15,000 kg
Date: 24/11/2009

Importer in Germany
In: 15,000 kg
Date: 15/12/2009
(via Amsterdam, through Rotterdam by ship)

Distributor in Germany
In: 400 kg
Date: 13/01/2010
Storage: 305 kg
Out: 95 kg (50 g packets)

Distributor in France
In: 95 kg (50 g packets)
Out: to ~200 shops

Sprout producer
In: 75 kg
Date: 10/02/2011
Out: 75 kg

10,000 kg Oct 2010

450 kg to Austria
375 kg to Spain

3,550 kg to 9 companies in Germany

Seed supplier/repacker in UK
In: 400 kg
Date: 13/01/2010
Storage: 305 kg
Out: 95 kg (50 g packets)

Distributor in Germany
In: 10,500 kg
Date: 21/12/2009, 01/03/2011
Out: 75 kg

One cluster in France
Date: 08/06/2011
8 cases of HUS and 4 O104:H4 +ve

41 clusters in Germany
Date: April/May 2011
>300 cases of HUS or O104:H4 +ve

From EFSA report, 2011
Economic impacts

Health impact
• Estimates of > $2.84 billion (US) for human health impact

Agricultural impact
• Compensation offer of $290 million (US)
• Losses claimed of $600 million (US) per week
• Farms closed, dumped produce, loss of trade, consumer confidence

Political impact
• Public health vs agricultural livelihood?
Timeline of the Open-Source Genomics Program

Major unique genome features of outbreak O104:H4 strain TY2482

Genetic Elements in Shiga-Toxin–Producing *Escherichia coli* O104:H4 strain TY2482

<table>
<thead>
<tr>
<th>Genetic Element</th>
<th>Notable Features or Functions</th>
<th>Size or S5989 Coordinates*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasmid</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pESBL TY2482</td>
<td>IncI1 plasmid, homologous to pEC.Bactec carrying bla CTX M.15</td>
<td>88 kb</td>
</tr>
<tr>
<td>pAA TY2482</td>
<td>Plasmid encoding aggregative adherence fimbriae I</td>
<td>76 kb</td>
</tr>
<tr>
<td>pG2011 TY2482</td>
<td>Plasmid with no obvious phenotype</td>
<td>1.5 kb</td>
</tr>
<tr>
<td><strong>Region of difference</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-ROD1</td>
<td>Degenerate prophage</td>
<td>296227 (tRNA-Thr)</td>
</tr>
<tr>
<td>I-ROD2</td>
<td>Stx2-encoding prophage</td>
<td>1176265 (wrbA)</td>
</tr>
<tr>
<td>I-ROD3</td>
<td>Micocin gene cluster; tellurite resistance gene cluster</td>
<td>1207704 (tRNA-Ser)</td>
</tr>
<tr>
<td>I-ROD4</td>
<td>Prophage</td>
<td>1811905 (wrFG)</td>
</tr>
<tr>
<td>I-ROD5</td>
<td>Prophage</td>
<td>2102453 (yceE)</td>
</tr>
<tr>
<td>I-ROD6</td>
<td>Molybdate metabolism regulator; yehL</td>
<td>2426442 (IS1)</td>
</tr>
<tr>
<td>I-ROD7</td>
<td>Multidrug-resistant gene cluster (dfpA 7, sul1, sul2, strA, strB, tetA); mercury resistance</td>
<td>4211244 (tRNA-Sec)</td>
</tr>
<tr>
<td>D-ROD1</td>
<td>Prophage</td>
<td>1094587–1140306</td>
</tr>
<tr>
<td>D-ROD2</td>
<td>Prophage</td>
<td>1413924–1446834</td>
</tr>
<tr>
<td>D-ROD3</td>
<td>Prophage</td>
<td>1754689–1800354</td>
</tr>
<tr>
<td>D-ROD4</td>
<td>Prophage</td>
<td>2688656–2701228</td>
</tr>
<tr>
<td>D-ROD5</td>
<td>Type VI secretion genes</td>
<td>3401720–3427357</td>
</tr>
<tr>
<td>D-ROD6</td>
<td>Prophage</td>
<td>4944269–5004333</td>
</tr>
</tbody>
</table>

*Coordinates from the genome of *E. coli* strain S5989 are given for predicted boundaries of regions of difference, with the gene carrying the insertion site shown in parentheses for a region of difference involving an insertion into S5989 (I-ROD). D-ROD denotes a region of difference involving a deletion.*
Phylogenetic placement of German EHEC O104:H4 outbreak strain

Emerging pathogenic *E. coli* O104:H4

- *Shigella dysenteriae*
- Enteropathogenic *E. coli* (EPEC)
- Enteroaggregative *E. coli* (EAEC)
- Enterohemorrhagic *E. coli* (EHEC)
- Shiga toxigenic EAEC
- *E. coli* O104:H4

http://www.giantmicrobes.com/us/products
O104:H4 may be viable but non-culturable
Can an ‘O104:H4’ outbreak happen in Australia?

<table>
<thead>
<tr>
<th>Condition</th>
<th>Yes/No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enteroaggregative <em>E. coli</em> (EAEC) present?</td>
<td>✓</td>
</tr>
<tr>
<td>Shiga toxigenic <em>E. coli</em> present?</td>
<td>✓</td>
</tr>
<tr>
<td>Unregulated antibiotic use?</td>
<td>✗</td>
</tr>
<tr>
<td>Human faecal contamination of crops?</td>
<td>✗</td>
</tr>
<tr>
<td>Sprout-source foodborne illness?</td>
<td>✓</td>
</tr>
<tr>
<td>Imported sprout seeds?</td>
<td>?</td>
</tr>
<tr>
<td>Overall likelihood?</td>
<td>↓</td>
</tr>
</tbody>
</table>
O104:H4 Summary

Rapid Genome Sequencing
• Allowed unprecedented speed and global teamwork in accurately identifying the pathogen
• Facilitated the development of molecular tools for specific screening and detection
• Informed appropriate therapy for infected patients
• Enabled understanding of the emergence(evolution of the specific strain
• Contributed to identification of the most probable food vehicle
**E. coli O157 variability**

*E. coli* O157 share many characteristics

- Virulence markers, phenotypic properties, genetically closely related

Differences in human epidemiology between countries

- Outbreaks vary in severity
- HUS and hospitalisation rates

No suitable animal model to determine virulence potential

Raises questions

- Are all strains equally capable of causing disease?
- Where do pathogenic strains come from?

Adapted from Whitworth (2008) AEM 74: 7447-7450, ESR 2006 and Rivas (pers. comm.)
Shiga toxin diversity

- O157 strains can harbour multiple stx types (combinations of stx\textsubscript{1}, stx\textsubscript{2} and stx\textsubscript{2c})
- Stx\textsubscript{2} subtype correlates with severity of disease
  - stx\textsubscript{2} > stx\textsubscript{2c}
- Stx associated with bacteriophage
  - stx\textsubscript{2c} and stx\textsubscript{2} carried by different phages
  - phages can insert at various points around the genome

$stx_1$ phage genome sequencing

- $\Phi14stx_1$
- $\Phi571stx_1$
- $\Phi3185stx_1$
- $\Phi3206stx_1$

DNA prep → Roche GS-FLX sequencing → Sequence Assembly → Bioinformatics analysis
Similarity of Australian $\textit{stx}_1$ phages to $\textit{stx}_2$ phages from Argentinean O157 isolates

- Australian $\textit{stx}_1$ bacteriophage genomes are similar to the $\textit{stx}_2$ bacteriophage genomes of US spinach outbreak and Argentinean $\text{E. coli}$ O157 strains
- Australian $\textit{stx}_1$ bacteriophage genomes insert at the same chromosome site (tRNA-$\text{argW}$) as the $\textit{stx}_2$ bacteriophage genomes of US spinach outbreak and Argentinean $\text{E. coli}$ O157 strains
Variable bacteriophage insertion sites in O157 chromosomes

Genome of Sakai strain of *E. coli* O157
Single nucleotide polymorphism (SNP) designation of virulence clades in *E. coli* O157

**Clade 2 & 3**
Overrepresented in clinical isolates
Clade 2 (47%)
Clade 3 (11%)

**Clade 7 (7%)**
↓ HUS
Less bloody diarrhea

**Clade 8 (26%)**
↑ HUS

Manning et al. (2008)
Distribution of *E. coli* O157 virulence clades in isolates from Argentina and Australia

### Summary – O157 comparative genomics

<table>
<thead>
<tr>
<th></th>
<th>Argentina</th>
<th>Australia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Most prev stx genotype</strong></td>
<td>$stx_2/stx_{2c}$</td>
<td>$stx_1/stx_{2c}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$stx_{2c}$ only</td>
</tr>
<tr>
<td><strong>Phage insertion sites</strong></td>
<td>$stx_2$ in $argW$</td>
<td>$stx_1$ in $argW$</td>
</tr>
<tr>
<td></td>
<td>$stx_{2c}$ in $sbcB$</td>
<td>$stx_{2c}$ in $sbcB$</td>
</tr>
<tr>
<td><strong>Virulence clade</strong></td>
<td>Clade 8 dominate in humans</td>
<td>Clade 7 dominate in humans</td>
</tr>
<tr>
<td></td>
<td>Clade 8 &gt; Clade 4 in cattle</td>
<td>Clade 7 &gt; Clade 6 in cattle</td>
</tr>
</tbody>
</table>
O157 Applied Genomics Summary

Genomics

- Allows rapid comparison of many pathogenic isolates
- Facilitates the development of molecular tools to characterise risk potential
- Has provided evidence for geographic/genetic segregation in the *E. coli* O157 populations of Argentina, Australia and other regions
- May allow the development of targeted management strategies
- May contribute to ‘evidence-based’ food trade considerations
Organic acid resistance in E. coli

Introduction

- Little is known about the genomic basis of organic acid resistance in E. coli
- Information has the potential to enable the:
  - design of more targeted interventions for food manufacturers to control this pathogen through the reformulation of foods to use acids that act synergistically
  - removal of preservatives deemed less favourable by consumers (i.e. salt)
  - development of products that not only ensure the effective preservation of the product but also minimization of the perception of unfavourable tastes/textures and colours

Aim

- To characterize the transcriptomic response of E. coli to organic (lactic, acetic, malic and gluconic) and inorganic (hydrochloric) acids
Organic acid resistance in E. coli

- A transcriptomic (cDNA microarray) study was undertaken to characterise the Acid Tolerance Response (ATR) of E. coli O157:H7 strain Sakai mounted upon induction of an ATR to lactic, acetic, citric, malic, gluconic and hydrochloric acid.

Acid resistance of K-12 and O157:H7 to BHI acidified to pH 3.5 with acetic, lactic, or hydrochloric acid

- Percentage of Survivors (%)
- Time (min) / Time (h)

Legend:
- O157:H7, adapted
- O157:H7, not adapted
- O157:H7 adapted + chloramphenicol
- K-12, adapted
- K-12, not adapted
- K-12, adapted + chloramphenicol
Organic acid resistance in E. coli

- *E. coli* elicits an acidulant- and pathotype-specific transcriptomic response to organic and inorganic acids
- The discovery of an acidulant-specific ATR indicates that more targeted interventions can be designed for food manufacturers to control *E. coli*
- The EHEC pathotype may possess additional molecular mechanisms which contribute to its acid resistance over the generic non-infectious *E. coli*
- The EHEC pathotype may possess a greater ability to survive in acidic environments in which low pH is associated with other environmental stresses

*Publication:*

The chilling and drying stress response of *E. coli* 

**Introduction and Aim**

- **Introduction**
  - The primary reservoir of *E. coli* O157:H7 is cattle
  - Foods of bovine origin are linked most frequently to major food-borne illnesses
  - In Australia and NZ, carcasses are cooled by refrigerated air
  - Air chilling is not specifically recognized as an intervention or critical control point, but can reduce the level of *E. coli* on carcasses by injury through a simultaneous decrease in temperature and water activity

- **Aim**
  - To gain a detailed understanding of the molecular response of *E. coli* O157:H7 to prolonged exposure to cold and osmotic stress
  - To identify potential targets for controlling or eliminating *E. coli* O157:H7 from carcasses and other chilled food products
The chilling and drying stress response of *E. coli*  

**Experimental Method**

- *E. coli* O157:H7 strain Sakai used throughout this study
- Transcriptomic (cDNA microarray) and proteomic (2D-LC/MS/MS) studies were conducted in parallel to determine the response of *E. coli* O157:H7 Sakai:
  - during exponential growth under steady state conditions
    - 35°C, $a_w$ 0.993 – optimal conditions
    - 25°C, $a_w$ 0.985 – intermediate conditions
    - 14°C, $a_w$ 0.985 – near growth-limiting temperature
    - 25°C, $a_w$ 0.967 - near growth-limiting water activity
    - 14°C, $a_w$ 0.967 – combination of near growth-limiting temperature and water activity
  - during dynamic changes
    - 35°C, $a_w$ 0.993 to 14°C, $a_w$ 0.993 - abrupt downshift in temperature
    - 35°C, $a_w$ 0.993 to 35°C, $a_w$ 0.967- abrupt downshift in water activity
    - 35°C, $a_w$ 0.993 to 14°C, $a_w$ 0.967 - abrupt downshift in temperature and water activity
The chilling and drying stress response of E. coli
Main findings and future work

- Differential expression of elements involved in oxidative stress resistance
  → Effect of oxidising agents on the cell (hypochlorite, $H_2O_2$)?

- Differential expression of elements involved in DNA damage repair
  → Effect of UV?

- Increased expression of elements involved in colanic acid biosynthesis
  → Importance of colanic acid biosynthesis and its regulation?

- Viable But Non Culturable (VBNC) state
  → Investigate morphological changes and whether cells are dividing or replicating DNA

Publication
Conclusions

‘Omics’ technologies for food safety
• Allow efficient ‘deep’ and ‘wide’ generation of data
• Provide digital data available for global comparison
• Facilitate the development of molecular tools to characterise risk potential and the design of control interventions
• May allow the development of targeted management strategies
• May contribute to ‘evidence-based’ food trade considerations
Thank you

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