Progress on Development of Field-Based Rapid Methods for Foodborne Pathogens

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Plan Objective 1 (of 3)

Rapid and Quantitative Sample Preparation, No Culture Enrichment…

• InnovaPrep Concentration Pipette

• New Class of Antibody-Associated Magnetic Particles (CRADA with Abraxis, Inc)

• Ferrofluid-based targeted concentration of foodborne pathogens (Tentative agreement with Ancera, Inc, Branford, CT)

• Novel capture of nucleic acid targets; High sample volume; Live/Dead (Tentative agreement & SBIR funding with VisuGen Global, LLC, Aurora, CO)
# InnovaPrep Performance

## Native Bacterial Load on Frozen Vegetables

<table>
<thead>
<tr>
<th></th>
<th>CFU g⁻¹ @ 30°C</th>
<th>CFU g⁻¹ @ 37°C</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>InnovaPrep</td>
<td>Control</td>
</tr>
<tr>
<td>3858</td>
<td>3239</td>
<td>2744</td>
<td>2434</td>
</tr>
<tr>
<td>1671</td>
<td>1071</td>
<td>1086</td>
<td>914</td>
</tr>
<tr>
<td>1500</td>
<td>1143</td>
<td>357</td>
<td>314</td>
</tr>
<tr>
<td>1490</td>
<td>1264</td>
<td>775</td>
<td>632</td>
</tr>
<tr>
<td>3900</td>
<td>2350</td>
<td>1282</td>
<td>679</td>
</tr>
</tbody>
</table>

1 "Signature Kitchens", Green Peas and Diced Carrots

\[ \bar{x} = 0.739 \pm 0.0504, \quad 0.791 \pm 0.0667, \quad P = 0.767 \]
Cell Capture by Antibody-coated Magnetic Particles

- Plate media after particle separation
- Colony count of cells remaining
- Extract DNA & amplify target

<table>
<thead>
<tr>
<th>Particles</th>
<th>Average CFU/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₀ culture</td>
<td>630</td>
</tr>
<tr>
<td>No cells - control</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>450</td>
</tr>
<tr>
<td>4</td>
<td>550</td>
</tr>
</tbody>
</table>
1) Add water that flows through capture chip (5 min)
2) Connect microparticle vial and wicking pad
3) Wait for wicking pad to turn blue (15 min)
4) Insert chip into reader to record results
Plan Objective 2 (of 3)

Develop rapid, quantitative detection methods…

- Piezoelectric membrane sensing (PEMS; MTRA with TBT Group Inc, Bellmawr, NJ)
- AlphaLISA (CRADA with Abraxis Inc, Warminster, PA)
- Flow-through immunoelectrochemistry
PEMS Biosensor Overview

- Antibodies are immobilized on the biosensors to provide selectivity.
- Resonance frequency ($f_i$) of the biosensors shift ($\Delta f_i$) upon antigen attachment due to a change in mass ($\Delta m$) and spring constant ($\Delta k$) of the cantilever.
- The quantity of the attached antigens can be calculated using calibration curves.

\[ \Delta f_i = f_i \left( -\frac{\Delta m}{2M_e} + \frac{\Delta k}{2K} \right) \]

E. coli O157:H7 Detection

Frequency shift (Hz)

Time (min)

-1800
-1500
-1200
-900
-600
-300
0
10
2
cells/ml
10
3
cells/ml
10
4
cells/ml
10
6
cells/ml

10^6 cells/ml

10^4 cells/ml

4 µm
AlphaLISA vs. ELISA Detection of Stx 2 in STEC-Inoculated Romaine Lettuce
Flow-through Enzyme-Linked Immunochemistry Detection
Flow-through Immunoimmunochemical Detection of *Salmonella*
Plan Objective 3 (of 3)

Develop and validate rapid identification methods…

- Agreement with BioRad (ddPCR) - comparing STEC detection versus IQ-Check and MLG
- MinION-field portable?
- Whole genome sequencing (Campylobacter spp.)
Sequencing of *Campylobacter* spp.
Isolates (35 total) from Beef Liver and Chicken

Whole genome sequencing-- PacBio [~12 isolates] and Illumina MiSeq

- ~12 isolates– Assembled complete genome
- 8 isolates– Type VI Secretion System
- ~14 isolates– Assessed for resistance against a panel of 13 antibiotics; Observed significant AMR for most isolates however not for gentamicin

*e.g., Campylobacter coli* YH502…

- A chromosome of 1,718,974 bp (CP018900.1)
- A mega-plasmid (pCOS502) of 125,964 bp (CP018901.1)
- GC content: 31.2%
- Contains 1931 coding sequences and 53 non-coding RNAs
- Multiple virulence genes (67) including a plasmid-borne type VI secretion system
- Antimicrobial resistance genes (beta-lactams, fluoroquinolones, and aminoglycoside)
- CRISPR sequences (14 repeats) and associated proteins (Cas1, Cas2, and Csn)
The closest neighbor of the YH502 strain was *C. coli* strain 14983A, which was isolated from a turkey farm housefly in North Carolina.

Multilocus sequence typing analysis of the complete genomes showed that *C. coli* YH502 and 14983A belonged to the same clonal complex (ST-828).
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