Development of a rapid DNA extraction protocol for qPCR detection of foodborne pathogens

Jeffrey D. Brewster
United States Department of Agriculture
Agricultural Research Service
Eastern Regional Research Center
Microbial Biophysics and Residue Chemistry Research Unit
ARS/Purdue Workshop 2010
High Speed Filtration - Goals

- Alternative to enrichment
- Handle ~ 10% solids (stomached hamburger)
- 10,000x concentration of 100 - 1000 ml
- 1 hour or less
- Cost < $10
- Disposable sterile module
- Conventional filters don’t work
  - clog
  - slow
  - high retention of bacteria
  - high cost
Leukocyte Removal Filter

Capacity >100 ml
10% ground beef slurry
> 10 ml/min flow rate
< 5% Retention of *E. coli*. O157:H7
PCR Detection Problems

- In theory detects 1 cfu
- In practice detects ~1000 cfu
- Fast methods discard >90% of DNA to avoid inhibition
- More elaborate methods are slow and exhibit poor recovery for very low DNA levels
- Recovery from gram positive << gram negative
## PCR inhibition by direct lysis reagents

<table>
<thead>
<tr>
<th>Reagent</th>
<th>qPCR copies</th>
<th>PicoGreen copies</th>
</tr>
</thead>
<tbody>
<tr>
<td>MicroLysis Plus</td>
<td>0.2</td>
<td>996,000</td>
</tr>
<tr>
<td>Yeast Protein Extraction Reag</td>
<td>0</td>
<td>872,000</td>
</tr>
<tr>
<td>Bacterial Protein Extraction Reag</td>
<td>4</td>
<td>617,000</td>
</tr>
<tr>
<td>HotShot</td>
<td>71</td>
<td>702,000</td>
</tr>
<tr>
<td>10x Modified HotShot + Tween</td>
<td>891</td>
<td>429,000</td>
</tr>
<tr>
<td>10x HotShot + Tween</td>
<td>122,059</td>
<td>281,000</td>
</tr>
<tr>
<td>UltraPrepMan</td>
<td>7</td>
<td>276,000</td>
</tr>
<tr>
<td>10x Modified HotShot + CTAB</td>
<td>0</td>
<td>212,000</td>
</tr>
</tbody>
</table>

DNA extracted from ~2x10⁶ cfu *L. monocytogenes*
Original Filtration Method
3 stage filtration/capture

RCQ pre-filter
Glass fiber pre-filter
Track-etched capture filter
10 ml/min max flow
(limit by TE filter)

Need to elute cells
Clogs with high levels of background flora
Modified Filtration Method

2 stage filtration + centrifugation

- RCQ pre-filter
- Glass fiber pre-filter
- Gravity flow (~1 m)
- 50-100 ml/min

2 centrifugations

30 min total
Sample Preparation

- Stomach 170 g ground beef + 360 ml isotonic dextrose
- Filter through RCQ and glass fiber filter
- Aliquot into ten 50 ml tubes
- Add 3 x 0 cfu, 3 x 13 cfu, 3 x 130 to tubes 1-9
- Add 1300 cfu to tube 10
- Centrifuge 8 min at 4200 x g, remove supernate
- Resuspend pellet, transfer to 0.6 ml tube
- Centrifuge 3 min at 21000 x g, remove supernate
Direct DNA Extraction and PCR

- Add 6 µl reagent to cell pellet in 600 µl tube
- Heat 10 min at 65° C
- Add 3 µl 2x neutralizer
- Transfer 8 µl to PCR tube
- Add 2 µl primers and 10 µl Master Mix
- Run qPCR assay (2 hour)
### E. coli O157:H7 in ground beef

<table>
<thead>
<tr>
<th>Sample</th>
<th>cfu/rxn</th>
<th>cfu/g</th>
<th>C_T</th>
<th>C_T</th>
<th>C_T</th>
<th>Copies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank 3° filtrate</td>
<td>0</td>
<td>0</td>
<td>U</td>
<td>U</td>
<td>U</td>
<td>0</td>
</tr>
<tr>
<td>Spiked 3° filtrate</td>
<td>13</td>
<td>1.5</td>
<td>37.2</td>
<td>35.6</td>
<td>36.0</td>
<td>10</td>
</tr>
<tr>
<td>Spiked 3° filtrate</td>
<td>130</td>
<td>15</td>
<td>32.8</td>
<td>32.5</td>
<td>32.1</td>
<td>104</td>
</tr>
<tr>
<td>Spiked 3° filtrate</td>
<td>1300</td>
<td>150</td>
<td>28.9</td>
<td>-</td>
<td>-</td>
<td>990</td>
</tr>
<tr>
<td>Bacteria standard</td>
<td>1.3</td>
<td>-</td>
<td>38.2</td>
<td>38.9</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Bacteria standard</td>
<td>13</td>
<td>-</td>
<td>36.5</td>
<td>35.7</td>
<td>-</td>
<td>11</td>
</tr>
<tr>
<td>Bacteria standard</td>
<td>65</td>
<td>-</td>
<td>32.7</td>
<td>34.1</td>
<td>-</td>
<td>64</td>
</tr>
<tr>
<td>Water</td>
<td>-</td>
<td>-</td>
<td>U</td>
<td>U</td>
<td>U</td>
<td>0</td>
</tr>
</tbody>
</table>
Acknowledgements

- Andrew Bigley
- Rebecca Linehart
- Aisha Abdul-Wakeel
- Chandi Vijay
- Ly Nguyen
- Ralph Mazenko