Objectives

1) To use human specific Bacteroides as indicator for human fecal contamination and risk for pathogenic viruses
2) To optimize extraction procedures for RNA and DNA from mollusks, raspberries and other contaminated foods
3) To develop a qPCR assay for quantifying the human-specific fecal bacterium, Bacteroides dorei in food samples
4) To develop protocols for Norovirus (NoV) detection and quantification
5) To evaluate the efficiency of the qPCR assay to detect B. dorei in raspberries compared to detection of viruses by RT-qPCR

Fecal source tracking by Bacteroides

A number of fecal source-specific markers

Investigation of microbial pollution in water

Viruses infecting humans come from human feces

B. dorei (Bacteroides HF8) is specific for human fecal pollution

Could this be a practical indicator for the presence of viruses?

Results

Sensitivity:

Virus/RNA extraction efficiency of controls:

<table>
<thead>
<tr>
<th>Virus RNA dilution</th>
<th>% Positive (Positive/Total)</th>
<th>Mean % recovery ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MCo</strong> 1:1</td>
<td>100 (61/61)</td>
<td>3.13 ± 5.73</td>
</tr>
<tr>
<td><strong>MCo</strong> 1:10</td>
<td>100 (61/61)</td>
<td>11.61 ± 12.35</td>
</tr>
<tr>
<td><strong>MNV</strong> 1:1</td>
<td>97 (29/30)</td>
<td>3.77 ± 3.58</td>
</tr>
<tr>
<td><strong>MNV</strong> 1:10</td>
<td>97 (29/30)</td>
<td>17.67 ± 8.67</td>
</tr>
</tbody>
</table>

Limit of detection (LOD):

<table>
<thead>
<tr>
<th>Agent</th>
<th>LOD of 50% of replicates at 1:10 dilution</th>
<th>LOD of 50% of replicates at 1:1 dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>NoV GI</td>
<td>68</td>
<td>94</td>
</tr>
<tr>
<td>NoV GII</td>
<td>68</td>
<td>7</td>
</tr>
<tr>
<td>MCo</td>
<td>181</td>
<td>4701</td>
</tr>
</tbody>
</table>

Reproducibility:

10^7 cells added to 25 g frozen raspberries

B. dorei retrieval from spilted raspberries

B. dorei were retrieved from fresh and frozen raspberries

Best retrieval was seen in fresh berries (~1%)

LOD (50% of replicates) = 505 cells in 25g

Quantiﬁcation of NoV in outbreak samples

<table>
<thead>
<tr>
<th>Estimated levels of NoV/g detected in 25 g of naturally contaminated raspberries</th>
<th>Mean levels of NoV/g ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>NoV GI</td>
<td>17</td>
</tr>
<tr>
<td>NoV GII</td>
<td>8</td>
</tr>
<tr>
<td>NoV GII (1:5 ± 1.7)</td>
<td>10^7</td>
</tr>
</tbody>
</table>

NoV/GI were determined by standard curves obtained using NoV RNA transcripts and corrected by taking into account the percentage of recovered MCo.

Project idea

- Food and drinking water serves frequently as vehicles for transmission of human enteric viruses like Noroviruses. Contamination of produce can take place during production by use of sewage polluted water or inefficient hygiene norms.
- Currently, there are no routine testing for viruses in foods and water due to the lack of validated methods and the methods used are still too labour-intensive and expensive to be incorporated in the quality control of most food industries.
- In this project we aim to develop a molecular indicator tool to determine the presence of human fecal pollution in relevant food sources and growth environments.

Challenges during extraction

- Raspberries = pH 2,5
- PCR inhibition
- Virus present in low numbers

Several extraction protocols were tested

Efficient extraction of both RNA (Norovirus) and DNA (from Bacteroides)

Final extraction protocol:

25 g of soft fruit or vegetables

Sample preparation

1. Elute by agitating in filter compartment bag with Tri-Glycine bath extract pH 9.5
2. Pellet and centrifuge eluate to separate from tissue debris
3. Precipitate virus with polyethylene glycol
4. Resuspended pellet in phosphate buffer saline
5. Clarify with chloroform/butanol extraction
6. Centrifuge at 3500 x g for 15 min
7. Precipitate phenolic compounds with Plant RNA aid

DNA/RNA extraction

- Lyse viral capsid and remaining tissue with lysobuffer
- Nucleic acid capture using paramagnetic silica

Detection

- Viral or bacterial DNA/RNA amplification by real-time PCR and TaqMan real-time RT-PCR, respectively

Acknowledgements

This work is funded by the Danish Ministry of Food, Agriculture and Fisheries

Conclusions

- qPCR assay for B. dorei developed and tested
- The abundance of B. dorei in human feces varies considerably
- Sample preparation for extraction of both viral RNA and B. dorei DNA was developed
- The limits of detection (LOD50’s) were found to be 505 B. dorei particles and below 100 RT-qPCR units of both genogroup I and II NoV’s per 25 g of raspberries
- The method was successfully applied to detect and quantify NoV in raspberries implicated in disease outbreaks