qPCR assay for detection of human fecal contamination in food samples

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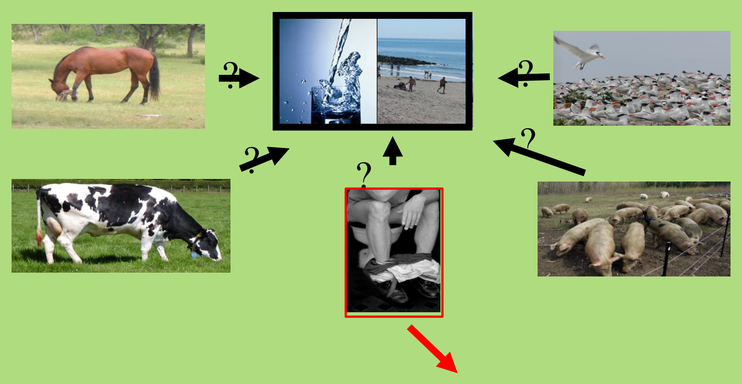
Objectives

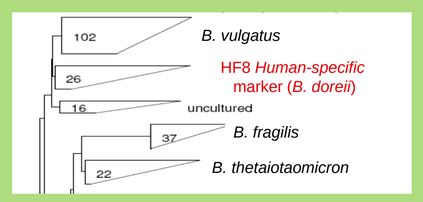
1) To use human specific *Bacteroides* as indicator for human fecal contamination and risk for pathogenic viruses
2) To optimize extraction procedures for RNA and DNA from mollusks, raspberries and other contaminated foods
3) To develop a qPCR assay for quantifying the human-specific fecal bacterium, *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?

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 Tris-Glycine beefextract pH 9.5
2. Retain and centrifuge eluate to separate from tissue debris (Pectinase treatment at neutral pH, for pectin containing berries only)
1. Precipitate virus with polyrthylene glycol
2. Resuspend pellet in phosphate buffer saline
3. Clarify with chloroform-butanol extraction
4. Centrifuge at 3500 x g for 15 min
5. Precipitate phenolic compounds with Plant RNA aid

DNA/RNA extraction

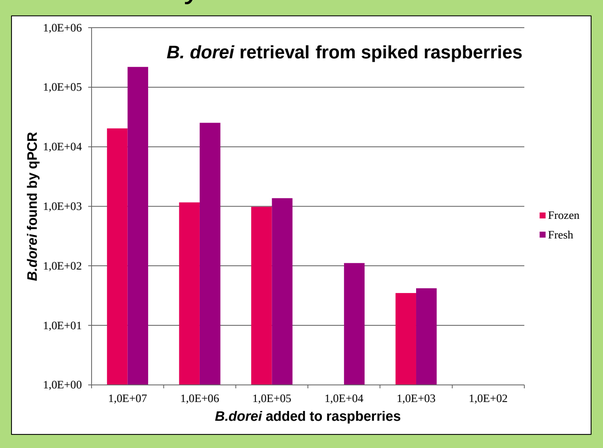
7. Lyse viral capsid and remaining tissue with lysisbuffer
8. Nucleotide capture using paramagnetic silica

Detection

9. Viral or bacterial DNA/RNA amplification by real-time PCR and TaqMan real-time RT-PCR, respectively (modified from Le Guyader et al., Appl. Environm. Microbiol. 2009)

Results

Sensitivity:



- *B. dorei were retrieved from fresh and frozen raspberries*
- *Best retrieval was seen in fresh berries (~1%)*
- *LOD_{50} (Limit of detection) = 505 cells in 25g*

Virus/RNA extraction efficiency of controls:

Virus	RNA dilution	Recovery of virus detected by RT-qPCR from 25 g raspberries	
		% Positive (Positive /Total)	Mean % recovery ± SD
MCo*	1:1	100 (61/61)	3.13 ± 5.73
	1:10	100 (61/61)	11.61 ± 12.35
MNV**	1:1	97 (29/30)	3.77 ± 3.58
	1:10	97 (29/30)	17.67 ± 8.67

** Mengovirus, ** Murine Norovirus*

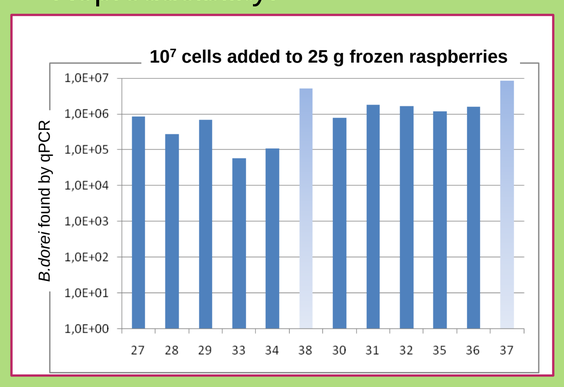
- *25 g samples were artificially contaminated with 2×10^6 RT-PCR units of MCo and MNV which could be recovered in most samples with approximately 12 and 18 percentage, respectively.*

Sensitivity:

	Limit of detection (LOD_{50})	
	RNA dilution	
Agent	1:1	1:10
NoV GI.1	68	94
NoV GII.7	68	7
MCo	181	4701

- *LOD_{50} at which 50% of replicates are positive determined by RT-qPCR units /25 g.*
- *Duplicate berry portions were artificially contaminated in two rounds with 10-fold serial dilutions of viral mixtures.*
- *Virus were detected on each extract (undiluted and 10-fold diluted) by RT-qPCR in duplicate, resulting in eight determinations per combination of method, virus and dilution.*

Reproducibility:



- *B. dorei were retrieved from frozen raspberries 10 times*
- *Light blue collums = retrieval without berries*

Quantification of NoV in outbreak samples

	Estimated levels of NoV/g detected in 25 g of naturally contaminated raspberries	
	No of extractions positive for NoV	Mean levels of NoV/g ± SD
NoV GI	17	$(3.1 \pm 5.2) \times 10^2$
NoV GII	8	$(1.6 \pm 1.7) \times 10^2$

- *Five of six different batches of imported frozen raspberries implicated in seven Danish NoV disease outbreaks during the winter months 2010-11 were found positive for NoV GI or GII.*
- *Estimated levels of NoV were determined by standard curves obtained using NoV RNA transcripts and corrected by taken into account the percentage of recovered MCo.*

Conclusions

- qPCR assay for *B. dorei* developed and tested
- The abundance of *B. dorei* in human feces varied 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

Acknowledgements

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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 hygienic 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