Evaluation of Multiple Human-Specific *Bacteroides* PCR Markers For Sewage Pollution Tracking

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Summary

The host specificity of the human-specific *Bacteroides* markers (i.e., HF183, BacHum, HuBac, BacH and Human-Bac) was evaluated in Southeast Queensland, Australia by testing fecal DNA samples from 11 animal species including humans. All human fecal samples were positive for all five markers indicating 100% sensitivity. The overall specificity of the HF183 markers to differentiate between humans and animals was 99%. The specificities of the BacHum and BacH markers were > 94%, suggesting that these markers are suitable for sewage pollution tracking in environmental waters in Australia. The BacHum (i.e., 63% specificity) and Human-Bac (i.e., 79% specificity) markers performed poorly in distinguishing between the sources of human and animal fecal samples.

Aim

The primary aim of the study was to evaluate the specificity of five human-specific *Bacteroides* PCR markers in fecal samples from 11 host groups collected from Southeast Queensland, Australia and to determine which marker is the best to identify the sources of sewage fecal pollution in environmental waters.

Methodology

•To determine the specificity of the human-specific markers, 196 fecal samples were collected from 11 animal species. Samples from human fecal sources (*n* = 50) were collected via influent to a STP. Individual fecal samples of cattle (*n* = 25), pigs, (*n* = 13), sheep (*n* = 17), goat (*n* = 4), horses (*n* = 9), and chickens (*n* = 10) were collected from an abattoir and various farms. Dog (*n* = 33), duck (*n* = 20) and pelican (*n* = 5), and kangaroo (*n* = 10) fecal samples were collected from various parks, ponds and lakes.

•Fecal samples were taken with a sterile swab from around the Brisbane Area (±100km). DNA were extracted from sewerage (i.e., 10mL) and feces (150mg-200mg) using the QIAmp Stool DNA kit (QIAgen, Valencia, CA, USA) following manufacturer's instructions.

•An experiment was conducted to determine the potential presence of PCR inhibitory substances in fecal samples collected from animals for specificity assay. DNA was extracted from 1 L of ultrapure DNase- and RNase-free sterile distilled water (Invitrogen) after concentrating the sample. A representative number of pooled animal fecal samples (*n* = 5) were spiked with a known gene copies of the HF183 marker. The threshold cycle (C_T) values of these spiked DNA samples were compared to those of the DNA samples from distilled water spiked with the same concentration of the HF183 marker.

Human-specific markers	Primer sequence (5´- 3´)	Amplicon size (bp)	Reference
HF183	ATC ATG AGT TCA CAT GTC CG GCC GTC TACT CT TGG CC	570	Bernhard and Field, 2000
BacHum	TGAGTTCACATGTCCGCATGA CGTTACCCCGCCTACTATCTAATG	81	Kildare *et al.* 2007
HuBac	GGGTTTAAAGGGAGCGTAGG CTACACCACGAATTCCGCCT	116	Layton *et al.* 2006
BacH	CTTGGCCAGCCTTCTGAAAG CCCCATCGTCTACCGAAAATAC	93	Reischer *et al.* 2007
Human-Bac	GTTGTGAAAGTTTGCGGCTCA CAATCGGAGTTCTTCGTGATATCTA	125	Okabe *et al.* 2007

For each PCR experiment, corresponding positive (i.e., plasmid DNA) and negative (sterile water) controls were included. Each DNA sample was tested in triplicate to obtain positive/negative results.

PCR detection of human-specific markers was done using previously published primers and PCR assays

Results

•To assess the PCR inhibitors, a representative number of pooled animal fecal samples were spiked with known gene copies of the HF183 marker and the C_T values of these samples were compared to those of the DNA samples from distilled water spiked with the same concentration of the HF183 markers. The results indicated that the undiluted DNA extracted from feces did not contain PCR inhibitory substances.

Samples	Threshold cycle (C_T) value for the Real-time PCR		
	Undiluted DNA	10-fold dilution	100-fold dilution
Distilled water	21 ± 0.3	-	-
Dog	21 ± 0.1	21 ± 1.2	21 ± 0.7
Pig	22 ± 0.7	22 ± 0.1	23 ± 1.2
Sheep	22 ± 0.1	22 ± 0.4	22 ± 0.9
Duck	21 ± 2.0	22 ± 0.3	22 ± 0.2
Cattle	22 ± 0.4	21 ± 7.0	21 ± 1.0

•Specificity is the ability to detect a source when it is not present and sensitivity is the ability to detect a source when it is present. Only one fecal sample from a sheep was found to be positive for the HF183 marker. However, the PCR band was very faint on the gel analysis.

•The overall specificity of the HF183 markers to differentiate between humans and animals was 99%. Similarly, the overall specificity of the BacHum, HuBac, BacH and Human-Bac markers were 96%, 63%, 94% and 79%, respectively. The overall sensitivity of all the human-specific markers was 100%.

Host groups	No. of samples tested/PCR positive results					
	UniBac	HF183	BacHum	HuBac	BacH	Human-Bac
STP (influent)	50/50	50/50	50/50	50/50	50/50	50/50
Cattle	20/20	20/0	20/0	20/8	20/0	20/4
Pigs	8/8	8/0	8/1	8/4	8/0	8/0
Sheep	17/17	17/1	17/2	17/5	17/1	17/4
Goat	4/4	4/0	4/0	4/0	4/1	4/0
Horses	9/9	9/0	9/1	9/3	9/0	9/4
Chickens	10/10	10/0	10/0	10/0	10/0	10/0
Dogs	33/33	33/0	33/2	33/22	33/6	33/14
Ducks	20/20	20/0	20/0	20/4	20/0	20/0
Pelicans	5/5	5/0	5/0	5/0	5/0	5/0
Kangaroos	10/10	10/0	10/0	10/4	10/0	10/3

Conclusions

•For the 5 human-specific markers tested in this study, the HF183 marker performed better than others. This marker showed 99% specificity to distinguish between the sources of human and animal fecal pollution. The performance of the five markers in terms of specificity was HF183 > BacHum > BacH > Human-Bac > HuBac.

•The BacHum and BacH markers showed 96% and 94% specificity suggesting that they could potentially be used to detect the sources of sewage pollution in environmental waters.

•HuBac marker was frequently detected in samples from animals, especially dogs followed by the Human-Bac marker which was also detected in a number of animal fecal samples including dogs. This suggests that these markers may not be suitable for the detection of sewage fecal pollution in Australian waters.