SOURCE IDENTIFICATION OF FAECAL POLLUTION IN A NON-SEWERED CATCHMENT BY MEANS OF HOST-SPECIFIC MOLECULAR MARKERS

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Summary

Multiple host-specific PCR markers [human-specific *Bacteroides* HF183 and HF134, cattle-specific *Bacteroides* CF128, dog-specific *Bacteroides* DogCan and human-specific enterococci surface protein (*esp*)] were used to detect faecal pollution in a mixed land use non-sewered catchment. The overall sensitivity (>85%) and specificity (>93%) of these markers were high indicating their suitability for detecting sources of faecal pollution. Of the 16 environmental samples tested from the study area, 14 (87%) were positive for at least one of the markers tested. Human-specific markers were consistently detected in samples collected from sites within close proximity to urban development. Based on the results, it appears that the host-specific markers are a sensitive measure of sources of faecal pollution in surface waters in Southeast Queensland, Australia

Objective

The primary objective of this study was to validate host-specific PCR markers for the detection of sources of faecal pollution and their application to identify the sources of faecal pollution in a non-sewered catchment in Southeast, Queensland, Australia.

Methodology

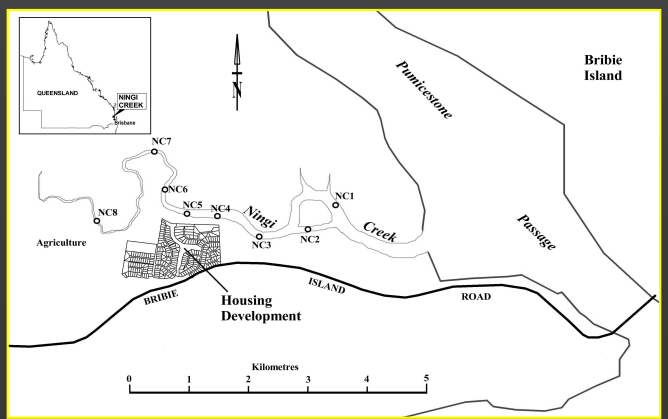
1.Sensitivity (s) and specificity (r) of the PCR primers were assessed by testing 197 faecal samples from 13 host groups. From faecal samples, DNA extraction was performed using either DNA stool kit or DNeasy blood and tissue kit (Qiagen).

2. Primers used for PCR assay

Primers	Oligonucleotide sequence (5´- 3´)	bp
Bac32F [a]	AAC GCT AGC TAC AGC CTT	700
Bac708	CAA TCG GAG TTC TTC GTG	
HF183 [a]	ATC ATG AGT TCA CAT GTC CCG	520
Bac708	CAA TCG GAG TTC TTC GTG	
HF134 [a]	ATC ATG AGT TCA CAT GTC CCG	570
Bac708	CAA TCG GAG TTC TTC GTG	
CF128F [a]	CAA ACY TTC CCG WTA ACT	580
Bac708	CAA TCG GAG TTC TTC GTG	
BacCanF [b]	GGA GCG CAG ACG GGT TTT	145
BacUni690R	CAA TCG GAG TTC TTC GTG ATA TCTA	
*esp*F [c]	TAT GAA AGC AAC AGC ACA AGTT	680
*esp*R	ACG TCG AAA GTT CGA TTT CC	

3. PCR assay

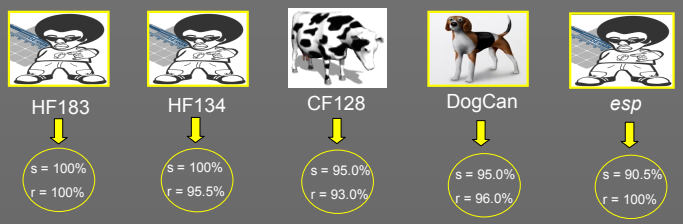
4. Map of the study area (Ningi-Creek catchment)



5. Sixteen water samples were collected on 2 occasions from 8 sampling sites (i.e. NC1-NC8) after storm events. The samples were analysed for the levels of *Escherichia* coli and enterococci. In addition, DNA was extracted from each water sample for PCR assay (presence/absence) of host-specific markers

Results

1. The overall sensitivity (s) and specificity (r) of the host-specific markers were high Indicating their suitability for detecting sources of faecal pollution



2. The levels of faecal indicator bacteria in water samples ranged between 9.1 X 10^2 and 1.2 X 10^4 CFU/100 mL (for *E. coli*) and 1.2 X 10^2 and 5.6 X 10^4 CFU/100 mL (for enterococci). At least one host-specific marker was detected in 14 (87%) out of 16 samples. Human-specific *Bacteroides* HF183 and HF134 markers were detected in 9 (56%) and 6 (37%) samples respectively. The *esp* marker was also detected in 6 (37%) samples. Cattle-specific marker CF128 was detected in 11 (69%) samples whereas dog-specific markers BacCan was detected in 5 (31%) samples. Human-specific markers were consistently detected in sites NC2-NC5 which are experiencing urban development and serviced by septic systems.

Water samples	Level of indicators (CFU/100 mL)		PCR results (presence/absence)				
	E. coli	Enterococci	HF183	HF134	CF128	BacCan	*esp*
Occasion 1							
NC1	2.1 X 10^3	4.1 X 10^3	+	-	-	-	+
NC2	3.6 X 10^3	3.2 X 10^3	+	-	+	+	-
NC3	4.9 X 10^3	1.3 X 10^4	+	+	+	+	+
NC4	4.1 X 10^3	1.9 X 10^4	+	+	-	+	+
NC5	1.2 X 10^4	4.3 X 10^4	+	+	+	-	-
NC6	3.9 X 10^3	2.8 X 10^4	+	-	+	-	+
NC7	3.1 X 10^3	3.9 X 10^3	-	-	+	-	-
NC8	3.4 X 10^3	1.4 X 10^3	-	-	+	-	-
Occasion 2							
NC1	3.1 X 10^3	3.7 X 10^3	-	-	+	-	-
NC2	9.1 X 10^2	1.0 X 10^2	+	-	-	-	-
NC3	4.9 X 10^4	3.9 X 10^3	+	+	-	+	-
NC4	4.4 X 10^4	5.6 X 10^4	+	+	+	+	+
NC5	4.2 X 10^4	3.9 X 10^4	+	+	+	-	+
NC6	1.1 X 10^3	2.1 X 10^3	-	-	+	-	-
NC7	1.6 X 10^3	3.1 X 10^2	-	-	+	-	-
NC8	2.1 X 10^3	1.2 X 10^2	-	-	-	-	-

Conclusions

1. Host-specific PCR markers were shown to be reliable for detecting faecal pollution from humans and animal sources in Southeast Queensland, Australia.

2. Among all markers, *Bacteroides* HF183 performed well in identifying the sources of human faecal pollution.

3. As a part of the on-going research, a real-time PCR assay for human-specific markers as well as multiplex PCR assay to detect faecal pollution from human and animal host groups is being undertaken

References

[a] Bernhard and Field 2000. *Applied and Environmental Microbiology*. 66 (4):1587-1594.

[b] Kildare *et al.* 2007. *Water Research*. 41(16): 3701-3715.

[c] Scott *et al.* 2005. Environmental Science and Technology. 39(1): 283-287