

A REAL-TIME PCR ASSAY FOR QUANTITATIVE DETECTION OF THE HUMAN-SPECIFIC ENTEROCOCCI SURFACE PROTEIN MARKER IN SEWAGE AND ENVIRONMENTAL WATERS



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Summary

A real-time PCR assay was developed to quantify the *E. faecium esp* marker in sewage and environmental waters. The concentration of culturable enterococci in raw sewage samples ranged between 1.3×10^5 and 5.6×10^5 cfu/100 ml. The real-time PCR detected 9.8×10^3 to 3.8×10^4 gene copies of the *esp* marker/100 ml of sewage. Surface water samples were collected from a non-sewered catchment after storm events. Of the 16 samples tested 6 (38%) were PCR positive and the concentration of the *esp* marker ranged between 1.1×10^2 and 5.3×10^2 gene copies/100 ml of water samples. The presence of the *esp* marker in water samples immediate after storm events not only indicated human faecal pollution but also provided evidence of the degree of human faecal pollution.

Objective

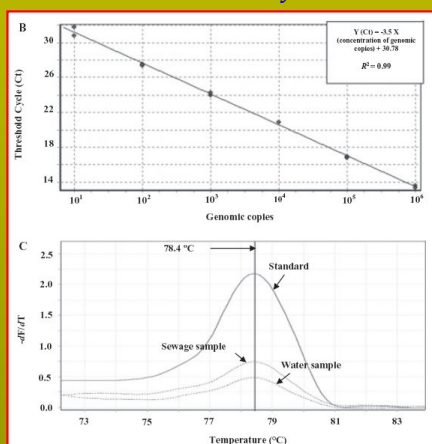
The aim of this study was to assess the host-specificity of the *esp* marker testing faecal samples from 13 animal species. A second aim was to develop a real-time PCR assay using SYBR Green I dye to quantify the marker in sewage and environmental waters to identify the magnitude of faecal pollution

Methodology

1. PCR primers : The forward primer specific for *E. faecium esp* gene was designed on the basis of the unique differences between the *E. faecalis* and *E. faecium esp* genes. The forward primer (TAT GAA AGC AAC AGC ACA AGT T) was used along with a conserved reverse primer (ACG TCG AAA GTT CGA TTT CC)

2. To determine the host-specificity of the *esp* marker, 197 faecal samples were collected from 13 animal species.

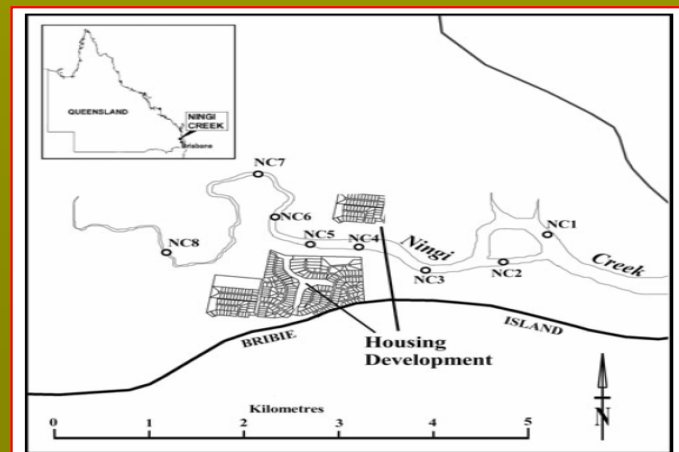
Real-time PCR assay



Standard curves generated using the *E. faecium* C68 DNA for the *esp* marker

Melting curves analysis of sewage and environmental DNA

3. Water sampling: Eight sampling sites (i.e., NC1-NC8) were chosen between upstream and downstream of the creek, and 16 grab samples were collected immediate after storm events.



Results

1. Specificity and sensitivity: The overall specificity of the *esp* marker to distinguish between sewage and animal faecal pollution was 100%. Overall, the *esp* marker was detected in 90.5% of combined sewage and septic tank samples and was not detected in any of the faecal samples from the animal species.

Host groups	PCR positive/no. of samples tested	Specificity%	Sensitivity%
Human waste	30/30	-	100%
Septic waste	8/12	-	67%
Total human	38/12	-	90.5%
Chickens	0/15	100%	-
Deer	0/10	100%	-
Dogs	0/20	100%	-
Ducks	0/15	100%	-
Goats	0/10	100%	-
Horses	0/14	100%	-
Kangaroos	0/15	100%	-
Pelicans	0/10	100%	-
Pigs	0/6	100%	-
Sheep	0/10	100%	-
Wild birds	0/10	100%	-
Cattle	0/20	100%	-
Total animals	0/155	100%	-

2. The concentrations of the human-specific *esp* markers in raw sewage.

Sewage samples	Number of enterococci (cfu/100 ml)	Real-time PCR results (gene copies/100 ml)
RS1-STP1	3.9×10^5	3.8×10^4
RS2-STP1	1.3×10^5	1.5×10^4
RS3-STP1	4.3×10^5	1.3×10^4
RS4-STP1	5.6×10^5	2.3×10^4
RS5-STP2	1.6×10^5	1.1×10^4
RS6-STP2	2.3×10^5	2.0×10^4
RS7-STP2	2.1×10^5	9.8×10^3
RS8-STP2	2.9×10^5	1.0×10^4
Geometric mean	2.6×10^5	1.6×10^4

3. Real-time PCR results of the human-specific *esp* marker in water samples collected from Ningi Creek on two events.

Water samples (events)	Number of enterococci (cfu/100 ml)	Real-time PCR results (gene copies/100 ml)
NC1 (event 1)	4.1×10^3	1.1×10^2
NC3 (event 1)	1.3×10^4	1.6×10^2
NC4 (event 1)	1.9×10^4	5.3×10^2
NC6 (event 1)	2.8×10^4	5.2×10^2
NC4 (event 2)	5.6×10^4	4.3×10^2

Conclusions

1. The *E. faecium esp* marker appears to be host-specific and promising for Human faecal pollution tracking in environmental waters in Southeast Queensland.

2. We successfully demonstrated the application of a newly developed real-time PCR assay to quantify the *esp* marker in sewage and environmental waters

3. Overall, the presence of high numbers of enterococci and the quantitative data on the human-specific *esp* marker provided evidence of the extent of faecal/human faecal pollution in an urban mixed land use catchment. Such study would provide valuable information for manager who are charged with protecting water quality