Microbial source tracking on the Colville River watershed – a test of library-independent genetic markers

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http://www.vetmed.wsu.edu/research_vmp/MicroArrayLab/

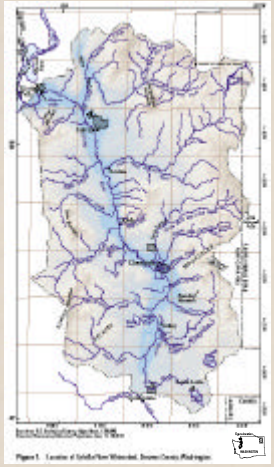
Background

Microbial source tracking (MST) is a process by which fecal water pollution is apportioned between host sources. If successful, this process should identify primary fecal contributors (e.g., humans or cows) and permit targeted mitigation. MST involves either library-dependent or library independent methods. The former requires expensive library development and has limited application across large spatial and temporal scales. Much effort is being devoted to identify library independent genetic markers for MST, but these tools remain largely untested.

In theory, application of library independent markers can be quantitative or qualitative. While quantitative approaches might seem most desirable, they can incur high cost and may not be necessary nor the most efficient option given high sampling variance that is characteristic of microbial contamination. For this reason we tested a scheme involving simple binary detection of multiple markers across multiple site visits. The underlying presence was that the most significant source of contamination will be detected most frequently.

Methods

Most of the genetic markers used in this study were developed as part of a recently completed microarray-based screen study (Soule et al. in review). PCR assays were developed for each of 15 markers and all markers were validated against fecal samples from a variety of sources. Standard methods were used to filter water samples, enumerate total enterococci, prepare template DNA, and conduct PCR tests.



Collect water; hold at 4°C until processed (within 8 h)

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Filter sample (0.1—1.0 L)
Incubate on mEi agar (41°C, 24 h)
Count colonies

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Incubate additional 24 h
Scrape colonies
Extract gDNA (Mobio soil kit)

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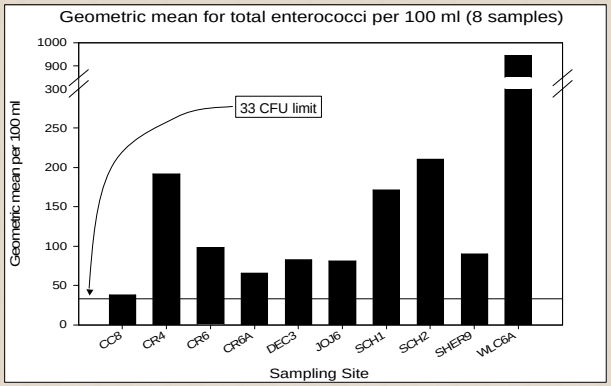
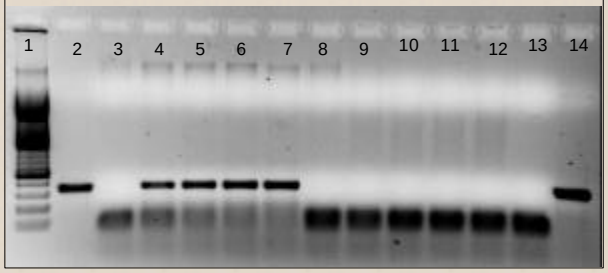
Conduct series of PCR tests for specific markers

Examples of collection sites. (A) SCH1, part of headwaters, (B) DEC3, part of headwaters, (C) CR4, intensive cattle grazing area.



Example of PCR test for marker Cow-15. Lane 1=size ladder, Lanes 2 and 14=positive controls; Lane 13=no-template-control; Lanes 3-12=samples.



References:
Scott, T. M., T. M. Jenkins, J. Lukasik, and J. B. Rose. 2005. Potential use of a host associated molecular marker in *Enterococcus faecium* as an index of human fecal pollution. Environ Sci Technol **19:**145-152.
Soule, M, EA Kuhn, F Loge, J Gay, and DR Call. *In review.* Using DNA microarrays to identify library-independent markers for bacterial source tracking.

Marker	Positive
Cow-15	34
Cow-19	56
Deer-40	30
Deer-48	46
Deer-89	30
Deer-90	30
Deer-91	30
Deer-93	30
Deer-94	30
ESP	22
Human-107	26
Human-66	2
Human-67	1
Human-68	0
Human-77	15
Human-81	0
Pos+7	80

Fifteen markers from Soule et al. (in review) and one marker from Scott et al. (2005) (ESP) plus a positive control were tested at 10 samples sites with 8 visits per site. Six of the deer markers were discontinued after the first three sample events because they were ubiquitous in this assay system. Four human markers were largely uninformative (66, 67, 68, and 81). The two cattle markers were significantly correlated (r=0.66, P=0.03) while remaining markers were not correlated.

Sites were ranked based on the total number of positive detection events. Rankings were lowest (=median score yellow), or highest (>median score; red).

Site	Activity	Avg Temp	Human	Cow	Deer
CR6	Concentrated grazing	17.0	10	12	6
CR4	Concentrated grazing	16.9	8	11	3
SHER9	Tributary, local septic	12.8	13	4	2
JOJ6	Cottage lake	18.9	7	7	5
CR6A	Concentrated grazing	17.8	6	14	5
SCH1	Headwater, grazed	16.1	3	12	5
SCH2	Headwater, grazed	16.9	4	9	7
WLC6A	Tributary, limited flow	16.2	4	4	6
DEC3	Headwater, grazed	14.4	6	8	4
CC8	USDA FS, grazed	10.5	5	9	3

Conclusions

- No gold standard exists by which to judge the diagnostic specificity and sensitivity of MST markers, but we can make some subjective judgments. JOJ6—mostly recreational homes—and SHER9—where we detected odor of raw sewage—scored high for human markers. CR6 is adjacent to a field that receives human effluent waste. Areas experiencing the highest cattle grazing pressure (CR4, CR6, and CR6A; estimated 3-4k head collectively) scored high for cattle markers.
- Presence of specific markers does not provide us with any information abou other sources of fecal pollution. For example, the high fecal indicator count for WLC6A may have originated from deer, but other livestock (e.g., horses) may have been present upstream from the sample location.
- Not all putative markers were suitable for a purely presence/absence detection scheme. Deer markers 89-94 originated from *Enterococcus mundtii* and this organism may be well suited to an environmental lifestyle considering that all five markers were ubiquitous in our first 3 sample efforts
- Absence of specific markers does not preclude any given source. Multiple markers may be needed to increase the probability of detecting a specific source. Three of seven human markers were uninformative in this study.
- Presence/absence detection of library-independent markers may be most useful for relative comparisons of fecal contamination between multiple sites or when one host is disproportionately represented across multiple sample visits. More work is needed to determine if PCR-based MST is cost effective

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