Applied and Environmental Microbiology, June 2010, p. 4116–4117 Vol. 76, No. 12
0099-2240/10/$12.00 doi:10.1128/AEM.00025-10

Hollow-Fiber Ultrafiltration and PCR Detection of Human-Associated Genetic Markers from Various Types of Surface Water in Florida▽

Stephaney D. Leskinen,[1] Miriam Brownell,[2] Daniel V. Lim,[1] and Valerie J. Harwood[2]*

Department of Cell Biology, Microbiology and Molecular Biology, University of South Florida, Tampa, Florida 33620,[1] and Department of Integrative Biology, University of South Florida, Tampa, Florida 33620[2]

Received 5 January 2010/Accepted 21 April 2010

Hollow-fiber ultrafiltration (HFUF) and PCR were combined to detect human-associated microbial source tracking marker genes in large volumes of fresh and estuarine Florida water. HFUF allowed marker detection when membrane filtration did not, demonstrating HFUF's ability to facilitate detection of diluted targets by PCR in a variety of water types.

Microbial source tracking (MST) uses analytical methods to determine the possible source(s) of fecal contamination in environmental waters (reviewed in reference 14). Human fecal pollution (i.e., sewage) is known to contain pathogens that pose serious health risks (6, 8). Identifying sewage impacts can prevent illness, through public warnings and environmental remediation. Detection of sewage has been demonstrated with genetic markers from microorganisms associated with humans (3, 4, 7, 11, 15), each of which has pros and cons (5, 14). A useful addition to MST would be the concentration of samples when the standard protocol (e.g., filtering 500 ml) (5) is not feasible due to turbidity of the water or dilution of the target(s). Hollow-fiber ultrafiltration (HFUF) concentrates particulates from water, with little increase in total assay time (9), while simultaneously retaining parasites, bacteria, and viruses (13). Dead-end HFUF of culturable enterococci in recreational water was successfully demonstrated in Florida (9) and California (10). The objective of this study was to assess the utility of HFUF for concentration of higher-volume samples that would permit detection of diluted MST markers from surface water samples by PCR. Three markers representing a variety of microbial types were targeted: (i) human-associated *Bacteroidales* (*Bacteria*), (ii) human polyomaviruses (HPyVs; virus), and (iii) *Methanobrevibacter smithii* (*Archaea*).

Water samples were collected in Hillsborough Country, FL. Freshwater originated from the unimpacted headwaters of the Hillsborough River (28°5′56.98″N, 82°18′44.05″W) and estuarine water from remediated Ben T. Davis Beach (27°58′5.71″N, 82°34′26.46″W). Sewage-impacted water samples were collected from Sweetwater Creek (27°59′57.27″N, 82°33′37.10″W), which received approximately 500,000 gallons of sewage from a sewer main break. Samples were collected within 5 days and 45 days of the spill. The 5-day sample was collected after the spill was reported, while the 45-day sample was collected to test a more diluted sample of the same water. Raw sewage (influent) for spiked samples was collected on the day of the HFUF experiment from a Hillsborough County wastewater treatment plant that processes 9 million gallons daily.

Initial HFUF samples consisted of Dulbecco's phosphate-buffered saline (D-PBS; pH 7.4) spiked with 5 ml of raw sewage (5×10^{-4} dilution). Spiked environmental samples included 10 liters of ambient water spiked with 5 or 2.5 ml of raw sewage. Fecal indicator bacterium (FIB) concentrations of the unamended samples were determined by membrane filtration using standard methods for *Enterococcus* spp., *Escherichia coli* (16), and fecal coliforms (2) to demonstrate the ambient microbial water quality at the sites. Water samples were first tested for existing MST markers before spiking with sewage. Ten-liter samples of unamended ambient water (Ben T. Davis Beach and Sweetwater Creek) were collected to assess the method's ability to detect diluted targets following method verification with spiked experiments. Subsequent sewage spikes into unamended samples were performed to ensure that any lack of detection using the HFUF protocol was not due to inhibition. Water samples were collected for processing by the standard protocols from each site in concert with HFUF samples (5). The Sweetwater Creek sample allowed only 300 ml to be filtered instead of the standard 500 ml due to its high turbidity (160 nephelometric turbidity units [NTU]).

The design and operation of the recreational dead-end concentrator (Rec DEC) have been previously described (9, 10). A new F80A Hemoflow polysulfone high-flux capillary dialyzer (Fresenius Medical Care North America, Lexington, MA) was used for each sample. Samples were fed into the filters by a peristaltic pump in approximately 6.5 min. All Rec DEC components were cleaned between sample feedings with 10% bleach followed by 10% sodium thiosulfate. Elution of each filter was performed using 250 ml of 4 M urea-50 mM lysine (pH 9.0) that was incubated in the filter fibers for 2 min before collection of the retentate.

The pH of each retentate was adjusted to 3.5 to retain HPyVs (11, 12), and then 25 ml was filtered through a 0.45-μm-pore-size nitrocellulose membrane. Each membrane was placed into a Mo Bio PowerSoil microcentrifuge tube (Mo Bio Laboratories, Carlsbad, CA), and extraction of total DNA was performed as previously described (5). Conventional PCR was used to determine the presence or absence of MST markers. Assays targeted a species-specific region of the 16S rRNA gene

* Corresponding author. Mailing address: Department of Integrative Biology, 4202 E. Fowler Ave., SCA 110, University of South Florida, Tampa, FL 33620. Phone: (813) 974-1524. Fax: (813) 974-1524. E-mail: vharwood@usf.edu.

▽ Published ahead of print on 30 April 2010.

TABLE 1. PCR detection of human-associated MST markers from surface water samples following HFUF with Rec DEC

Sample type	Date (mo/day/yr)	Sewage spike (ml)	Detection of human-associated markers[c]		
			Human *Bacteroides*	*M. smithii*	HPyV
D-PBS	02/05/08	5	P	P	P
	02/20/08	5	P	P	P
Hillsborough River	08/06/08	5	P	P	P
	09/08/08	5	P	P	P
	10/01/08	2.5	P	P	P
Ben T. Davis Beach	03/27/09	0	A	A	A
		2.5	P	P	P
Sweetwater Creek	04/02/09	0	P[a]	P[b]	P[a]
	05/05/09	0	A[b]	P[b]	P[b]
		2.5	P	P	P

[a] Marker detected using previously published membrane filtration protocol.
[b] Marker absent using previously published membrane filtration protocol.
[c] P, present; A, absent.

of human-associated *Bacteroidales* (3) with slight modifications (5), the conserved T antigen of human BK and JC polyomaviruses (1, 5), or the *nifH* gene of *Methanobrevibacter smithii* (5, 15).

Each marker was detected in all HFUF spiked samples (Table 1). The procedure was also successful in detecting the markers with the reduced sewage spike volume (2.5 ml) for the Ben T. Davis Beach and Sweetwater Creek samples (Table 1). Detection of smaller amounts of spiked sewage was not attempted in this study; however, previous work determined detection limits of 10^{-3} to 10^{-4} dilution for HPyVs and *M. smithii* (5) and 10^{-5} to 10^{-6} dilution for *Bacteroidales* (3, 5).

Ambient FIB levels were highest at Sweetwater Creek followed by Ben T. Davis Beach and the Hillsborough River, as expected. No markers were detected with the ambient beach water alone; however, the combined procedure was able to detect each marker in the first unamended Sweetwater Creek sample (5-day sample). PCR of samples processed using the standard protocol failed to detect *M. smithii*. HPyVs and *M. smithii* were detected in the second unamended Sweetwater Creek sample (45-day sample) following HFUF alone.

HFUF combined with PCR detected human-associated MST markers in both fresh and estuarine waters. The combination was particularly advantageous over membrane filtration because much higher volumes could be sampled when dilution of sewage had occurred. The absence of the human *Bacteroidales* marker on 5 May 2009 coupled with the presence of the other markers may be due to different decay rates of the targets or to the physical fate of the markers when they enter the water. Sweetwater Creek is tidal, and microbes that tend to be transported to sediments would likely persist longer than those remaining in the water column. HFUF's ability to permit detection of diluted PCR targets in impacted waters can increase the sensitivity of PCR-based methods in environmental waters, which could contribute to applications such as total maximum daily load (TMDL) assessments and implementation and beach monitoring. Future studies utilizing HFUF combined with PCR for MST makers should focus on determining the public health significance of detecting these markers from higher-volume samples in relation to the presence of microbial pathogens in such samples.

This study was funded in part by a grant from the U.S. Army Research, Development, and Engineering Command and by U.S. EPA Gulf of Mexico Alliance Regional Partnership Project Grant MX-96478707-0.

REFERENCES

1. **Aksamit, A. J.** 1993. PCR detection of JC virus, p. 361–366. *In* T. F. Smith, D. H. Persing, F. C. Tenover, and T. J. White (ed.), Diagnostic molecular microbiology: principles and applications. Mayo Foundation, Rochester, MN.
2. **APHA.** 2005. Standard method for the examination of water and wastewater, p. 66–69. *In* Fecal coliform membrane filter procedure, 21st ed., vol. 9. American Public Health Association, Washington, DC.
3. **Bernhard, A. E., and K. G. Field.** 2000. A PCR assay to discriminate human and ruminant feces on the basis of host differences in *Bacteroides-Prevotella* genes encoding 16S rRNA. Appl. Environ. Microbiol. **66:**4571–4574.
4. **Brownell, M. J., V. J. Harwood, R. C. Kurz, S. M. McQuaig, J. Lukasik, and T. M. Scott.** 2007. Confirmation of putative stormwater impact on water quality at a Florida beach by microbial source tracking methods and structure of indicator organism populations. Water Res. **41:**3747–3757.
5. **Harwood, V. J., M. Brownell, S. Wang, J. Lepo, R. D. Ellender, A. Ajidahun, K. N. Hellein, E. Kennedy, X. Ye, and C. Flood.** 2009. Validation and field testing of library-independent microbial source tracking methods in the Gulf of Mexico. Water Res. **43:**4812–4819.
6. **Harwood, V. J., A. D. Levine, T. M. Scott, V. Chivukula, J. Lukasik, S. R. Farrah, and J. B. Rose.** 2005. Validity of the indicator organism paradigm for pathogen reduction in reclaimed water and public health protection. Appl. Environ. Microbiol. **71:**3163–3170.
7. **Korajkic, A., B. D. Badgley, M. J. Brownell, and V. J. Harwood.** 2009. Application of microbial source tracking methods in a Gulf of Mexico field setting. J. Appl. Microbiol. **107:**1518–1527.
8. **Lee, D. Y., H. Lauder, H. Cruwys, P. Falletta, and L. A. Beaudette.** 2008. Development and application of an oligonucleotide microarray and real-time quantitative PCR for detection of wastewater bacterial pathogens. Sci. Total Environ. **398:**203–211.
9. **Leskinen, S. D., and D. V. Lim.** 2008. Concentration and biosensor detection of enterococci from recreational water. Appl. Environ. Microbiol. **74:**4792–4798.
10. **Leskinen, S. D., V. J. Harwood, and D. V. Lim.** 2009. Rapid dead-end ultrafiltration concentration and biosensor detection of enterococci from beach waters of Southern California. J. Water Health **7:**674–684.
11. **McQuaig, S. M., T. M. Scott, V. J. Harwood, S. R. Farrah, and J. O. Lukasik.** 2006. Detection of human-derived fecal pollution in environmental waters by use of a PCR-based human polyomavirus assay. Appl. Environ. Microbiol. **72:**7567–7574.
12. **McQuaig, S. M., T. M. Scott, J. O. Lukasik, J. H. Paul, and V. J. Harwood.** 2009. Quantification of human polyomaviruses JC virus and BK virus by TaqMan quantitative PCR and comparison to other water quality indicators in water and fecal samples. Appl. Environ. Microbiol. **75:**3379–3388.
13. **Morales-Morales, H. A., G. Vidal, J. Olszewski, C. M. Rock, D. Dasgupta, K. H. Oshima, and G. B. Smith.** 2003. Optimization of a reusable hollow-fiber ultrafilter for simultaneous concentration of enteric bacteria, protozoa, and viruses from water. Appl. Environ. Microbiol. **69:**4098–4102.
14. **Stoeckel, D. M., and V. J. Harwood.** 2007. Performance, design, and analysis in microbial source tracking studies. Appl. Environ. Microbiol. **73:**2405–2415.
15. **Ufnar, J. A., S. Y. Wang, J. M. Christiansen, H. Yampara-Iquise, C. A. Carson, and R. D. Ellender.** 2006. Detection of the *nifH* gene of *Methanobrevibacter smithii*: a potential tool to identify sewage pollution in recreational waters. J. Appl. Microbiol. **101:**44–52.
16. **U.S. Environmental Protection Agency.** 2000. Improved enumeration methods for the recreational water quality indicators: enterococci and *Escherichia coli*, EPA/821/R-97/004. EPA Office of Science and Technology, Washington, DC.