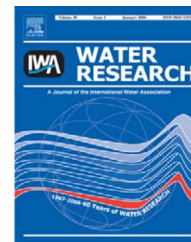
WATER RESEARCH 40 (2006) 3719–3724

Available at www.sciencedirect.com

journal homepage: www.elsevier.com/locate/watres



A rapid reverse transcription-PCR assay for F^+ RNA coliphages to trace fecal pollution in Table Rock Lake on the Arkansas–Missouri border

S.K. Dryden[1], *B. Ramaswami*[1], *Z. Yuan, D.E. Giammar, L.T. Angenent**

Department of Energy, Environmental & Chemical Engineering, Washington University in St. Louis, St. Louis, Missouri 63130, USA

A R T I C L E I N F O

Article history:
Received 13 February 2006
Received in revised form
24 July 2006
Accepted 4 September 2006
Available online 27 October 2006

Keywords:
Bacteriophages
Fecal indicators
F^+ RNA
MPN
RT-PCR

A B S T R A C T

Source determination of fecal contamination is imperative to efficiently reduce the fecal material load to environmental waters. This study developed primer pairs targeting three F^+ RNA bacteriophages and a simple filtration sampling method to enumerate and identify coliphages in environmental waters. Water samples were collected seasonally for one year from the watershed of Table Rock Lake on the Arkansas–Missouri border in areas predisposed to fecal contamination. Collected samples were analyzed quantitatively with most probable number and plaque assays and qualitatively with reverse transcription-PCR. We demonstrated the usefulness of F^+ RNA coliphages as an indicator of fecal contamination, but were unable to distinguish between human and non-human sources. F^+ coliphage numbers in Table Rock Lake showed seasonal variation with the highest level of coliphage presence during the January sampling event.

© 2006 Elsevier Ltd. All rights reserved.

1. Introduction

Elucidating the source of a fecal contaminant is imperative to controlling pollution. A variety of methods targeting bacteria, viruses, and biological macromolecules have been used to distinguish between fecal pollution of human and non-human origin. For example, workers have developed microbiological and molecular methods that include an initial bacterial culture originating from mammalian and bird intestines. These methods include: fecal coliforms to fecal streptococci ratios (Geldreich and Kenner, 1969); *Bacteroides* sp. presence (Kreader, 1995); *Escherichia coli* ribotypes (Carson et al., 2001) and antibiotic resistance patterns (Harwood et al., 2000). Such assays, however, are time consuming, labor intensive, and require extensive culture collections.

Detection of certain host-specific markers with molecular biology assays does not require culturing of bacteria, and therefore are a more precise and rapid methodology for identifying sources of fecal pollution. Host molecular markers include specific nucleic acid sequences of bacteriophages (i.e., viruses of bacteria) infecting *Bacteroides fragilis* and *Bacteroides thetaiotaomicron*, which are mammalian gut bacteria (Blanch et al., 2004; Puig et al., 1999; Puig et al., 2000; Tartera et al., 1989). The absence of *B. fragilis* phages in polluted waters in the United States; however, limit the usefulness of this marker (Havelaar et al., 1993; Jagals et al., 1995; Puig et al., 1999; Scott et al., 2002). Researchers identified specific RNA coliphages (i.e., bacteriophages that infect *E. coli*) from human and non-human fecal material, suggesting that these phages can also be used to distinguish between human and non-human fecal sources of pollution (Cole et al., 2003; Havelaar and Hogeboom, 1984; Luther and Fujioka, 2004).

Coliphages have been characterized into two groups: somatic, which infect through the cell wall, and male-specific

*Corresponding author. Tel.: +1 314 935 5663; fax: +1 314 935 5464.
E-mail address: angenent@seas.wustl.edu (L.T. Angenent).
[1] Contributed equally.
0043-1354/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.watres.2006.09.003

(F^+), which infect through sex pili. To discriminate between fecal sources, workers have also typed F^+ coliphages into four sub-groups that are either highly associated with humans (groups II and III) or non-humans (groups I and IV) (Scott et al., 2002). Historically, typing was performed with serotyping assays after several viral isolation steps, including concentration of phages from environmental samples, isolation and purification of phages with single or double agar layer plaque assay methods, and propagation in broth (Havelaar and Hogeboom, 1984; Havelaar et al., 1993; Sobsey et al., 1990). However, the methods of F^+ RNA coliphage serotyping have been shown to produce ambiguous results (Beekwilder et al., 1996). For this reason, researchers have developed a method that utilizes reverse transcription-polymerase chain reactions (RT-PCR) or PCR and a subsequent reverse-line blot hybridization technique for genotyping of F^+ RNA or F^+ DNA coliphages, respectively (Vinje et al., 2004). Both serotyping and genotyping suffer from the need to isolate viruses, which can be time consuming.

The overarching goal of this study was to develop a simple and reliable technique to detect and identify the source of fecal contamination in environmental waters. The study developed primers for a RT-PCR technique to differentiate between fecal sources without the need for viral isolation and membrane hybridization. A suite of three PCR primer pairs specific for F^+ RNA coliphages was designed to discriminate between human and non-human fecal pollution after a propagation step. We tested this method with samples collected from source-rich surface waters in the Table Rock Lake watershed on the Arkansas–Missouri border. The RT-PCR technique was used to identify bacteriophages, while single agar layer (SAL) Petri dish techniques and a traditional most probable number (MPN) assay were used to quantify bacteriophages. Samples gathered for microbiological analysis were collected via direct sampling with filtration to discard bacteria and debris.

2. Materials and methods

2.1. Bacterial strains and bacteriophages

Bacteriophages MS2, GA and SP were used as a reference in all experiments for groups I, II and IV, respectively. *E. coli* strain C-3000 (ATCC 15597) was used as a host strain and grown in minimal media (6.0-g Na_2HPO_4, 1.0-g NH_4Cl, 3.0-g KH_2PO_4, 10-mL 10% glucose in 1 L) supplemented with 1-mL 1 M $MgSO_4$, and 100-mg thiamine. *E. coli* and bacteriophage MS2 (ATCC 15597-B1) were obtained from the American Type Culture Collection (ATCC; Manassas, VA). Bacteriophages GA and SP were provided by Dr. Mark Sobsey, University of North Carolina, Chapel Hill, NC.

2.2. Sample collection

Sample sites (see web-based supplementary data) for specific contaminants from chicken farms, private septic systems, and municipal treatment facilities, and a background site were selected through a multicriteria geospatial information systems (GIS) analysis (data not shown). Sampling locations and events for October 2004 through January 2005 were the same as described in Yuan et al. (2006). In addition to the full sample sets gathered from October 2004 to January 2005, a subset of samples was collected in May and August 2005. We sampled once per season to assess the effects of seasonal variation in source loadings and lake dynamics. During each sampling event, environmental samples were directly collected into sterile 250-mL polypropylene bottles and stored at 4 °C for subsequent laboratory analysis. An additional protocol using granular activated carbon (Jothikumar et al., 1995) was also performed during the October 2004 and January 2005 sampling events (see web-based supplementary data).

2.3. Sample preparation

The directly collected samples were filtered through 0.22 μm pore-size filters (Stericup, Millipore, Billerica, MA). A SAL plaque assay was used to enumerate the presence of bacteriophages. First, 100 mL of the filtered sample was mixed with tepid minimal media agar and 2 mL of host bacteria, and then plated into Petri dishes and incubated for 24 h. Translucent areas in the SAL plates were counted. A modified MPN assay (Eaton et al., 1998) was also conducted for each sample. A serial dilution of a 10-fold gradient was performed into minimal media with the bacterial host. For the October 2004 and January 2005 samples, triplicate MPN vials with 2, 20, and 200 $\times$ dilutions of the sample were assessed. Following an insufficient dilution of the January samples, five replicates of MPN vials with 2, 20, 200, and 2000 $\times$ dilutions of the sample were assessed for May and August samples.

2.4. RT-PCR bacteriophage identification

Positive MPN tubes were extracted using a TRI REAGENT LS protocol for RNA isolation according to the manufacturer's specifications (Sigma, St. Louis, MO). The resulting RNA pellet was suspended in 5 μL of TE Buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0), 1 μL was used for reverse transcription and the remaining solution was stored at −20 °C.

Multiple primer alignments based on the complete genomic sequences of F^+ RNA phages (MS2, GA and SP) were constructed using Clustal X Multiple Sequence Alignment Program version 1.81 (University of British Columbia Bioinformatics Centre, Vancouver, Canada). Primer pairs were designed using Primer3 (Whitehead Institute for Biomedical Research, Cambridge, MA), NetPrimer (Premier Biosoft International, Palo Alto, CA), and OligoAnalyzer 3.0 (IDT-DNA, Coralville, IA) primer evaluation software. The primers's alignment and specificity were also checked with Amplify 3.1 (University of Wisconsin, Madison, WI).

Extracted environmental samples and bacteriophage standards were used to identify the bacteriophages present via a RT-PCR procedure. Extracted RNA was transcribed into cDNA with reverse transcriptase. A 10-μL solution with 1 μL of extracted sample and 1 μL of random primers was thermally denatured for 10 min at 70 °C and then chilled on ice. These templates were added to a 10-μL solution composed of 100-μM dNTP mix, 5 U of placental RNase inhibitor, 1.5 U of avian myeloblastosis virus reverse transcriptase, and reverse transcriptase reaction buffer. The suspension was transcribed at

Download English Version:

https://daneshyari.com/en/article/4485821

Download Persian Version:

https://daneshyari.com/article/4485821

Daneshyari.com