Available online at www.sciencedirect.com

SciVerse ScienceDirect

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

Fecal source tracking in water using a mitochondrial DNA microarray

Nguyet-Minh Vuong [a,c], Richard Villemur [b], Pierre Payment [b], Roland Brousseau [a,c], Edward Topp [d], Luke Masson [a,c,*]

[a] Biotechnology Research Institute, National Research Council of Canada, Montreal, QC H4P 2R2, Canada
[b] INRS-Institut Armand-Frappier, 531 Boulevard des Prairies, Laval, QC H7V 1B7, Canada
[c] Departement of Microbiology and Immunology, University of Montreal, Montreal, QC H3T 1J4, Canada
[d] Agriculture and Agri-Food Canada, 1391 Sandford Street, London, ON N5V 4T3, Canada

ARTICLE INFO

Article history:
Received 11 April 2012
Received in revised form
28 July 2012
Accepted 3 September 2012
Available online 4 October 2012

Keywords:
Mitochondria
DNA microarray
Source tracking
Fecal
Peptide nucleic acid
PCR clamping
Quantitative PCR

ABSTRACT

A mitochondrial-based microarray (mitoArray) was developed for rapid identification of the presence of 28 animals and one family (*cervidae*) potentially implicated in fecal pollution in mixed activity watersheds. Oligonucleotide probes for genus or subfamily-level identification were targeted within the 12S rRNA – Val tRNA – 16S rRNA region in the mitochondrial genome. This region, called MI-50, was selected based on three criteria: 1) the ability to be amplified by universal primers 2) these universal primer sequences are present in most commercial and domestic animals of interest in source tracking, and 3) that sufficient sequence variation exists within this region to meet the minimal requirements for microarray probe discrimination. To quantify the overall level of mitochondrial DNA (mtDNA) in samples, a quantitative-PCR (Q-PCR) universal primer pair was also developed. Probe validation was performed using DNA extracted from animal tissues and, for many cases, animal-specific fecal samples. To reduce the amplification of potentially interfering fish mtDNA sequences during the MI-50 enrichment step, a clamping PCR method was designed using a fish-specific peptide nucleic acid. DNA extracted from 19 water samples were subjected to both array and independent PCR analyses. Our results confirm that the mitochondrial microarray approach method could accurately detect the dominant animals present in water samples emphasizing the potential for this methodology in the parallel scanning of a large variety of animals normally monitored in fecal source tracking.

1. Introduction

Fecal pollution monitoring in water quality assessments is of great significance for public health as the major source of waterborne pathogens is related to the large number of pathogenic microorganisms associated with fecal material (Lemarchand et al., 2004; Santo Domingo et al., 2007; USEPA, 2007). In addition, over 60% of emerging infectious disease events are caused by the transmission of an infectious agent from animals (zoonoses), with 75% of these originating from wildlife (Atlas et al., 2010). By ingestion or contact with contaminated water, humans can contract gastroenteritis, as

* *Corresponding author*. Biotechnology Research Institute, 6100 Royalmount Avenue, National Research Council of Canada, Montreal, QC H4P2R2, Canada. Tel.: +1 514 496 3123; fax: +1 514 496 7251.
E-mail address: luke.masson@nrc-cnrc.gc.ca (L. Masson).
0043-1354/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.watres.2012.09.011

well as ear, urinary tract, eye, nose and skin infections (Brunkard et al., 2011; Griffin et al., 2003; Hrudey et al., 2003; Stewart et al., 2007). Aside from the threat to public health, fecal contamination of water can have broad economic impacts through beach closures, closures of fisheries, and public health costs associated with waterborne disease.

Due to the plethora of fecal-associated pathogens, the microbiological quality of water is primarily monitored using fecal indicator organisms, such as total coliforms, fecal coliforms, *Escherichia coli*, enterococci and *Clostridium perfringens* (Edge and Schaefer, 2006; Lemarchand et al., 2004; Stewart et al., 2007; Stoeckel and Harwood, 2007). *E. coli* is the most commonly used indicator as it is believed to be present only in the intestine of warm-blooded animals, and cannot survive for extended periods of time outside its host. However, the utility of this indicator is being questioned because of recent data suggesting its adaptation and growth within environmental matrices (Keramas et al., 2003; Kon et al., 2007; Luo et al., 2011). Another important consideration is that fecal coliform indicators do not identify the source of the fecal matter, a crucially important criterion for implementing effective, preventive, or remedial strategies as well as helping to mitigate conflicts between stakeholder groups (Field and Samadpour, 2007). In mixed activity watersheds, fecal pollution is generally diffuse, with potential sources including runoff from feedlots, manure-amended crop land or pastures, mammalian or avian wildlife, malfunctioning septic systems, urban runoff, and sewage discharge (Santo Domingo et al., 2007; Simpson et al., 2002).

Numerous microbial source tracking (MST) methods have been developed over the last two decades to address the issue of host-specific contributions of fecal contamination to watersheds (Field and Samadpour, 2007; Meays et al., 2004; Simpson et al., 2002; Stoeckel and Harwood, 2007). Labour intensive library-dependent methods involve comparing a microbial reference library representative of the fecal sources found within a specific aquatic ecosystem to microorganisms from suspected fecal pollution sources. However, drawbacks of this approach rest with the large number of isolates required to generate reference banks, high costs associated with routine monitoring, and sensitivity to the mode of statistical analysis (Stoeckel and Harwood, 2007; Lasalde et al., 2005).

Library-independent MST methods that exploit host-specific genetic markers to indicate the presence of fecal contamination from a specific human or animal host are slowly replacing library-dependent methods (Edge and Schaefer, 2006). Although some success has been found with various bacterial (and viral) genes as human and some animal-specific fecal indicators (Hsu et al., 1995; Bernhard and Field, 2000b), the search for specific reliable markers has become a challenge and consequently only a few animal markers are currently available; wildlife species and domestic animals being poorly represented.

The use of mitochondrial DNA (mtDNA) for discriminating between fecal pollution sources has emerged as a promising animal-specific marker due to three key factors: (i) mtDNA is highly specific to animals with low intra-species variation; (ii) it is abundant in feces and is excreted in the environment (Martellini et al., 2005; Iyengar et al., 1991); and (iii) mitochondrial sequence information is readily available in DNA databases for many animals. Indeed various methods exploiting the direct detection of animal gut contents have been developed for mtDNA of several animal species in water, such as conventional and nested-PCR (Martellini et al., 2005), multiplex-PCR (Martellini et al., 2005; Caldwell et al., 2007), quantitative-PCR (Caldwell et al., 2007; Schill and Mathes, 2008; Caldwell and Levine, 2009) and macroarray dot blots (Kortbaoui et al., 2009).

In the present study, we describe the development of a mitochondrial genome-targeted detection DNA microarray (mitoArray) to simultaneously screen for the presence of mtDNA from 28 animals and one family (*cervidae*) in water. As DNA detection microarrays do not possess sufficient sensitivity to detect low concentration targets in environmental samples directly, the utilization of two conserved regions in the 12S and 16S ribosomal RNA regions of the mitochondrial chromosome allowed the development of two universal primer sets and the amplification of animal specific-amplicons from water samples to increase sensitivity. Two of these universal primers were also validated in order to quantify the total mtDNA concentration within a sample by Q-PCR. The animals under investigation represent potential sources of fecal pollution, including human, wildlife, waterfowl, domestic and livestock animals and the use of the detection array could provide an easy pre-screening of environmental samples as a prelude to targeting quantitative determination of positive animal inputs. The microarray's performance was validated using DNA extracted from animal tissues as well as fecal and water samples. A complete protocol for water analysis using this approach is described.

2. Materials and methods

2.1. *Fecal coliform enumeration and target DNA source*

Fecal coliforms were enumerated using the membrane filtration protocol by Clescerl et al. (1999). Basically, 100 ml of different sample dilutions were vacuum-filtered through a sterile 0.45-μm filter. The filters were placed on FC medium to determine fecal coliform bacteria after incubation at 44.5 °C for 24 h.

2.1.1. *Total DNA*

DNA from human, cow, pig and poultry blood samples were purchased from Novagen (WI, USA). Mouse total DNA was extracted from a C2C12 skeletal myoblast cell line (provided by the Gene therapy group, BRI-NRC, Montreal, QC). Canada goose, gull, pigeon and cormorant total DNAs were extracted from liver or blood samples provided by Environment Canada (Burlington, Ontario, CA). White-tailed Deer, muskrat, beaver, raccoon, and coyote DNA were extracted from various tissues provided by Agriculture and AgriFood Canada (London, Ontario, CA). Cat and dog DNA were extracted from saliva samples. Fish DNA samples were extracted from various fish species purchased from local merchants. All other animal DNAs: horse, sheep, red deer, quail, turkey, ostrich, emu, moose, goat, rabbit, caribou, and duck were extracted from