

# A DGGE- based *E. coli* community profiling method for bacterial source tracking.



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## ABSTRACT

### OBJECTIVES:

Current bacterial source tracking (BST) methods are limited in their ability to match contaminated sinks with potential sources. The objective of this study was to identify target genes that could differentiate potential sources and match them to polluted sinks. Thirteen gene fragments common to *E. coli* were screened for their:

- distribution in 176 environmental *E. coli* isolates.
- sensitivity of detection in pure cultures of *E. coli*.
- fingerprint-based differentiation of *E. coli* communities.
- ability to match sources to contaminated sinks.

### METHODS:

The distribution of 13 gene fragments (23S rRNA, *gadAB*, *lacZ*, *lamB*, *uidR*, *mdh*, *phoE*, 2 fragments of 16S rRNA and 4 of *uidA*) was assessed by PCR of DNA extracted from 176 environmental *E. coli* isolates. The sensitivity of detection was determined by PCR following serial dilution of an *E. coli* culture. Each gene fragment was screened with DGGE for its ability to differentiate (i) artificial *E. coli* assemblages of differing composition and (ii) total *E. coli* communities grown from animal fecal material. The genes that showed higher differentiation profiles were tested for their ability to match single sources of contamination to their sinks. For this purpose, we collected water and fecal material samples from confined small lakes dominated by birds and effluent from a waste water treatment plant.

### RESULTS:

PCR detection of the 13 gene fragments showed that all fragments were 100% distributed among the *E. coli* isolates except for *uidA1066* (97.1%) and *16SE1/E2* fragments (95.4%). Three fragments of *uidA* (298, 754 and 1939) and the 16S rRNA gene fragment provided the lowest detection limit ( $10^3$  cells  $ml^{-1}$ ) in pure cultures. Cluster analysis of DGGE fingerprints showed that the *phoE* and *uidA1939* gene fragments most effectively differentiated mixed *E. coli* communities and indicated the potential of these two gene fragments to match pollution sinks to potential sources.

### CONCLUSIONS:

- All gene fragments were 100% distributed except for *16SE1/E2* and *uidA1066*.
- The lowest detection level ( $10^3$  cell  $ml^{-1}$ ) was achieved by: 16S, *uidA* 298, 754 and 1939.
- Both *phoE* and *uidA1939* showed high differentiation and matching capabilities.
- Targeting *phoE* or *uidA1939* for *E. coli* community-based DGGE could be used to differentiate *E. coli* communities from different animals and to match sources to sinks. Hence, the method developed here has a potential application in BST studies.

## METHODS

### Distribution, Sensitivity and differentiation

*E. coli* (n = 176) isolated from Lake Erie Beach (OH) by specific media and confirmed to be *E. coli* by PCR for *E. coli*-specific 16S rRNA gene segment.

Distribution and sensitivity of detection for the 13 gene fragments was assessed by PCR.

The ability of the gene fragments to differentiate mixed *E. coli* communities was assessed by DGGE of:

Artificial assemblages of *E. coli* DNA



"Whole" *E. coli* communities grown from different animal fecal material



### Matching specific sources to contaminated sinks

Matching was performed by PCR-DGGE on total DNA extracted from "whole" *E. coli* communities grown from:

➔ Bird fecal material and lake water

➔ Waste water treatment effluent and creek water



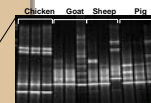
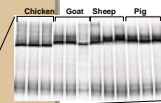
## RESULTS

### Distribution, Sensitivity and differentiation

The table below shows the distribution, sensitivity of detection and differentiation of *E. coli* communities (artificial and natural communities grown from different animal fecal material) for the thirteen gene fragments:

- ➔ All gene fragments were 100% distributed in 176 *E. coli* isolates except for *16SE1/E2* and *uidA1066*
- ➔ The lowest detection ( $10^3$  cells  $ml^{-1}$ ) was achieved by: 16S, *uidA298*, 754 and 1939 gene fragments.
- ➔ The highest ability to differentiate "whole" *E. coli* communities (both artificial assemblages and Communities grown from fecal material of different animals) was achieved by: *phoE* and *uidA1939*

Gene Fragment	Distribution (% out of 176 <i>E. coli</i> )	Sensitivity (cells $ml^{-1}$ )	Differentiation of "whole" <i>E. coli</i> communities	
			Artificial Assemblages	Animal fecal material
16S E1/E2	95.4	$10^4$	Low	Low
<i>uidA1066</i>	97.1	$10^4$	Low	Low
23S	100	$10^4$	Low	Low
<i>gadA/B</i>	100	$10^4$	Low	Low
<i>lamB</i>	100	$10^4$	Low	Low
<i>lacZ</i>	100	$10^4$	Intermediate	Intermediate
<i>mdh</i>	100	$10^4$	Low	Low
<i>phoE</i>	100	$10^4$	High	High
<i>uidR</i>	100	$10^4$	Intermediate	Intermediate
16S	100	$10^3$	Low	Low
<i>uidA298</i>	100	$10^3$	Intermediate	Intermediate
<i>uidA754</i>	100	$10^3$	Intermediate	Intermediate
<i>uidA1939</i>	100	$10^3$	High	High



### Matching specific sources to sinks

Both *PhoE* and *uidA1939* were able to show that:

- ➔ *E. coli* communities from bird fecal material are contributing to water contamination in the lake. Dendrogram analysis showed that these communities are similar to each other at 90% threshold (Fig.1).

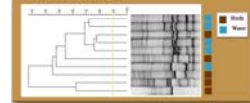


Figure 1: Dendrogram analysis of *phoE* DGGE fingerprints obtained from "whole" *E. coli* communities isolated from bird fecal material and lake water.

- ➔ *E. coli* communities from the waste water treatment plant effluent were identical to the communities found downstream of the effluent discharge, however they can be separated from the upstream communities (Fig 2). Thus, the effluent appears to be contributing new *E. coli* to the original *E. coli* communities found in the creek.

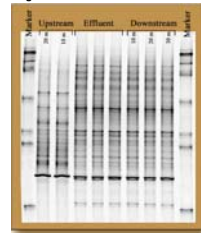


Figure 2: *uidA1939* DGGE gel showing fingerprints of "whole" *E. coli* communities isolated from effluent, upstream (10 and 20 m) and downstream (10, 20 and 30 m) water samples.