Screening Potential *Escherichia coli* Gene Targets for DGGE-Based Bacterial Source Tracking

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Esseili M.A, Sigler W.V., and Kassem I.I.
Laboratory for Microbial Ecology
University of Toledo, Toledo, OH

Contact information:
Email: m_esseili@yahoo.com
Lab phone: 419-530-4581

ABSTRACT

BACKGROUND

The frequent occurrence of bacterial pollution has been the concern of those that depend on natural waters for recreation and commerce. Detection of fecal pollution indicators has been limited by inconclusive methodologies that are resource-intensive or limited in their ability to match pollution sources with sinks. Our overall aim is to develop an *E. coli* community fingerprinting method that can rapidly differentiate pollution sources while potentially matching true sources with sinks. Therefore, this study aimed to (i) assess the environmental distribution of genes commonly used to detect *E. coli*, (ii) select the genes that provided the best fingerprint-based detection and differentiation of mixed *E. coli* communities, and (iii) test the sensitivity of these genes.

METHODS

Water samples were collected from three different locations at Lake Erie Beach (Ohio), which has experienced frequent episodes of elevated *E. coli* levels. One hundred and Seventy-six *E. coli* isolates were identified using modified m-TEC and eosin methylene blue media and confirmed by PCR using *E. coli*-specific 16S rDNA primers. The distribution of 13 gene fragments (16S rRNA, 23S rRNA, *gadAB*, *lacZ*, *lamB*, *uidR*, *mdh*, *phoE*, and 4 fragments of *uidA*) was assessed by performing gene-specific PCR assays on all *E. coli* isolates. Each gene fragment was screened for its ability of to differentiate mixed *E. coli* communities by first constructing three artificial *E. coli* assemblages, which were generated by combining DNA from 8 *E. coli* isolates determined to be of differing phylotype by BOX-PCR. The assemblages were subjected to PCR-DGGE using primers specific for each of the 13 gene fragments to determine the gene most effective to discern mixed *E. coli* assemblages. To determine the resolving capability of each gene in natural *E. coli* communities, PCR-DGGE was performed on DNA extracted from total *E. coli* grown from animal fecal material. The sensitivity of each of the 13 gene fragments to detect *E. coli* was determined following serial dilution of *E. coli,* and direct PCR amplification.

RESULTS

PCR detection of the 13 gene fragments showed that except for *uidA* 1066 (97.1%) and *16S* E1/E2 (95.4%), all fragments were distributed among 100% of the *E. coli* isolates. PCR-DGGE showed that *lacZ, phoE* and *uidA*1939 gene fragments were useful to show clear differences among artificial *E. coli* assemblages as well as among *E. coli* communities grown from animal fecal material. The three fragments of *uidA (298,754 and 1939)* and the *16S* rRNA gene provided the greatest sensitivity of *E. coli* detection in pure cultures. PCR of theses genes detected as little as 10^2 *E. coli* cells.

CONCLUSIONS

We conclude that among the different gene fragments tested, *lacZ, phoE* and *uidA 1939* performed best to generate the most discerning and representative profiles. However, *16S rRNA, uidA 1939, uidA 754, uidA 298* gene fragments were found to be the most sensitive for *E. coli* detection in pure cultures.

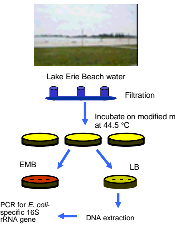
Therefore, the *uidA 1939* gene fragment was found to give the best results when the three criteria: distribution, sensitivity and differentiation were collectively considered. This study strongly suggests the need for further investigations to identify further targets to detect pollution indicators such as *E. coli*.

OBJECTIVES

- Assess the environmental distribution of gene fragments commonly used to detect *E. coli*.
- Select the gene fragment that provides the best fingerprint-based detection and representation of mixed *E. coli* communities using DGGE.
- Determine the sensitivity of detection of the targeted gene fragments in serially diluted *E. coli* cultures.

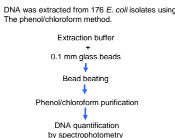
MATERIALS and METHODS

Sampling and *E. coli* isolation



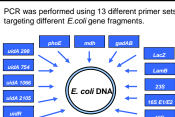
DNA Extraction

DNA was extracted from 176 *E. coli* isolates using The phenol/chloroform method.



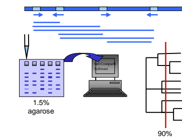
PCR to study gene distribution

PCR was performed using 13 different primer sets targeting different *E.coli* gene fragments.



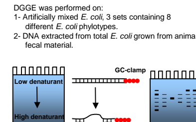
BOX-PCR

BOX-PCR analysis was performed on all *E. coli* isolates using BOX A1R primers. Fingerprints were considered to have derived from identical *E. coli* strains if they were at least 90% similar.



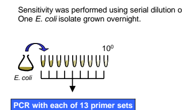
DGGE

DGGE was performed on:
1- Artificially mixed *E. coli*, 3 sets containing 8 different *E. coli* phylotypes.
2- DNA extracted from total *E. coli* grown from animal fecal material.



Sensitivity

Sensitivity was performed using serial dilution of One *E. coli* isolate grown overnight.



RESULTS

Gene distribution

All gene fragments were distributed among 100% of the 176 *E. coli* isolates except:

- *uidA* 1066: 97.1 %
- 16S E1/E2: 95.4%

BOX PCR

Cluster analysis of 176 BOX-PCR fingerprints at a threshold of 90% similarity (as determined by the Pearson correlation coefficient) revealed:

- 66 unique *E.coli* phylotypes **(Fig. 1)**.

DGGE: artificial assemblages

DGGE of 3 sets (A,B and C) of 8 artificially mixed *E.coli* (different phylotypes) revealed:

- Low discriminatory profiles with: 23S,16S, *lamB*, *mdh*,16SE1/E2, *uidA 1066* and *gadAB* **(Fig. 2)**.
- Intermediate discriminatory profiles with: *uidR, uidA 754* and *uidA 298*
- High discriminatory profiles with: *lacZ, phoE* and *uidA 1939* **(Fig. 3)**.

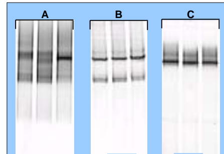


Fig. 2

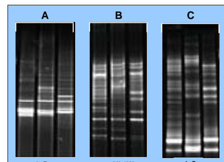


Fig. 3

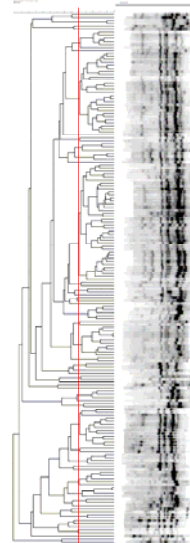


Fig. 1 Dendogram analysis of BOX-PCR fingerprints generated from 176 *E. coli* isolates. The number *E. coli* phylotypes was determined at the 90% similarity threshold

DGGE: fecal material

DNA was extracted from fecal samples of the following animals: chicken, goat, sheep and pig.
DGGE for each of the 13 gene fragments revealed the following discriminatory profiles :

- Low discrimination of *E. coli* communities in different animals: *23S, lamB,16S,16SE1/E2, uidA1066, gadAB* and *uidA 298* **(Fig. 4)**.
- Intermediate discrimination of *E. coli* communities in different animals : *mdh, uidR* and *uidA 754*.
- High discrimination of *E. coli* communities in different animals : *lacZ, phoE* and *uidA1939* **(Fig. 5)** .

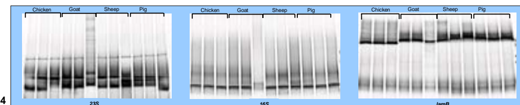


Fig. 4

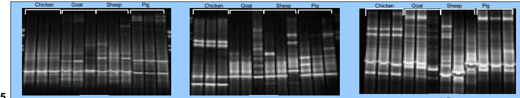


Fig. 5

Gene detection sensitivity (Table 1)

PCR detection of serially diluted *E. coli* cells revealed:

- High sensitivity: *16SE1/E2*, and *uidA 1066*
- Intermediate sensitivity : *23S, gadAB, lamB, lacZ, mdh, phoE* and *uidR.*
- Low sensitivity : *16S, uidA 298, 754* and *1939.*

Table1: Summary of primer sensitivity for *E. coli* detection

Gene fragment	16S E1/E2	UidA 1066	23S	gadAB	lamB	lacZ	mdh	PhoE	UidR	16S	UidA 298	UidA 754	UidA 1939
Detection limit / cell	10[illegible]	10[illegible]	10[illegible]	10[illegible]	10[illegible]	10[illegible]	10[illegible]	10[illegible]	10[illegible]	10[illegible]	10[illegible]	10[illegible]	10[illegible]

CONCLUSIONS

- ***lacZ, phoE*** **and** ***uidA1939*** **performed best to generate the most discriminatory fingerprint profiles in artificially mixed** ***E. coli*** **and total** ***E. coli*** **grown from animal fecal material.**
- ***uidA*** **(298,754 and 1939) and the** ***16S*** **rRNA gene fragments yielded the greatest sensitivity of** ***E. coli*** **detection in pure cultures.**
- **The** ***uidA1939*** **gene fragment was found to give the best results when the three criteria: distribution, sensitivity and differentiation were collectively considered.**