

# DGGE analysis of *E. coli* community structure as a potential bacterial source tracking tool: a comparison with BOX-PCR

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## INTRODUCTION

In 1986, the USEPA adopted recreational water quality criteria for bacteria based on levels of *E. coli* as an indicator of fecal pollution (USEPA, 1986). In response to the continual need to identify sources of bacterial pollution, library independent and -dependent methods have evolved that compare and characterize *E. coli* populations. Recently, it was shown that library independent methods outperformed library-based methods in their ability to identify/exclude any of five different fecal sources artificially added to a water sample (Griffith et al., 2003). As currently utilized, library independent methods provide information concerning only the presence or absence of a given genetic marker. However, library independent methods have not been used to gather information regarding the structure of the bacterial community, an attribute of bacterial pollutants that might be useful for rapid sample comparisons to match potential sources with sinks.

Recent work showed that denaturing gradient gel electrophoresis (DGGE) could differentiate laboratory isolates of *E. coli* based on polymorphisms in the sequence of the *uidA* gene (Farnleitner et al., 2000). *uid* codes for the  $\beta$ -glucuronidase gene, which has been shown to be an appropriate molecular target for *E. coli* detection (Bej et al., 1991). Therefore, it follows that DGGE of *uidA* might be useful to rapidly compare *E. coli* populations originating from point and non-point pollution sources without the need for isolate library development.

The objectives of this study were to:

1. Compare the resolution of *E. coli* isolates by DGGE of *uidA* with that of an established, library dependent method, BOX-PCR.
2. Determine the ability of DGGE to identify differences in mixed *E. coli* populations between two matrices at two natural sites.

## MATERIALS AND METHODS

- Water (W) and sediment (S) samples were collected from three locations at Lake Erie Beach (LEB) and the Inland Lake (IL) at Maumee Bay State Park (Oregon, Ohio, Figure 1) and pooled. Sample identification was established according to beach name and collection location (e.g. LEB-W, Lake Erie Beach water; IL-S, Inland Lake sediment, etc.).

Figure 1. Lake Erie Beach (left) and the beach at the Inland Lake (right).



- Presumptive *E. coli* were cultured from water samples according to the modified *E. coli* method (USEPA, 2000). Sediment samples were also processed with the USEPA method following dilution in buffer.

- Isolates were designated as *E. coli* that were magenta in color on modified m-TEC agar, metallic green on eosin methylene blue agar, and whose DNA produced a PCR product of proper size following amplification using *E. coli*-specific PCR primers (Sabat et al., 2000).

- BOX-PCR analysis was performed on all *E. coli* isolates using BOX A1R primers as previously described (de Bruijn, 1992).
- BOX-PCR fingerprint pattern similarity was determined by calculating the Pearson correlation coefficient. Fingerprints were considered to have derived from identical *E. coli* strains if they were at least 90% similar.
- PCR of *uidA* genes was performed with primers UAL-1939 and UAR-2105 according to the method of Bej et al. (1991).

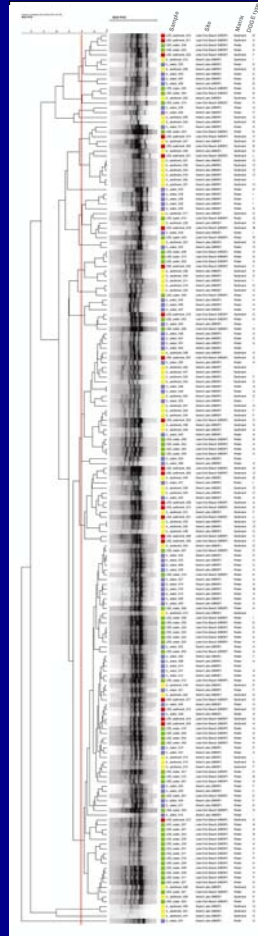


Figure 2. Dendrogram analysis of BOX-PCR fingerprints generated from Lake Erie Beach and Inland Lake water and sediment. The red line indicates the 90% similarity threshold used to determine the number of *E. coli* phylotypes.

- DGGE of the *uidA* gene amplified from each isolate was performed as described previously (Sigler and Zeyer, 2002) in a denaturant concentration range of 47 to 54%.
- The ability of DGGE to effectively discriminate differing populations of mixed *E. coli* phylotypes was assessed on two levels;
  - (i) Four artificial "communities" generated by combining DNA from eight, randomly-picked *E. coli* isolates from LEB W, LEB S, IL W, or IL S.
  - (ii) Natural communities comprised of cultured *E. coli* washed from membranes of filtered water and sediment dilution incubated on modified m-TEC medium.

## RESULTS AND DISCUSSION

- Cluster analysis of 180 *E. coli* BOX-PCR fingerprints revealed 21 unique phylotypes (assuming a 90% similarity threshold, Figure 2), while DGGE of *uidA* genes revealed 17 unique phylotypes (Figure 3). These results suggest that the resolution of *E. coli* phylotypes by DGGE of *uidA* is comparable to that of BOX-PCR.

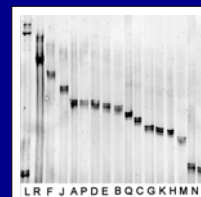


Figure 3. DGGE separation of the 17 *E. coli* phylotypes detected in this study. Letters correspond to "DGGE type" in Figure 2.

- Using BOX-PCR, between 67.1% (IL W) and 47.8% (LEB S) of the isolates were correctly classified into their respective environments (Table 1). The mixture of diffuse sources represented by the non-host environment can at least partially explain the low rates of correct classification as compared with those established in previous host-based studies (Dombek et al., 2000; Carson et al., 2003).

Table 1. Jackknife analysis of BOX-PCR-based classification of *E. coli* isolates.

Host class	<i>E. coli</i> patterns (%) assigned to class			
	LEB W	LEB S	IL W	IL S
LEB W	56.5	17.4	12.1	13.7
LEB S	5.7	47.8	8.6	9.8
IL W	17.0	8.7	67.1	21.6
IL S	18.9	26.1	12.1	54.9

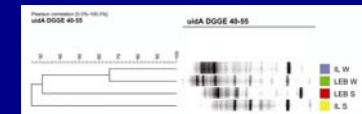


Figure 4. Dendrogram analysis of DGGE fingerprints generated from *uidA*-PCR of artificial *E. coli* communities.

- Dendrogram analysis revealed that DGGE of *uidA* effectively differentiated the four artificial communities (Figure 4) as well as the mixed *E. coli* communities cultured from LEB and IL water and sediment (Figure 5).

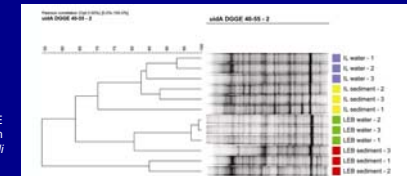


Figure 5. Dendrogram analysis of DGGE fingerprints generated from *uidA*-PCR of natural *E. coli* communities.

## CONCLUSIONS

BOX-PCR and DGGE analyses indicated that LEB- and IL- water and sediments harbored diverse populations of *E. coli*.

Both analyses detected a similar number of *E. coli* phylotypes (BOX-PCR, 21; DGGE, 17), suggesting that DGGE of *uidA* provided comparable isolate resolution to BOX-PCR.

The effectiveness of BOX-PCR to classify *E. coli* strains to non-host environments appeared to be limited. In contrast, DGGE of *uidA* was effective in differentiating and classifying mixed *E. coli* populations.

According to our results, future efforts should be directed toward optimizing the use of DGGE as a tool to rapidly compare potential bacterial pollution sources and sinks on the basis of *E. coli* population structure.

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