DGGE-based assessment of structural differences in environmental *Escherichia coli* communities

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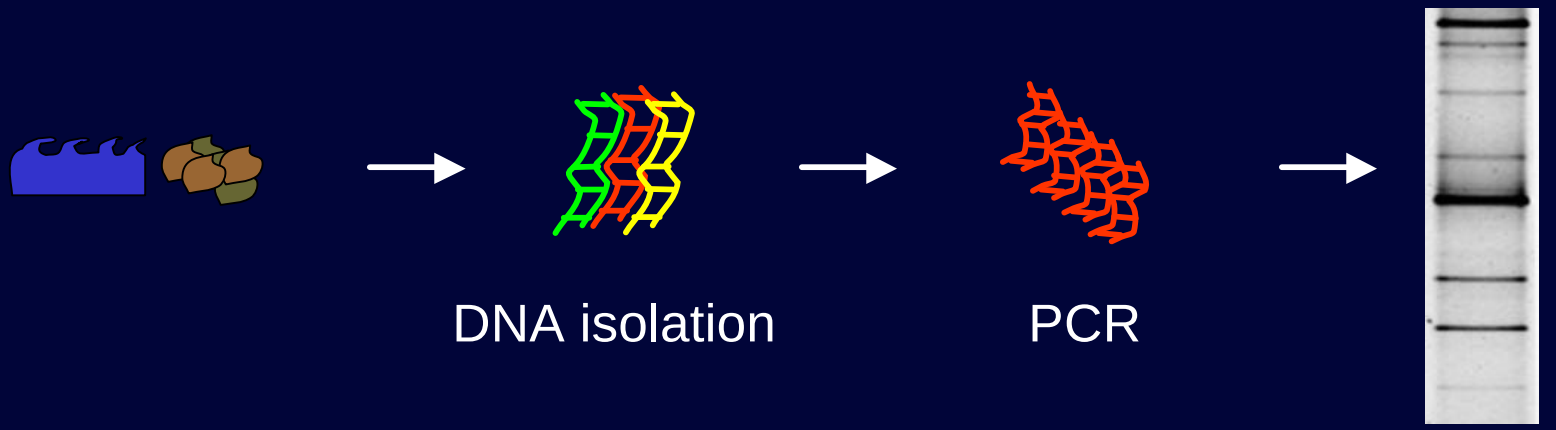
WATER QUALITY ADVISORY
BACTERIAL LEVELS HERE CURRENTLY
EXCEED STATE STANDARDS.
CHILDREN, ELDERLY & THOSE IN
ILL HEALTH ARE ADVISED NOT TO SWIM.

Methodology to study *E. coli* host origins and ecology

- Library-dependent
  - Match phenotypic or genotypic patterns in bacteria occurring in libraries generated from polluted sites with those from known sources.
    - Match source isolates to sink isolates
    - Resource-consuming
- Library-independent
  - Detection of genetic sequences in DNA isolated directly from environmental bacterial populations.
    - No library necessary
    - Qualitative

Can the best attributes of both classes of methodology be harnessed in one tool?

Denaturing gradient gel electrophoresis (DGGE)



- Fingerprints can be used to rapidly match sink communities to those sources most likely contributing to the pollution.

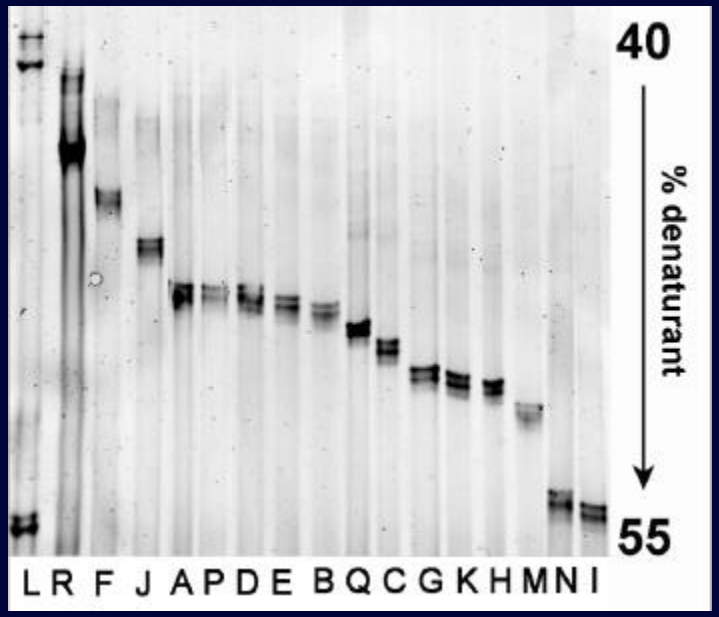
Research Objectives

- Test the ability of DGGE to rapidly distinguish mixed *E. coli* communities of differing composition.
- Apply DGGE to determine the role of sediments in *E. coli* transport throughout Maumee Bay (OH).

Experimental outline: *E. coli* library

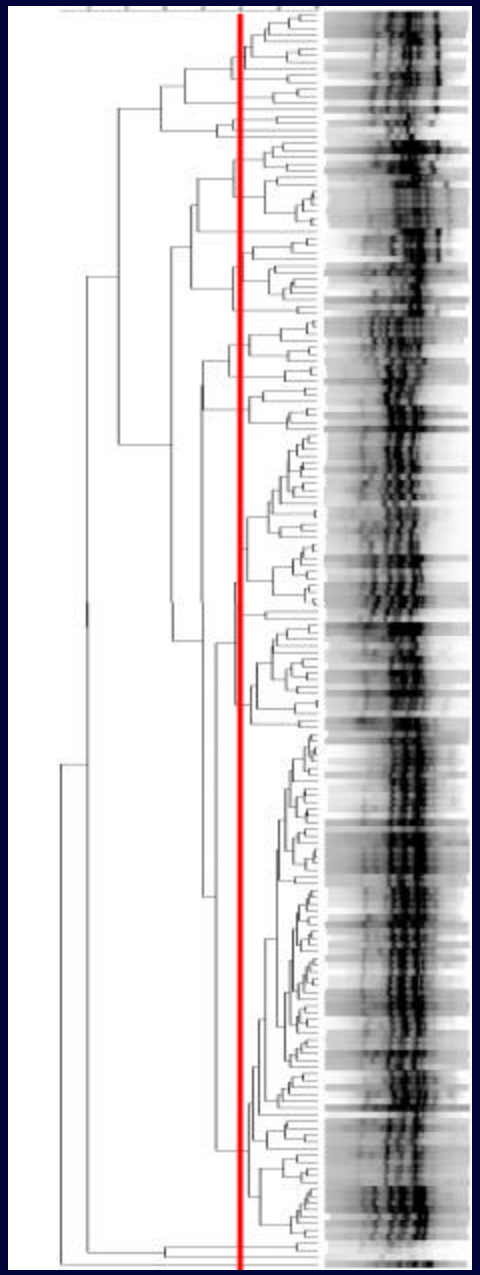
- Maumee Bay State Park (OH)
  - Water and sediment
- 184 confirmed *E. coli*
  - Modified m-TEC
  - EMB agar
  - 16S rRNA PCR

- Determine the number of distinct *E. coli* phylotypes detected by DGGE.
  - PCR-amplify the *uidA* gene from each *E. coli* isolate.
    - *uidA* exhibits sequence polymorphism (Farnleitner et al., 2000).
- DGGE of each isolate resulted in detection of 18 unique phylotypes.

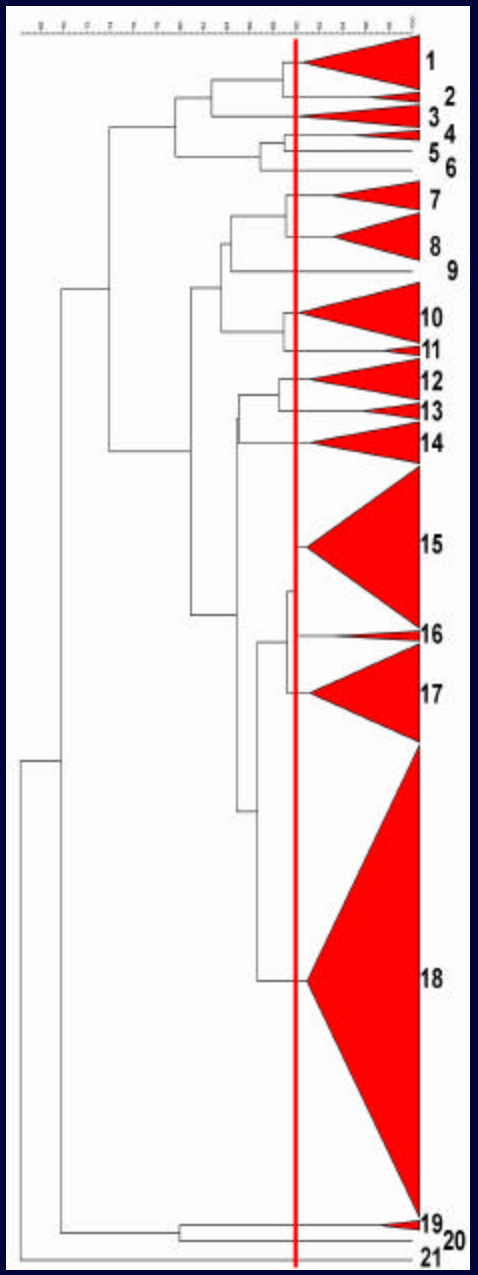


Good or bad?

- BOX-PCR performed on each *E. coli* isolate.
  - Fingerprints were compared to determine phylotype similarity.
  - Clusters that formed above 90% similarity were collapsed to yield phylotype number.

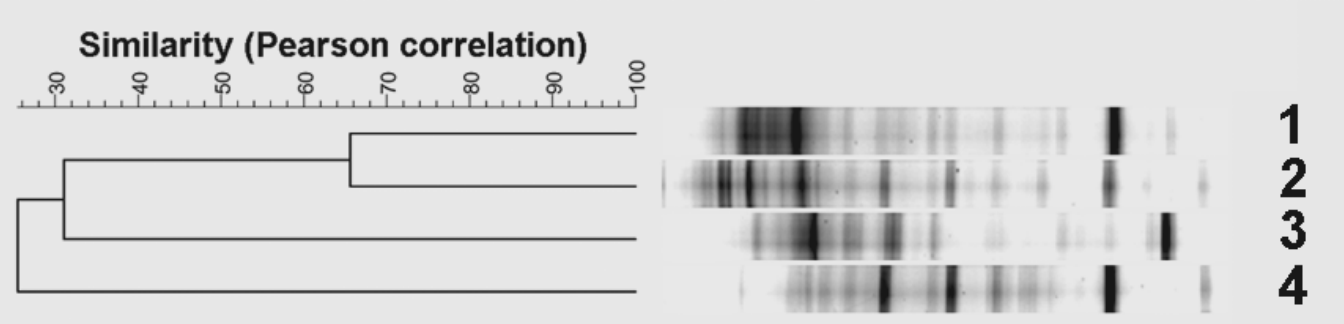


- BOX-PCR identified 21 *E. coli* phylotypes.
  - Compare with 18 phylotypes detected by DGGE of *uidA* from isolates.
  - What about mixed communities?



Experimental outline: lab-scale analysis

- Four artificial communities were constructed from DNA isolated from random *E. coli.*
  - PCR of *uidA*
  - DGGE

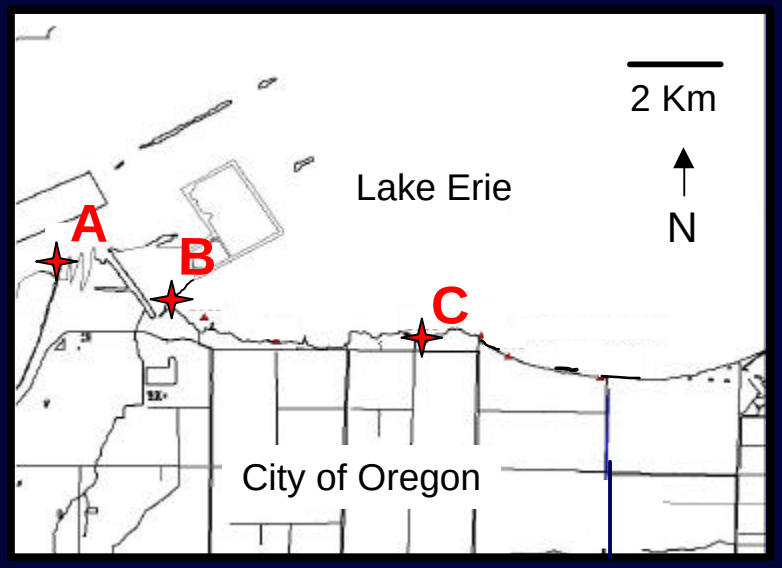


Therefore, DGGE of *uidA:*

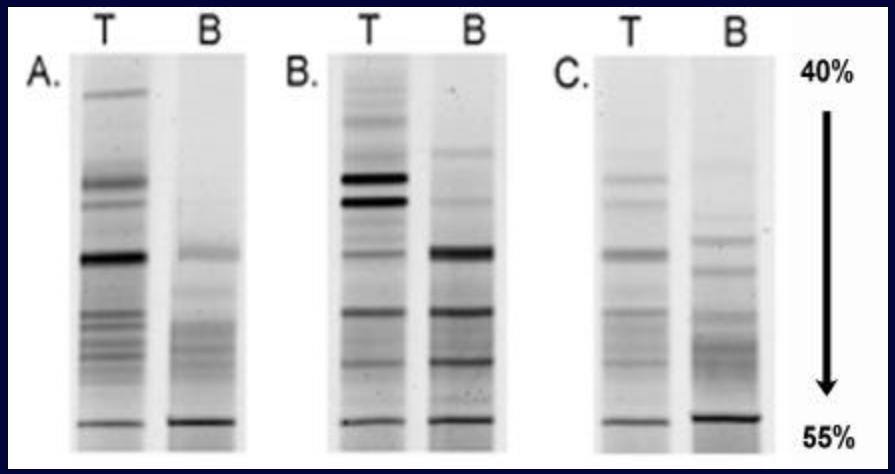
- Resolution is similar to BOX-PCR at the isolate level.
- Useful to differentiate mixed communities.

Application: *E. coli* transport

- Determine the role of sediments in *E. coli* transport throughout Maumee Bay (OH).
  - Suspended sediments transport *E. coli* communities to new locations throughout the bay.



- Isolate DNA
  - Trapped sediment
  - Bottom sediment
- PCR-DGGE of *uidA*
- *E. coli* communities in trapped sediments are different than those in bottom sediments.
  - Supports sediment-mediated transport
  - Trapped sediments appear to harbor a greater number of *E. coli* phylotypes.

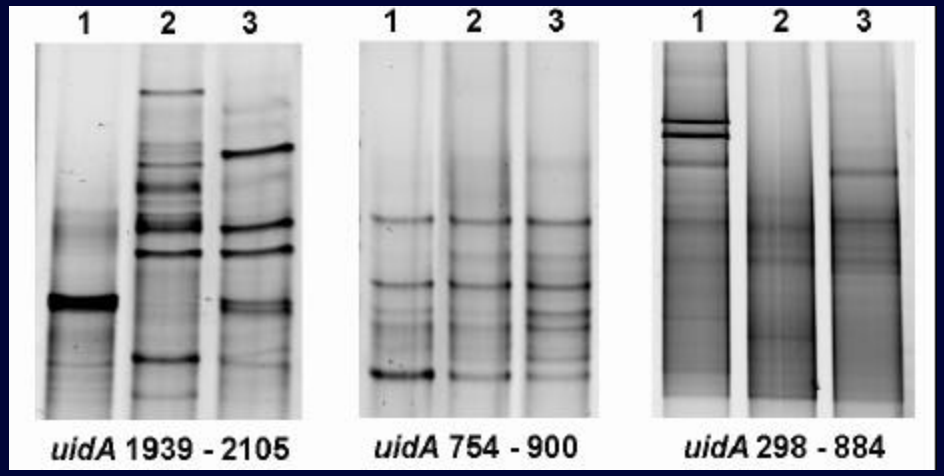


Older, less viable inputs

Newer, more viable inputs

Further studies and applications

- Screen other *E. coli* gene targets



- Develop DGGE as a screening tool
  - Couple sink *E. coli* populations with those from the most likely sources.
- Facilitation of ecological studies
  - Fate and survival of *E. coli*
  - Genetic markers to identify species-specific *E. coli* strains

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