Molecular fingerprinting and bacterial source tracking: small victories and future promise

Von Sigler
Laboratory for Microbial Ecology
University of Toledo

LME
laboratory for microbial ecology

Acknowledgements/further information

- Malak Esseili - Ph.D. student
  - Poster OT9 - A DGGE- based *E. coli* community profiling method for bacterial source tracking
- Issmat Kassem - Ph.D. student
  - Poster OT15 - Multiplex PCR-based detection and genotyping of Staphylococcus aureus in environmental matrices
- Xixi Huang – M.S. student

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PARADE

Must Our Beaches Disappear?

A Report By Don Harrison

Plus... A School That's Breaking The Cycle of Poverty • Building Friendships Online • Actor Stanley Tucci

Outline

- Library dependent techniques
  - Requirements
  - Case study

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- Library dependent techniques
  - Requirements
  - Case study
- Library independent techniques
  - General information
  - DGGE
  - Case studies
- Ideas for thought

Case studies

- Library-dependent
  - Is a common source contributing to bacterial pollution in two Ohio lakes?

Case studies

- Library-dependent
  - Is a common source contributing to bacterial pollution in two Ohio lakes?
- Library-independent
  - Can community fingerprinting pinpoint the origins of bacterial pollution?
  - Identifying proximal bacterial pollution sources in Maumee Bay
  - Identifying proximal bacterial pollution sources in central Lake Erie (Cleveland)

Library dependent techniques

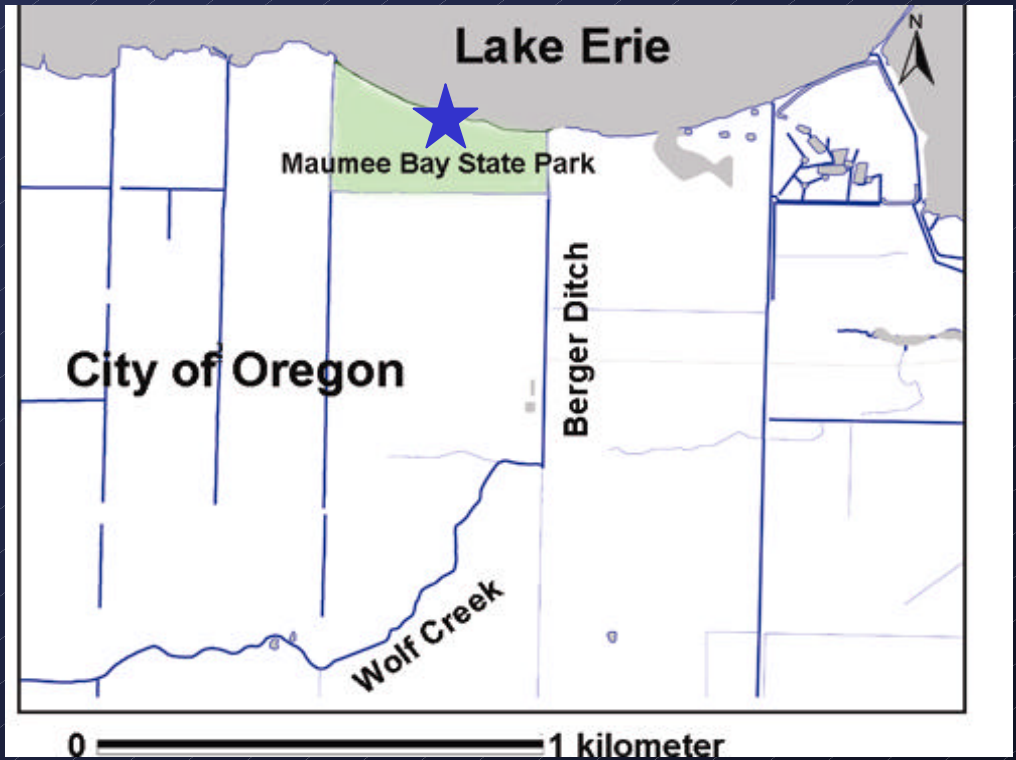
- Based on the matching of traits in bacteria isolated from the pollution sink with those from potential sources

Library dependent techniques

- Based on the matching of traits in bacteria isolated from the pollution sink with those from potential sources
- Requires the development of a host-origin database
  - Total number of isolates needed for statistical validation is unclear (from several hundred to a few thousand, per site)
  - Traits can be phenotypic…
    - Antibiotic resistance analysis (ARA)
  - …or genotypic
    - DNA fingerprinting

Library construction

- Consider the prominent point and non-point sources



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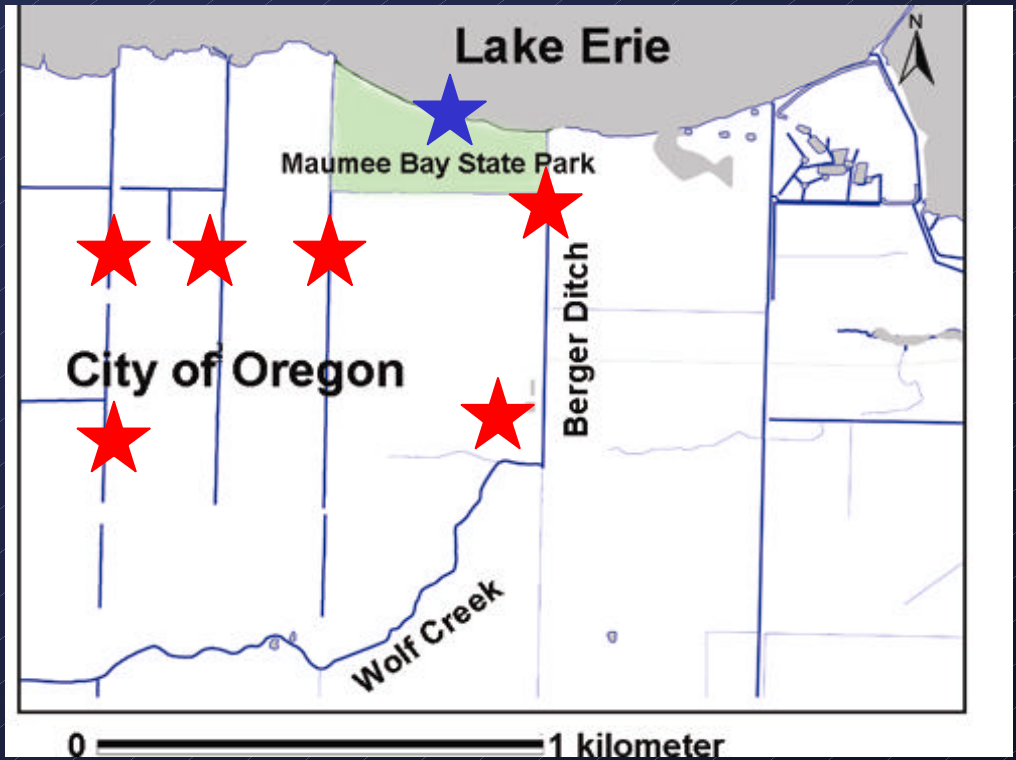
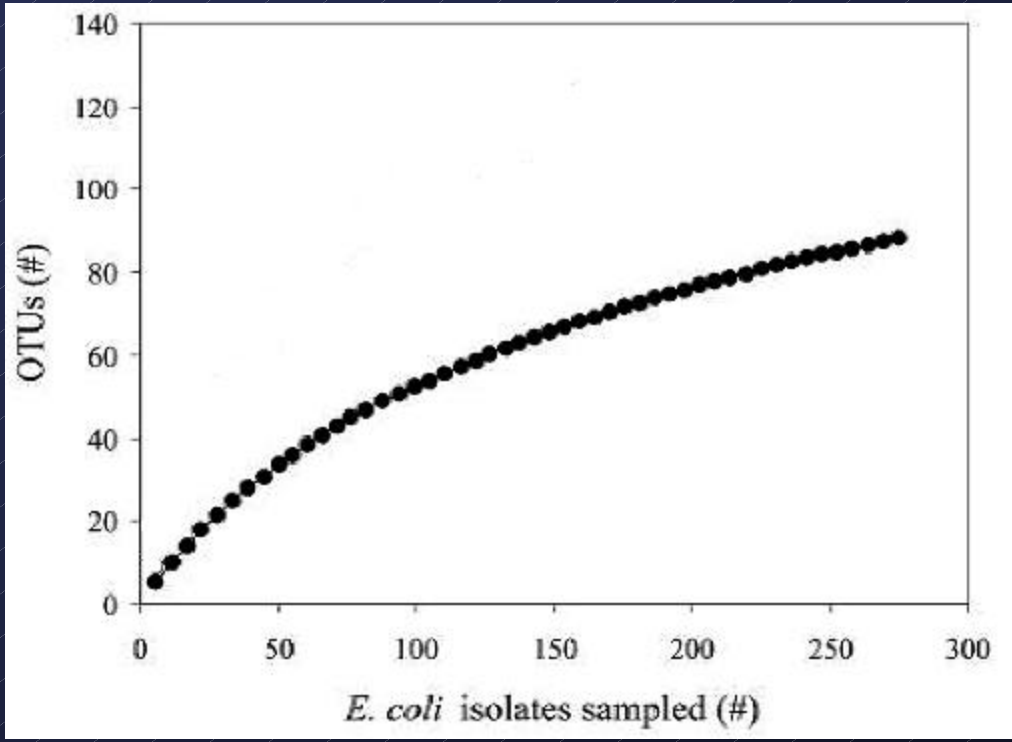


Library construction

- Consider the prominent point and non-point sources
- How many isolates are necessary?



From: Yang et al., 2004

Library construction

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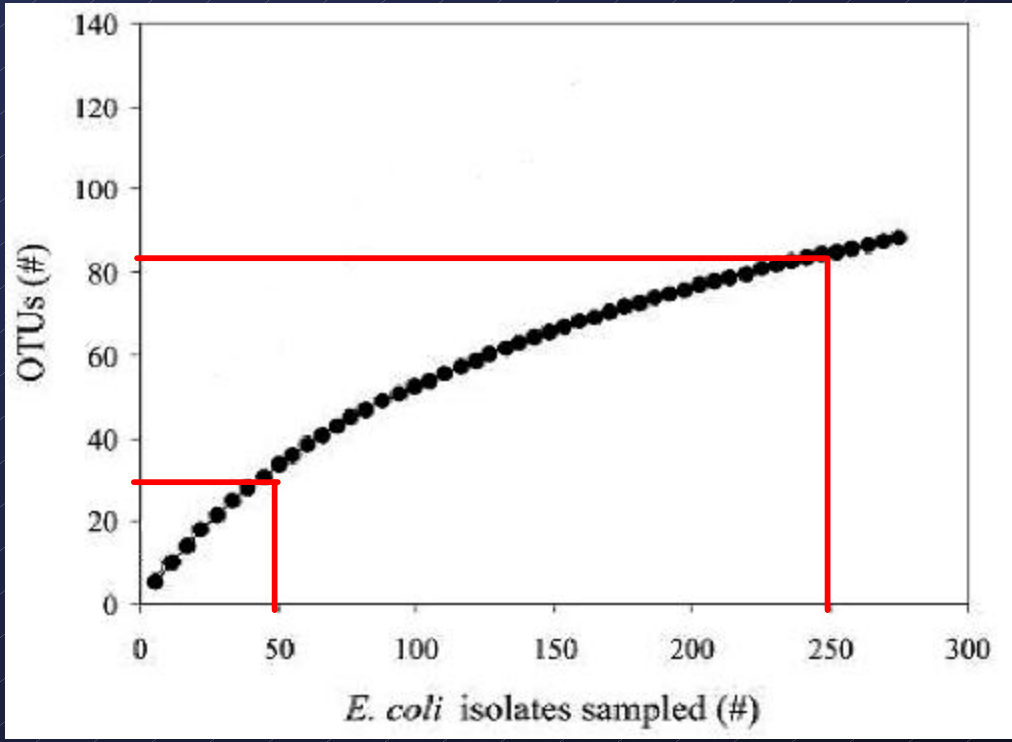

From: Yang et al., 2004

Library construction

- Consider the prominent point and non-point sources
- How many isolates are necessary?
  - Wiggins et al. (2003) suggested that at least 2900 fecal enterococci were necessary for a true representation of the enterococci community in a Virginia watershed

Library construction

- Consider the prominent point and non-point sources
- How many isolates are necessary?
- Sampling distribution
  - Many isolates from a few individuals

    or
  - A few isolates from several individuals

Library construction

- Consider the prominent point and non-point sources
- How many isolates are necessary?
- Sampling distribution
- Geographic / temporal variability
  - Are the phenotypic/genotypic characteristics of *E. coli* from Ohio cows genetically similar to those isolated in Texas?

Library construction

- Consider the prominent point and non-point sources
- How many isolates are necessary?
- Sampling distribution
- Geographic / temporal variability
  - Are the phenotypic/genotypic characteristics of *E. coli* from Ohio cows genetically similar to those isolated in Texas?
  - Antibiotic resistance assays can be geographically limited (Wiggins et al., 2003)…
    - Virginia human: 71% correct classification
    - Florida human: 45%
  - …as can ribotyping (North, Central, and South Florida) (Scott et al., 2003)

Case study I: Is a common source contributing to bacterial pollution in two Ohio lakes?

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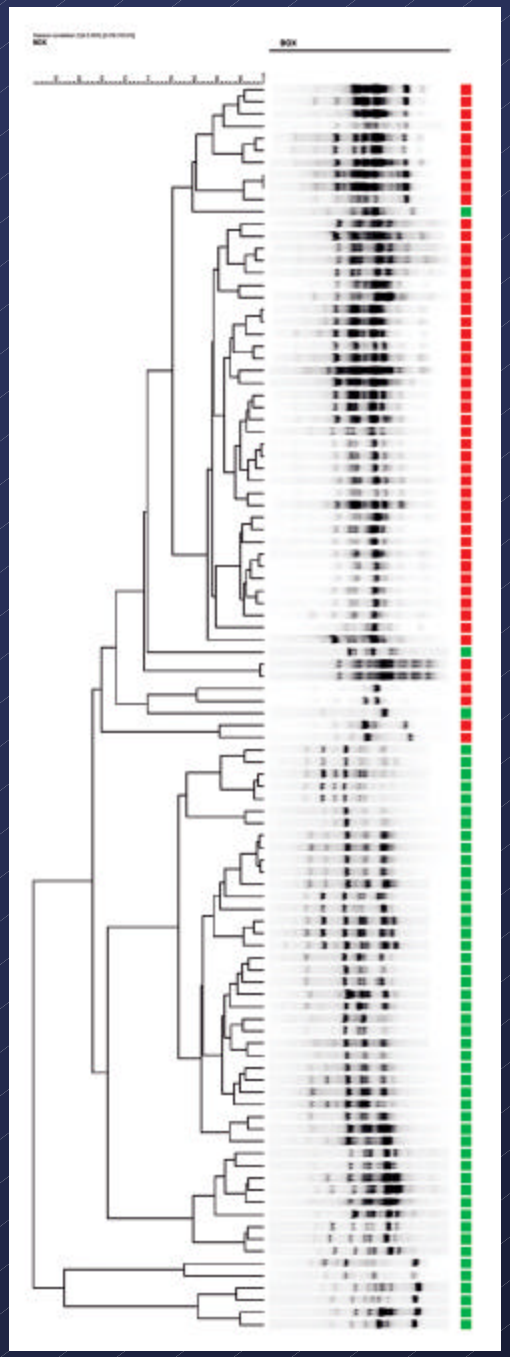
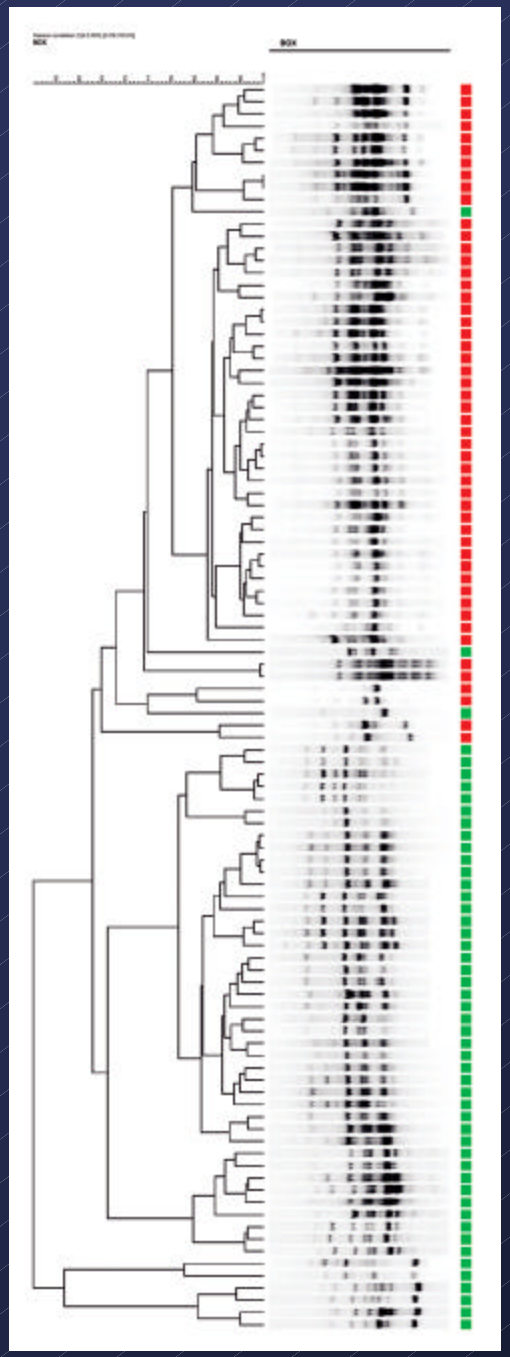
- *E. coli* collected from two contaminated lakes at MBSP
  - Lake Erie (n = 53)
  - Inland Lake (n = 57)
- DNA isolated from each isolate and subjected to BOX-PCR
- Computerized analysis was used to compare the similarity of the two collections



Lake Erie Beach

Inland Lake



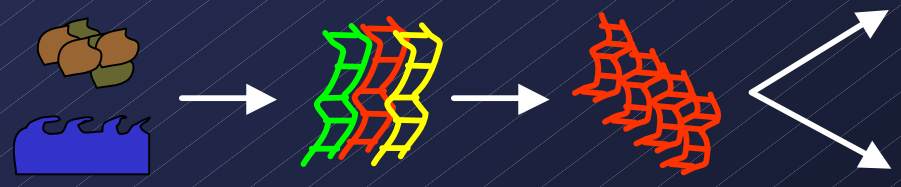
Lake Erie Beach

Inland Lake

From: Amie Brady, U.S. Geological Survey

Library-independent techniques

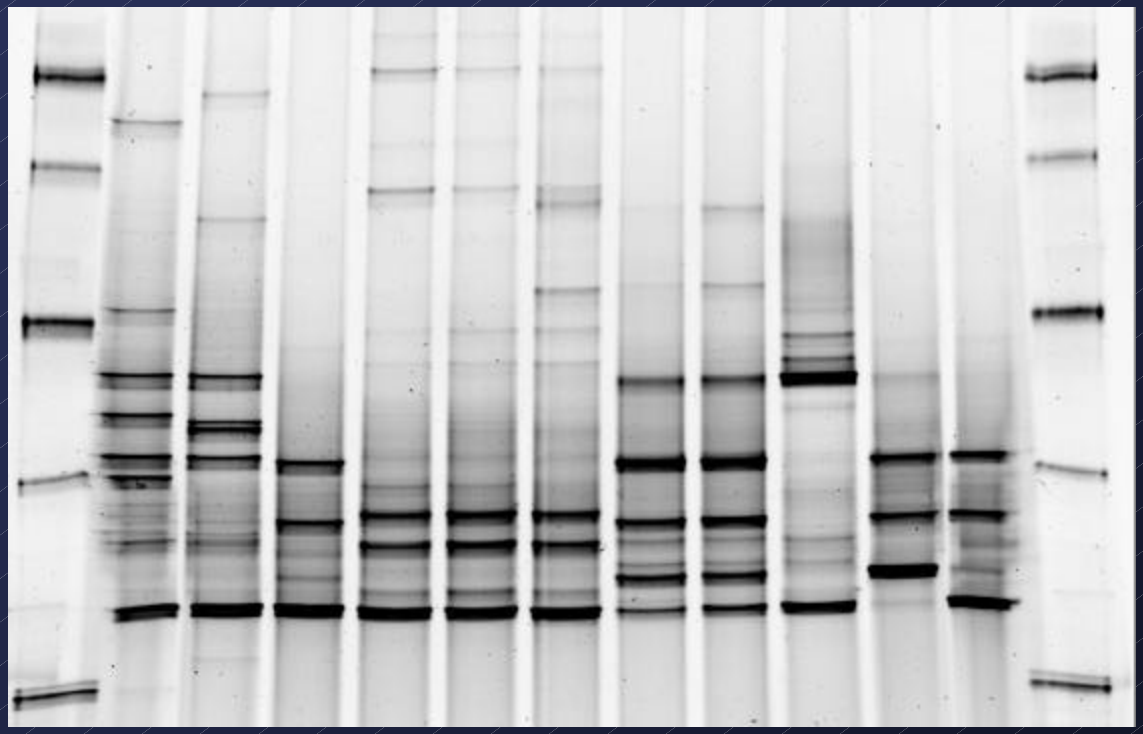
- Genetic methods that do not require the development of bacteria libraries
- Detection of genetic sequences in DNA isolated directly from environmental bacterial populations



<u>Detection</u> of species-specific pathogens

<u>Description</u> of communities of specific pathogens

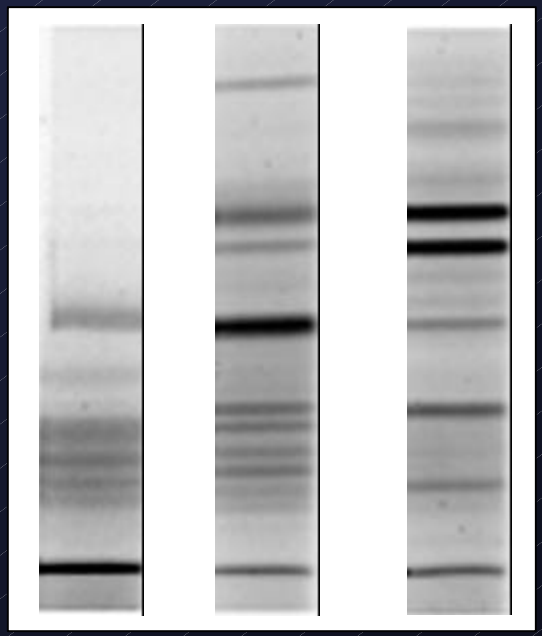
Denaturing Gradient Gel Electrophoresis (DGGE)



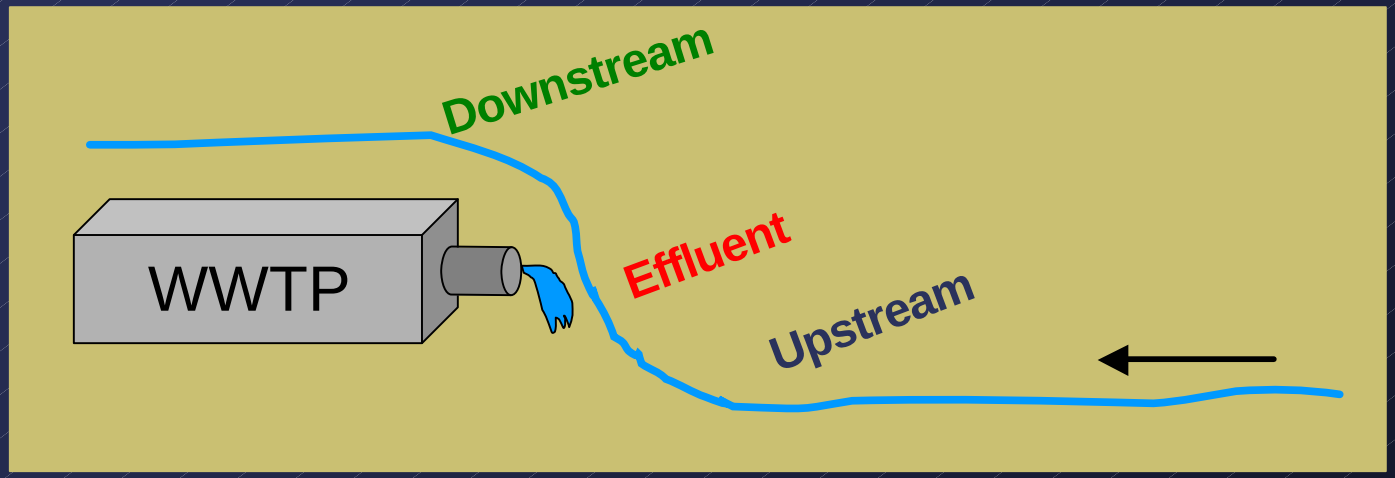
- Bacterial <u>communities</u> are represented by banding patterns that can be compared to determine similarity

Library-independent fingerprinting

- Whole community fingerprints are generated from potential sources and the pollution sink.
  - Similarity between fingerprints implies contribution from the potential sources.



Case study II: Can community fingerprinting pinpoint the origins of bacterial pollution?



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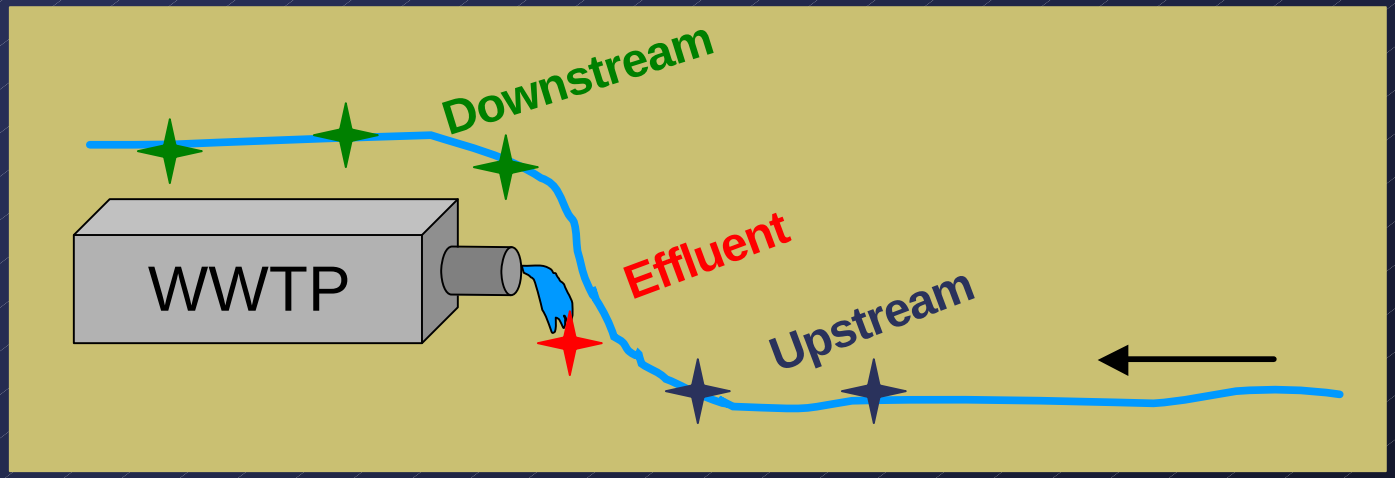

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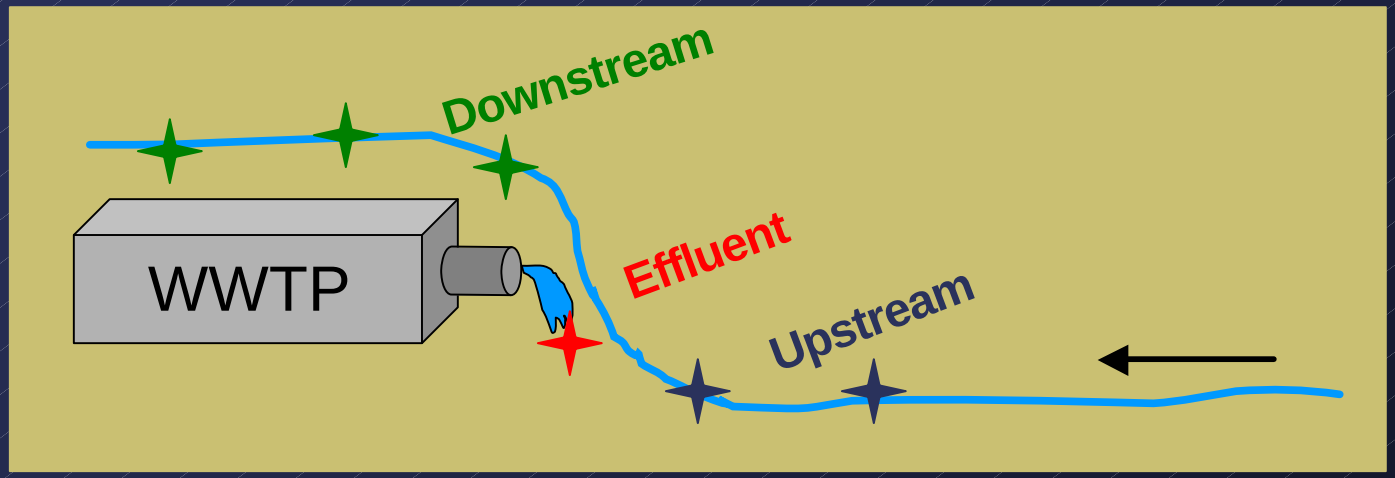

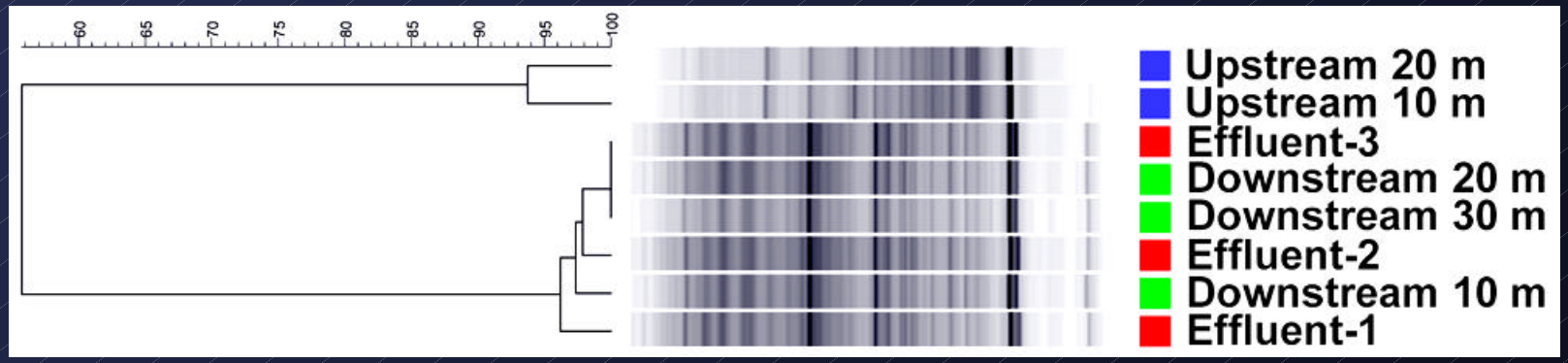

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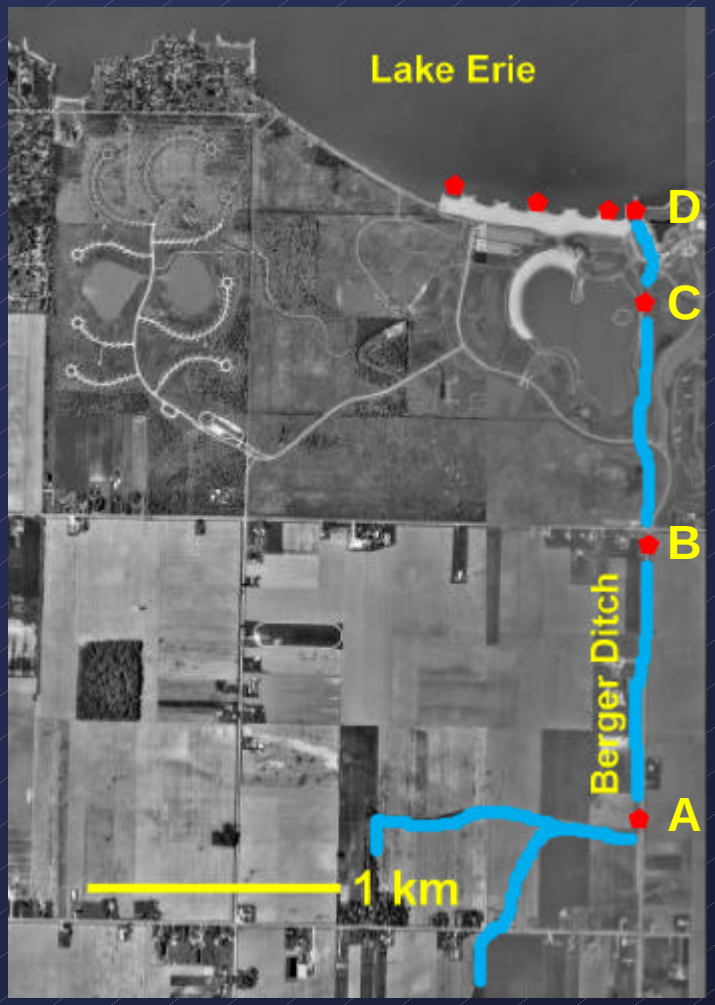

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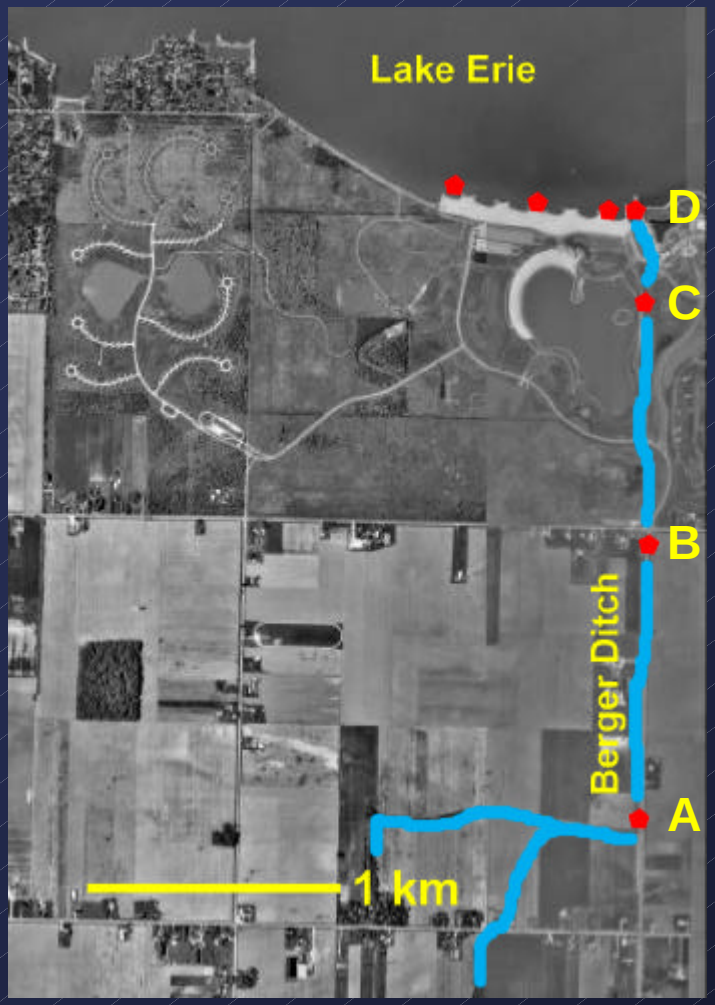

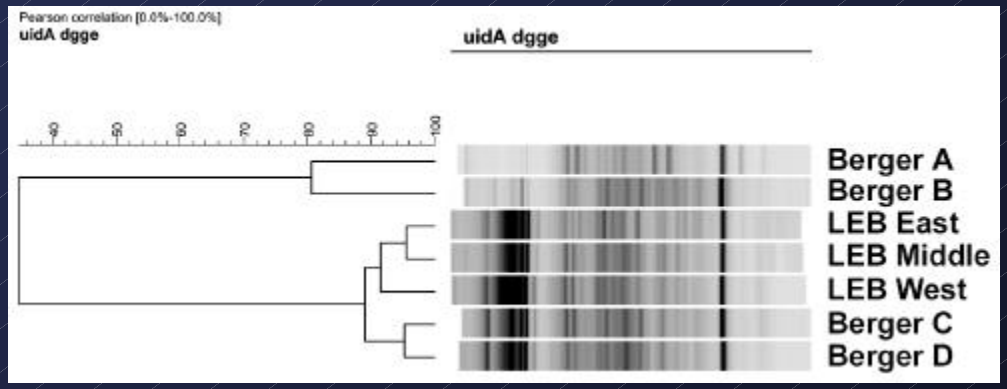

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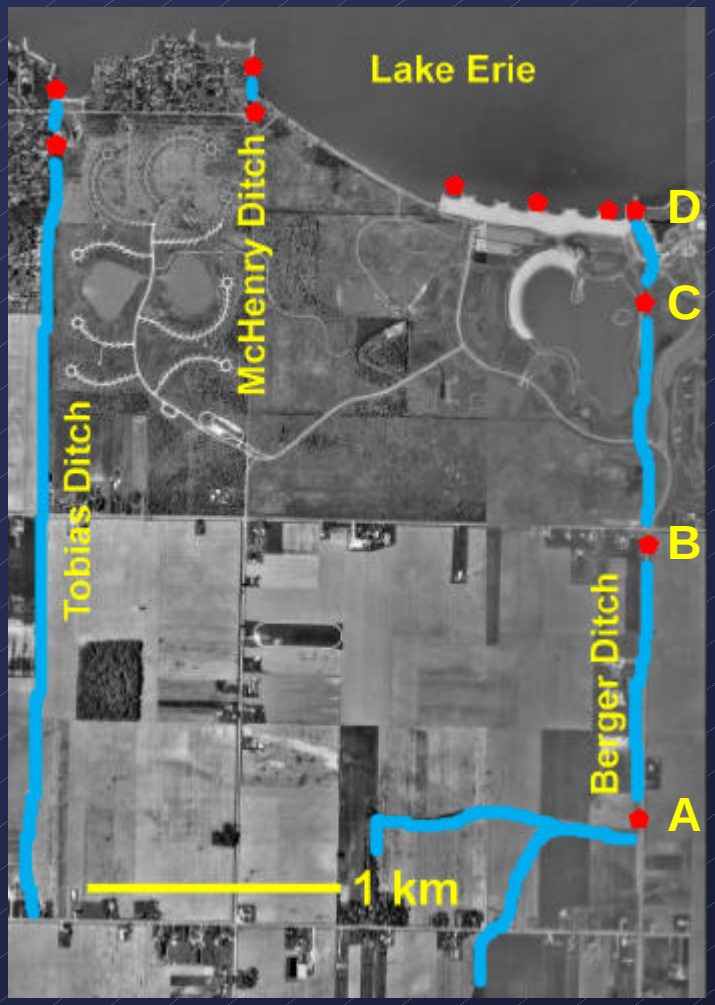

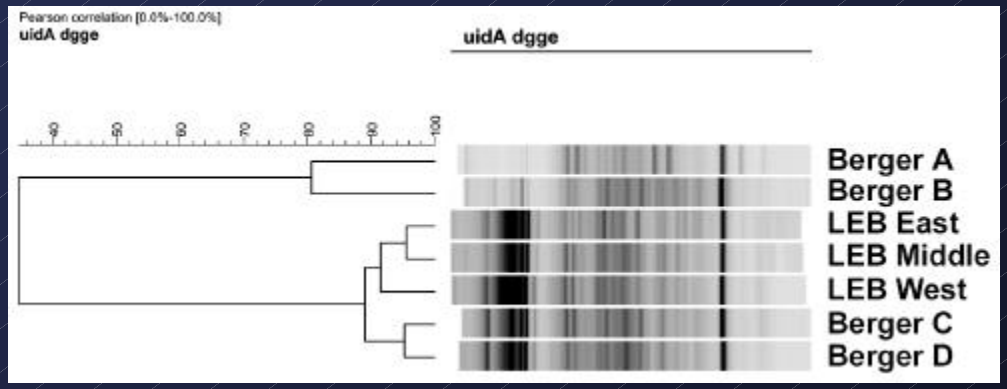

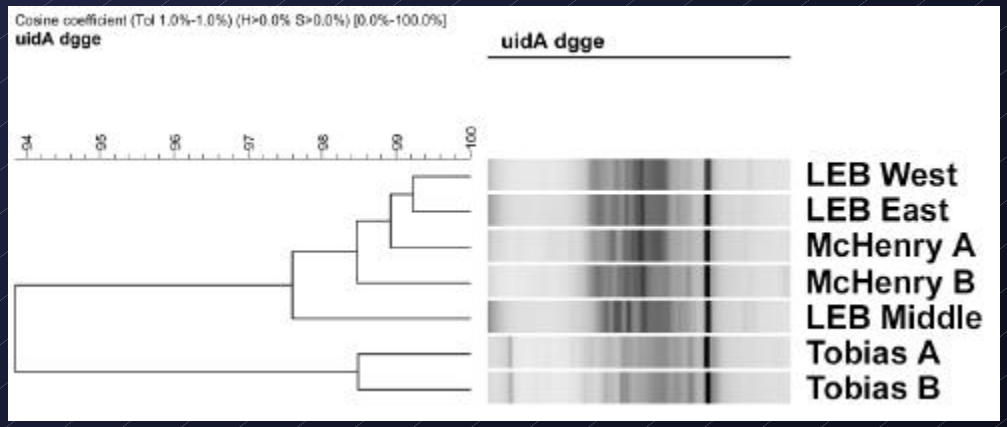

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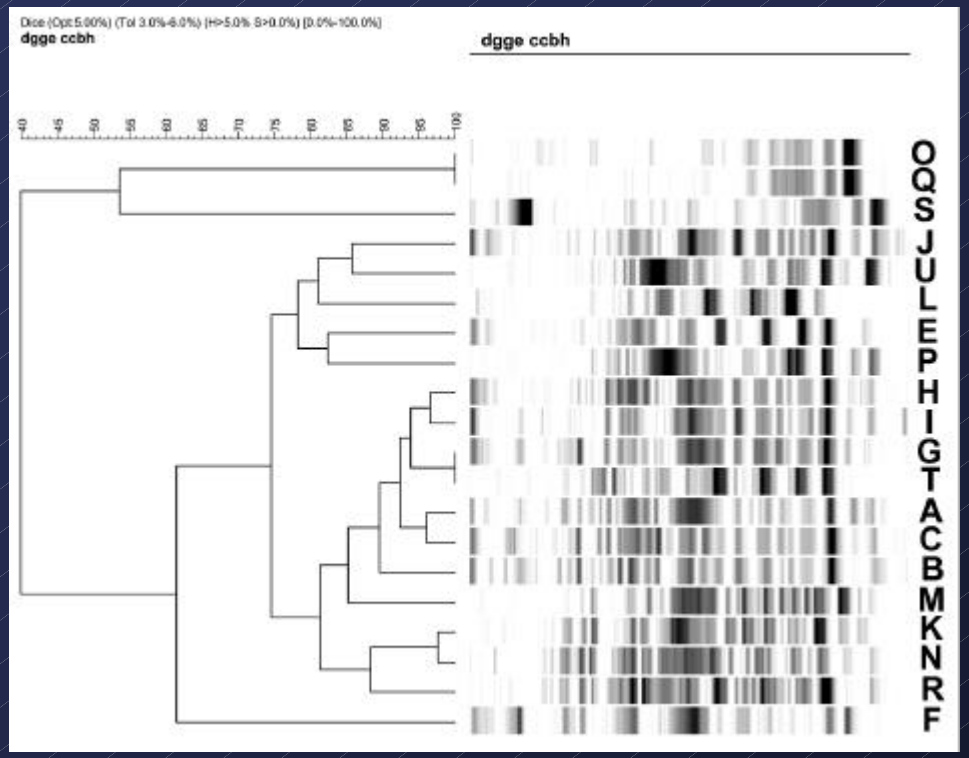

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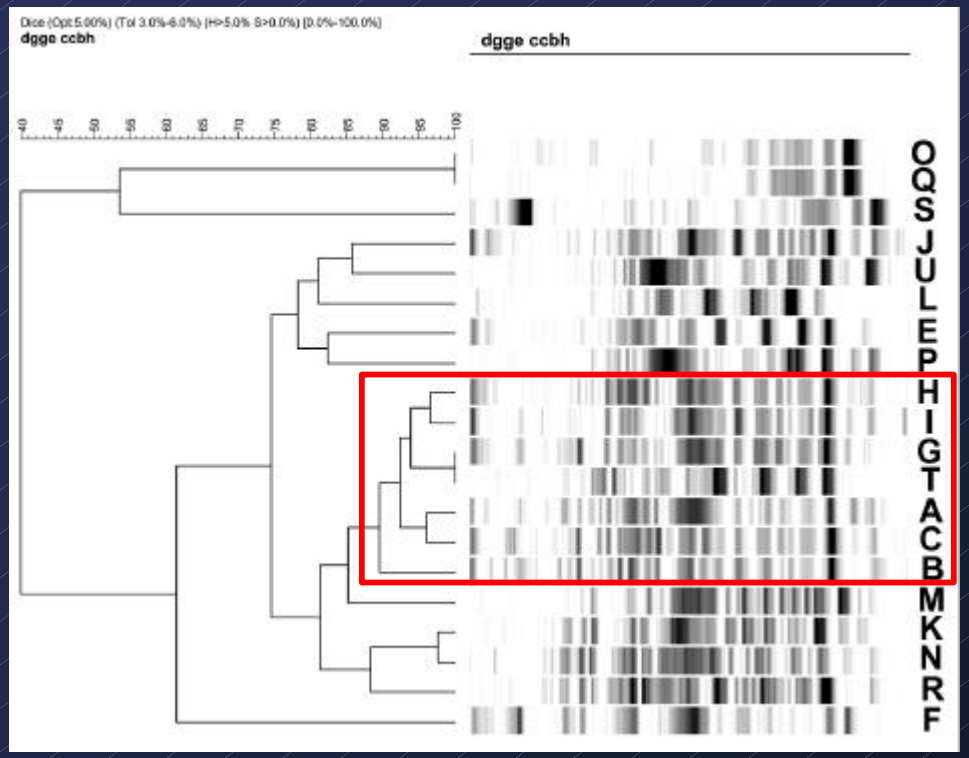

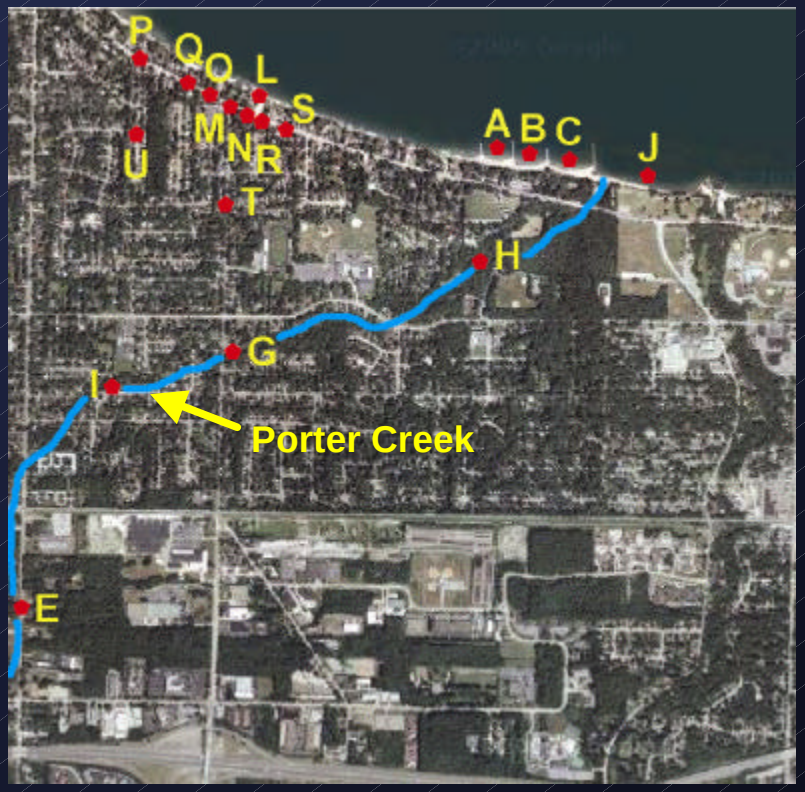

Conclusions/ideas for thought

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- Community fingerprinting shows promise for relatively rapid characterization of *E. coli* communities.
- Future research should include targeting actual pathogen communities.
  - *Salmonella spp*., *Campylobacter spp*., *S. aureus*
- Polymorphic genes (e.g. for *E. coli*: *uidA*, *phoE*) might be useful in emerging technologies such as microarray analyses.