Microbial Pollution of Water - Old Problems New Approaches

Sheridan Kidd Haack

USGS, Lansing, MI

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science for a changing world

Microbial Pollution of Water — Old Problems, New Approaches

- The players
- The issues
- The technologies
- Examples from my research

  - Collaborators: D. Long, D. Hyndman, Michigan State U; L. Alm, Central Michigan U; M. Wolcott, R. Whitman, USGS
  - Technical Support: L. Fogarty (NRP), T. West (CMU), J. Underwood, N. Frantz, C. Frasz (MSU-USGS)

The Players: Bacteria, Viruses and Protozoa

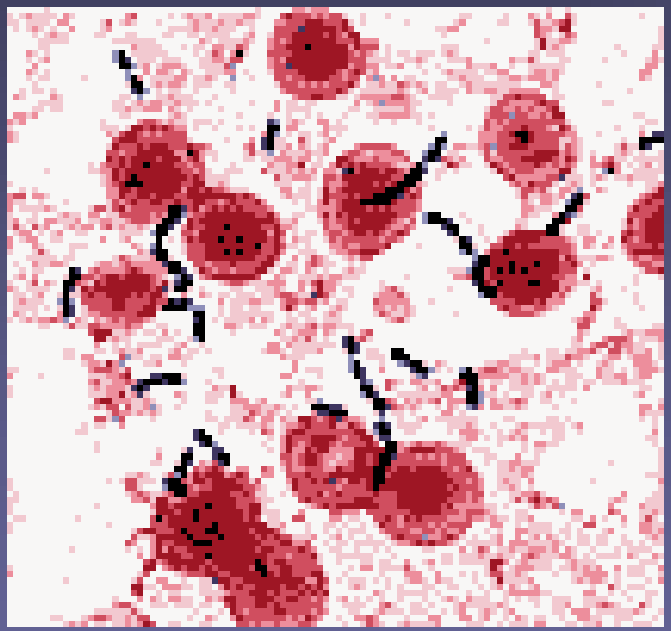
Bacteria: Single-celled microorganisms lacking a nucleus.

Viruses: A genetic element, having either DNA or RNA, and being able to alternate between a "non-living" state outside the cells of other organisms or a "living" state inside their cells.

Protozoa: Microorganisms that are typically single-celled but usually possess a nucleus.

All organisms with unique life styles, habitats and ecology

Enterococcus faecalis in a Blood Culture



Samples of blood from patients suspected of having bloodstream infections are sent to the microbiology laboratory for culture. The blood is drawn from the patient and placed immediately into a broth medium or blood culture bottle. This bottle is incubated. If bacteria are present in the blood, they will grow, allowing them to be detected in the laboratory by a variety of methods. Once it has been determined that there are organisms growing, a Gram stain from a drop of broth from the bottle is made to aid in determining the type of organism. In this case, the gram-positive or bluish-purple cocci resemble streptococci or enterococci due to the chaining formations. These organisms are subcultured on solid media for further identification.

A Biofilm From an Alpine Lake

- All natural surface waters harbor numerous and diverse microorganisms
- Biofilms concentrate microorganisms in a structured and protected environment

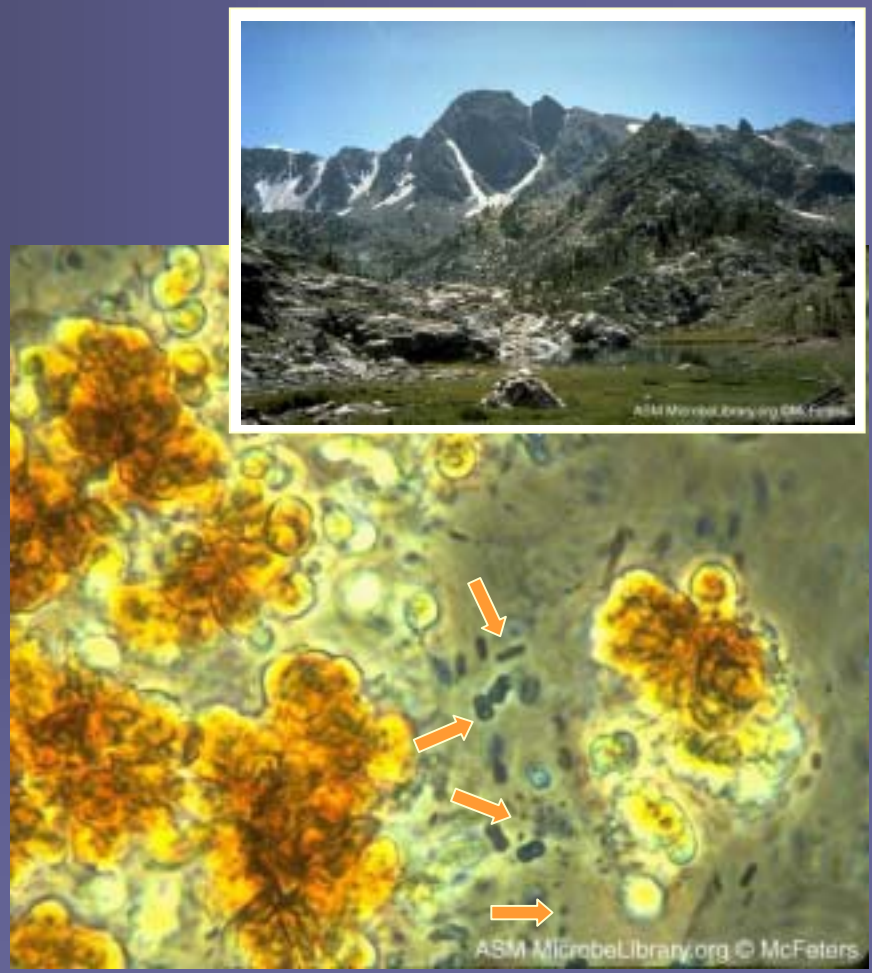
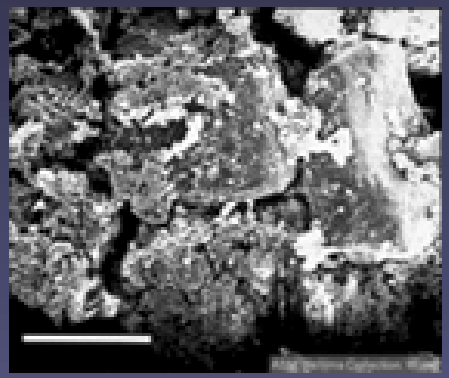


Photo Credits: Gordon McFeters, Department of Microbiology, Center for Biofilm Engineering, Montana State University, Bozeman, Mont., USA. Licensed for use, ASM MicrobeLibrary (linked to http://www.microbelibrary.org)

A Biofilm From a Septic System

1



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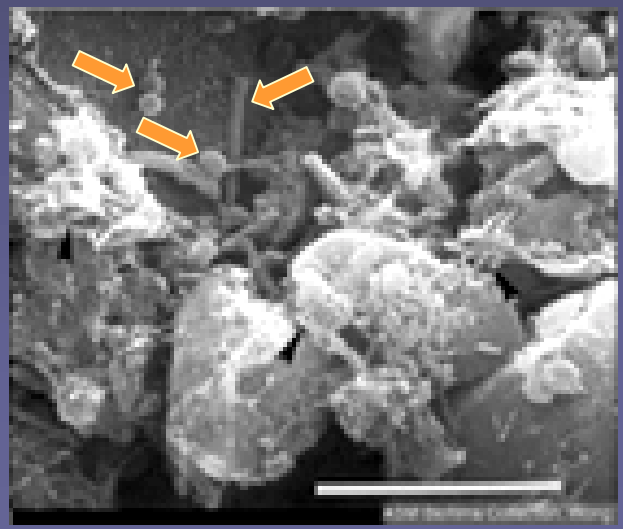


Figure 1 is a scanning electron micrograph (SEM) of the naturally occurring biofilm on sand grains in the clog mat of a septic system infiltration mound. The biofilm is composed of mineral particles, a variety of microorganisms, and a network of slime, or glycocalyx, that binds the microorganisms and particles together. Scale bar is 150 µm.

Figure 2 is a SEM of the naturally occurring biofilm on sand grains in the clog mat of a septic system infiltration mound. The biofilm is composed of mineral particles, a variety of microorganisms, and a network of slime, or glycocalyx (indicated by the arrows), that binds the microorganisms and particles together. Scale bar is 4.3 µm.

Giardia and Cryptosporidium

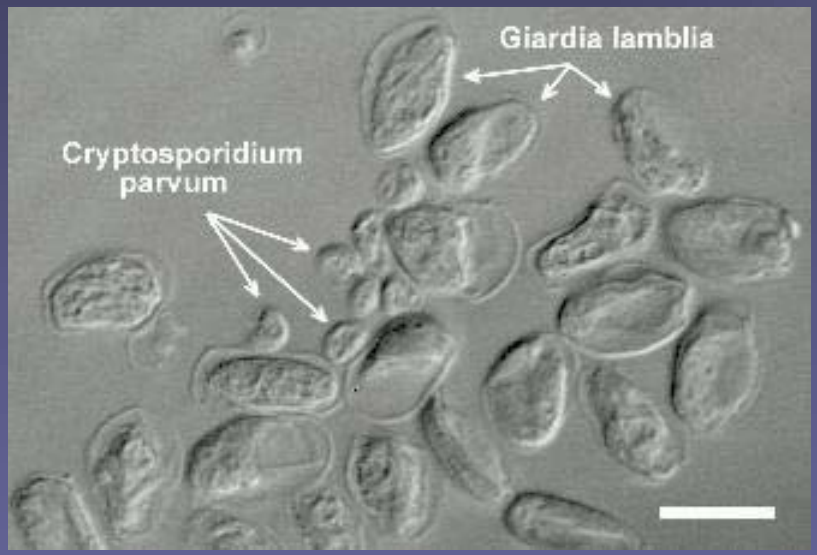
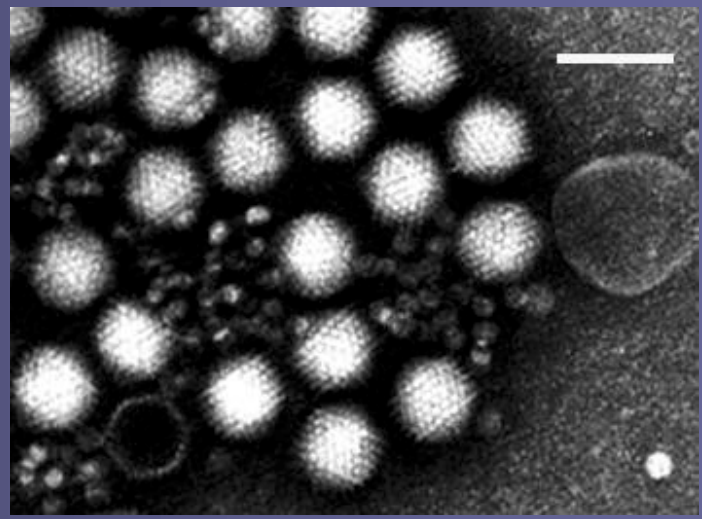


Photo Credit: H.D.A. Lindquist, U.S. EPA

Cryptosporidium parvum oocysts and *Giardia lamblia* (intestinalis) cysts imaged together for purposes of comparison. In the photomicrograph, the *C. parvum* oocysts are distinguished from neighboring *G. lamblia* cysts by their smaller size. Bar = 10 microns.

http://www.epa.gov/nerlcwww/cpt_gda.htm

Viruses



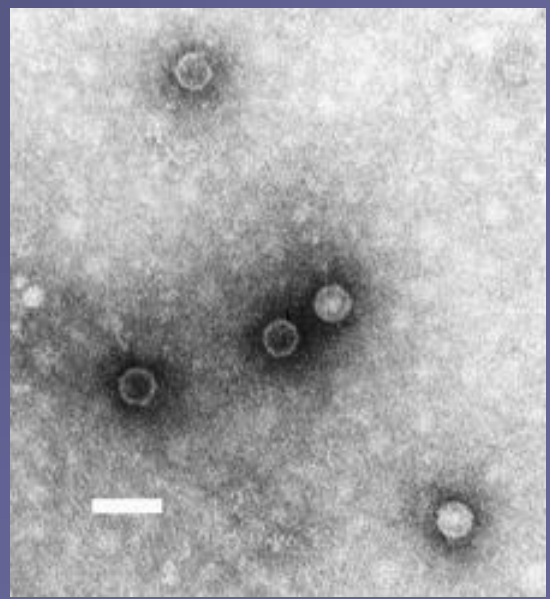


Photo Credit: F.P. Williams, U.S. EPA
Note the two virus types present in the adenovirus photograph. The larger virus particles with prominent capsomeric detail on their surface are adenovirus particles. The small featureless particles seen mostly clumped between the adenovirus particles are parvovirus particles. Bar = 0.1 micrometer

Note that poliovirus particles exhibit a basically featureless appearance in comparison to other small viruses such as astrovirus, Norwalk virus and other SRSVs, and typical (non-SRSV) calicivirus. Bar = 0.05 micrometers

http://www.epa.gov/nerlcwww/adeno.htm

Microbial Pollution - the Issues

- Human Health
  - Emerging human and animal microbial pathogens and algal toxins : GW sustainability (ASR, recharge, reclamation, GW/SW interactions), recreational water quality, drinking water quality; aquifer geochemistry
- Ecosystem Health and Integrity
  - Emerging wildlife disease, zoonotic disease, agriculture/aquaculture
- Technology
  - Reliance on centuries-old methods; promise of new

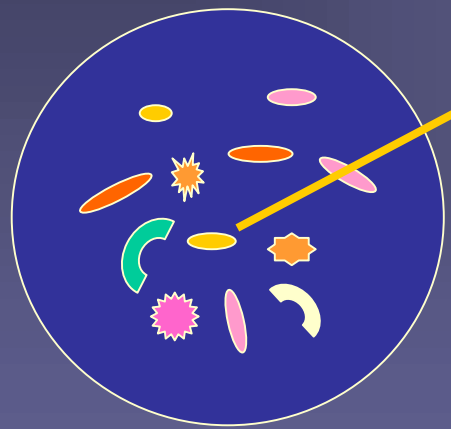
How Do We Evaluate Microbial Pollution?

- Water must be free of “fecal pollution”
  - Not necessarily free of pathogens
- How do we define “free of fecal pollution”?
  - Quantify the numbers of “fecal indicators”
    - Microbes primarily found in intestines of animals or humans
    - do not cause disease themselves
    - presence in water indicates fecal pollution

The Traditional Fecal Indicator Bacteria

Total Coliform Bacteria

A group of bacteria - some occur naturally in water



E. coli

- A specific bacterium in the coliform group
- Native to intestinal tract
- Most are not pathogens (BUT: *E. coli* O157:H7!)

Fecal Coliforms are a subset of total coliforms that grow at 44.5 ºC

The Traditional Fecal Indicator Bacteria

- Old: Fecal Streptococci
  - *Streptococcus* and *Enterococcus*
  - *Staphylococcus, others*
- New: Enterococci
  - Some enterococci are pathogens themselves

Some New Fecal Indicators

- *Clostridium perfringens*
  - Resistant spore form may better represent protozoa
- Coliphage
  - Viruses that infect coliform bacteria
  - Indicators of survival and transport of viruses

What Do Fecal Indicators Indicate?

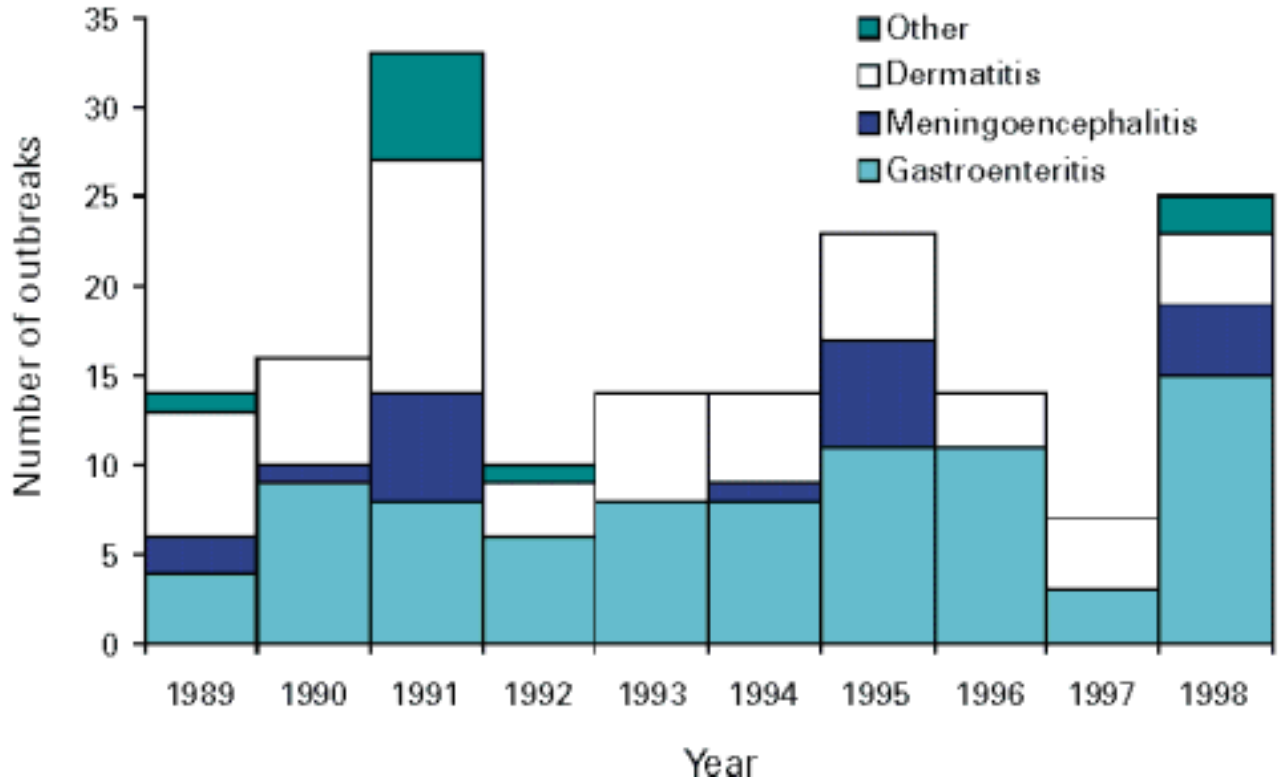
- Feces are in, or have recently been introduced to, the water
  - Nutrients – e.g. nitrate, ammonia
  - Organic Carbon – oxygen demand
  - Various microorganisms
    - Cows: *E. coli* O157:H7
    - Birds: *Campylobacter*, *Salmonella*
    - Wild Animals: various protozoa such as *Giardia*
    - Domestic animals: various animal or human pathogens
    - Humans: enteric viruses, a variety of bacteria and protozoa
  - Chemicals - pharmaceuticals, biogenic and synthetic hormones

What Do Fecal Indicators NOT Indicate?

- That any SPECIFIC pathogen is present
- The presence of viruses or protozoa
- The presence of bacteria with different survival or transport characteristics
- The presence of non-fecal pathogens
  - *Aeromonas*
  - *Pseudomonas*
  - Toxic algae

Waterborne Disease-Recreational Water

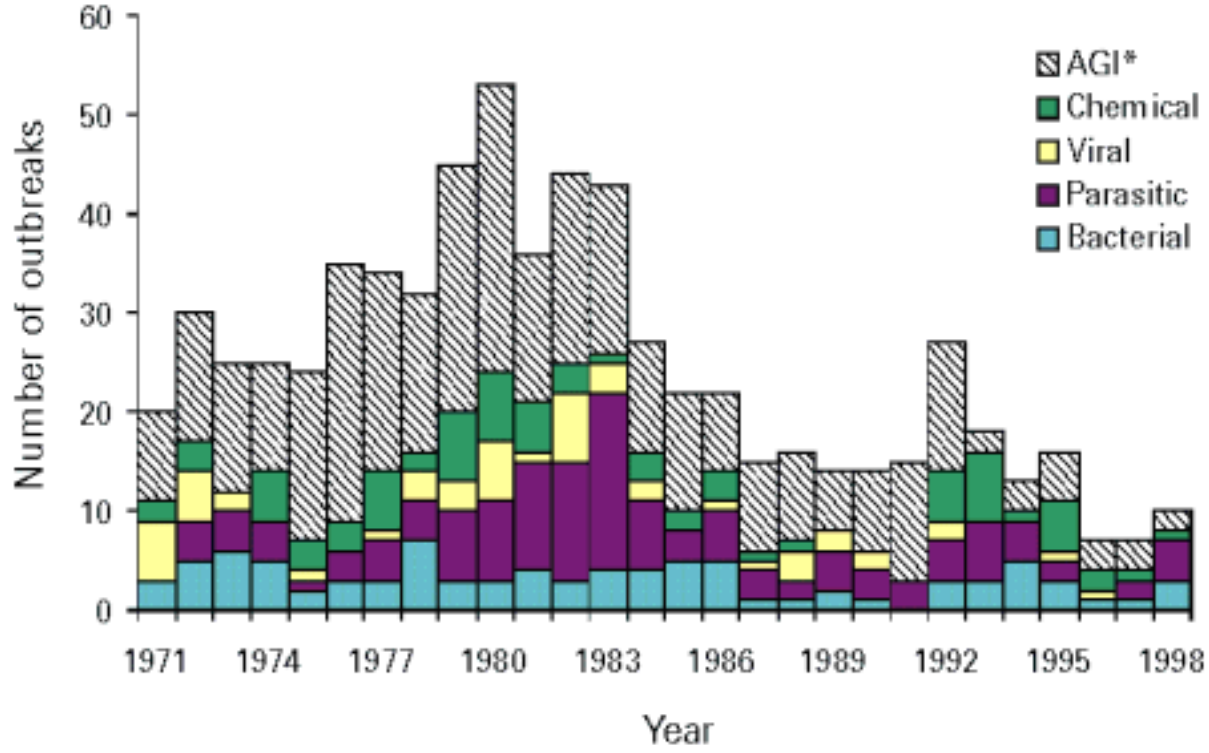
FIGURE 7. Number of waterborne-disease outbreaks associated with recreational water, by year and illness — United States, 1989–1998 (n = 171)



http://www.cdc.gov/epo/mmwr/preview/mmwrhtml/ss4904a1.htm
Morbidity and Mortality Weekly Report, May 26, 2000(49) SS04, pages 1-35

Waterborne Disease - Drinking Water

FIGURE 5. Number of waterborne-disease outbreaks associated with drinking water, by year and etiologic agent — United States, 1971–1998 (n = 691)



*Acute gastrointestinal illness of unknown etiology.

http://www.cdc.gov/epo/mmwr/preview/mmwrhtml/ss4904a1.htm
Morbidity and Mortality Weekly Report, May 26, 2000(49) SS04, pages 1-35

Ground Water Protection

- 250 disease outbreaks between 1971-1994 from contaminated ground water (Craun and Calderon, 1997)
- *Cryptosporidium* in 5%, infectious virus in 4.8% of wells (Macler and Merkle, 2000)
- Most modelling of transport based on indicators, and lab-based (Macler and Merkle, 2000; Harvey and Harms, 2002).
- Set-back distances and time-of-travel to wells rarely field-verified (Macler and Merkle, 2000)
- States with approved wellhead protection programs did not have lower rates of microbial contamination (TCR MCL violations) than states without (Macler and Merkle, 2000)
- However, states using hydrogeological siting criteria had fewer violations than those without (Macler and Merkle, 2000).

Macler, BA and Merkle, JC. 2000, *Hydrogeo. J.*, 8, 29

Emerging Microbial Issues

- Emerging diseases of humans
  - Microsporidia, *Cyclospora*, *Helicobacter*, *E coli* O157:H7
  - All in the last decade
- Emerging diseases of wildlife
  - Storm drains
  - Domestic animals/agriculture
- Antibiotic resistance of traditional pathogens

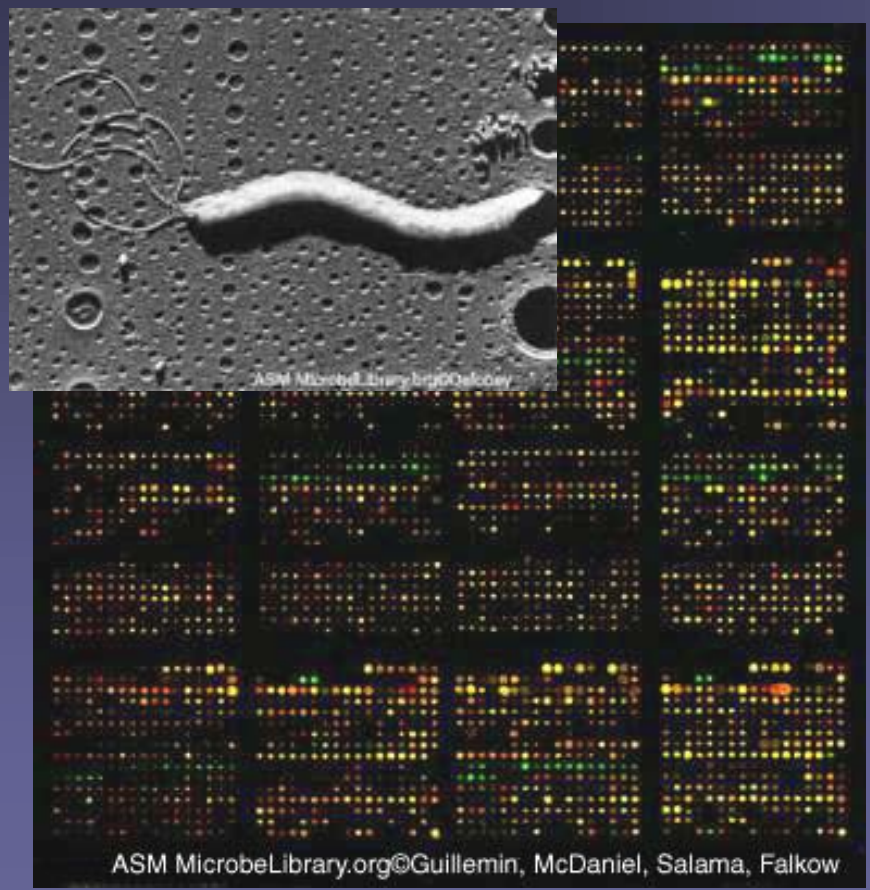
Microbial Pollution - New Technologies

- Exciting times for microbiologists!
- Promise

"Of the hundreds of cows that graze in the saturated fields, several harbor *E. coli* O157:H7, a bacterium harmless to livestock but potentially deadly to people. The bacterium, shed in the cows' manure, is washed into a stream that feeds a public water district 20 miles away. Hours later, a red light flashes on an electronic watershed map mounted on the control panel of the water district's monitoring station. Microbial contaminants—detected by gene chips affixed to stationary stream posts (or implanted in the gills of fish sentinels) and inserted into wells—have entered the system. Other lights flash, indicating the identity of the microbe. The lights alert the district later manager. She tracks the contaminants..."

JB Rose and DJ Grimes. 2001. Reevaluation of microbial water quality: powerful new tools for detection and risk assessment. AAM

Genome of *Helicobacter pylori* on Gene Chip



Helicobacter pylori electron micrograph; causative agent of chronic gastritis, peptic ulcers and gastric cancer. Average size: 1 micron by 2-5 microns. The microarray contains 99% of the *Helicobacter pylori* genes. The microarray is printed on a 20 mm^2 area.

Promise:

- **Technology can be read electronically**

Reality:

- **First consensus *H. pylori* was a pathogen = 10 years ago**
- **Many currently unknown/un-growable microorganisms**
- **We can't put this chip in the alpine lake biofilm**

Photo Credits: Electron micrograph of *Helicobacter pylori*. Cindy R. DeLoney, Loyola University of Chicago. *Helicobacter pylori* gene microarray, Karen Guillemin, Timothy McDaniel, Nina Salama, and Stanley Falkow, Department of Microbiology and Immunology, Stanford University School of Medicine. Licensed for use, ASM MicrobeLibrary.

Reality –Technical Challenges

- Occurrence, distribution, population dynamics and ecology knowledge base is poor
  - Sampling issues
  - Matrix effects, unknown environmental distributions and numbers
- Laboratory/technical challenges
  - M. Enserink. 2001. Science, vol 294:1810-1812. Can lab sleuths clinch the [anthrax] case? *"...At the moment few researchers would bet on it, because microbial genetic analysis is not as standardized as human DNA analysis is."*

Reality – New Methods Are Providing Valuable Information

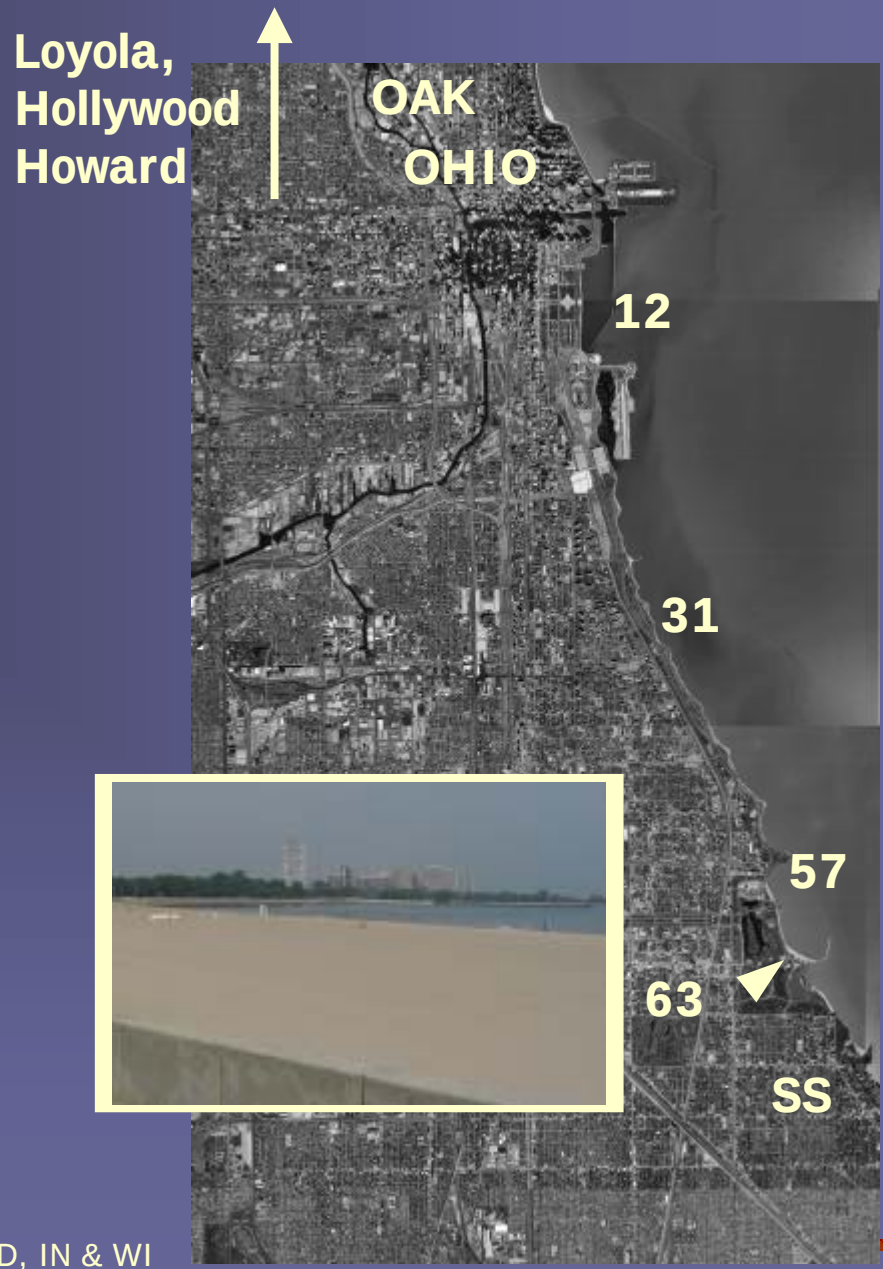
- **DeSerres et al. 1999:** DNA-fingerprints of HAV isolated from contaminated wells, a cesspool and residents showed all shared the same form of the virus.
  - At variance with ground water flow direction
  - Presence of virus in the absence of indicators
  - *J. Infectious Disease*, 1999, 179: 37
- **Chee-Sanford et al. 2000:** Genes coding resistance to tetracycline tracked swine lagoon waste into ground water
  - Genes from lagoon isolates found in ground water 250 m down gradient
  - Different from genes in environmental isolates from the same area
  - *Applied & Environmental Microbiology*, 2001, 67: 1494

Overview

- **Fundamental information on microbial sources, fate, transport and ecology is needed for:**
  - Effective environmental modelling and decision making
  - Effective development and application of new technologies
- **Studies must be conducted in an ecosystem, watershed and/or hydrogeologic context**
- **Examples of the promise and the reality of technologies**
  - Every DNA-based method could be standardized, electronically detected
  - Fundamental microbiological questions remain to be answered

Sources, Fate & Transport

- 63rd Street Beach, Chicago
- No direct human sources
- Large gull population
- Studied
  - *E. coli* DNA fingerprints
  - enterococci
  - wastewater chemicals

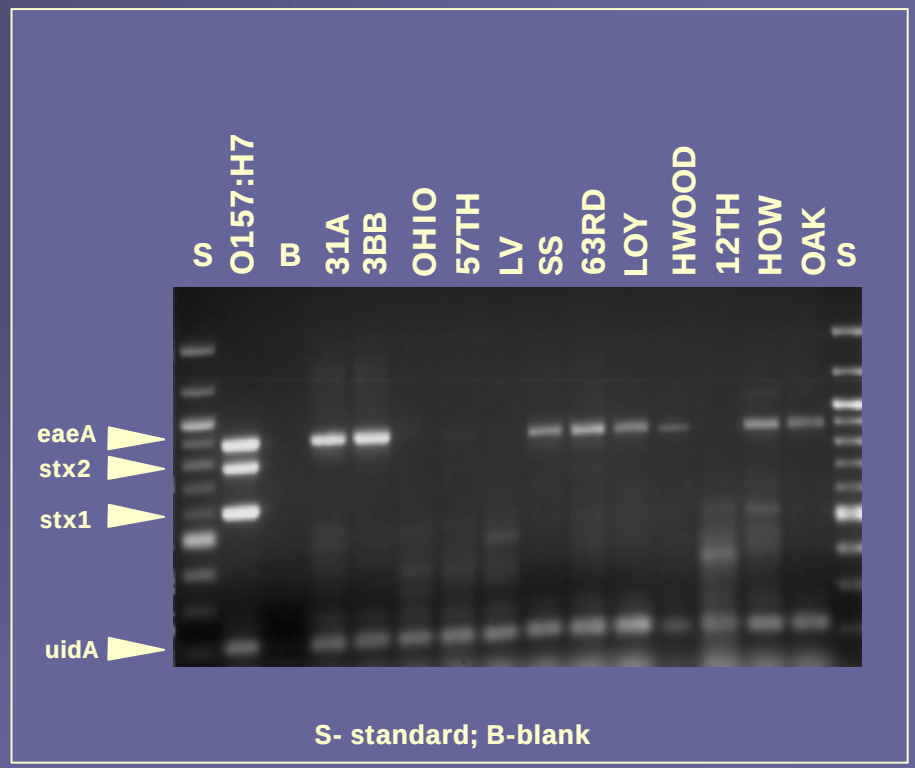


Collaborators: Richard Whitman, Mark Wolcott, USGS, BRD, IN & WI

Is *E. coli* O157:H7 Present?

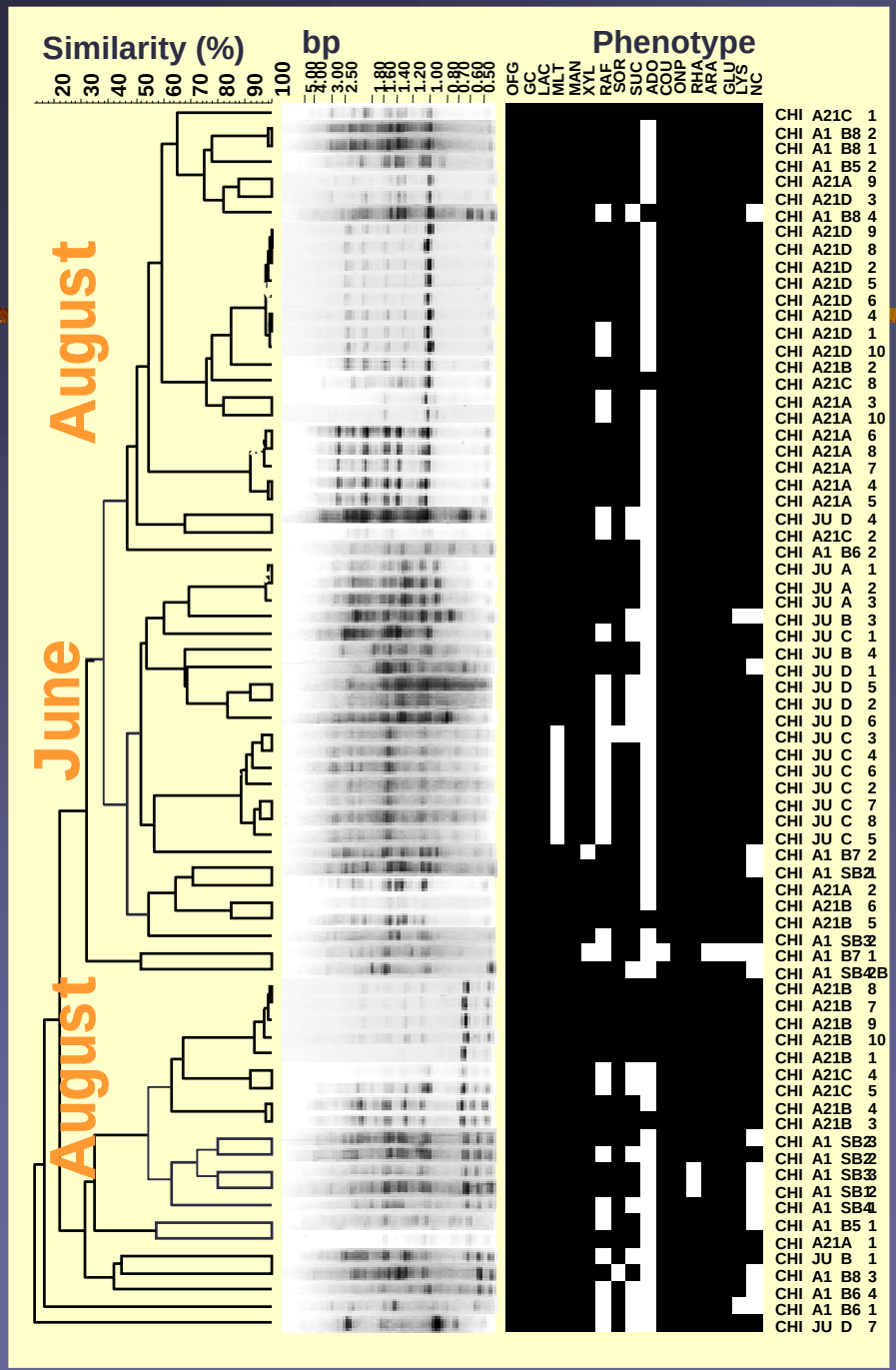
- No. No evidence of *stx*1 or *stx*2 genes
- However, *eae*A gene was detected
  - Not related to numbers of *E. coli*
  - Varied with location

Multi-Plex PCR



Sources at 63rd Street Beach

- *E. coli* DNA fingerprints
  - June – 0 water and 2 sand match gulls
  - August – about ½ water and sand match gulls
- Enterococci
  - June -Gull isolates different species and different antibiotic resistances than water or sand isolates (August – few enterococci)
- Wastewater Chemicals
  - August –napthalene, etc.; bisphenol A
  - September (rain) – caffeine, DEET, triclosan



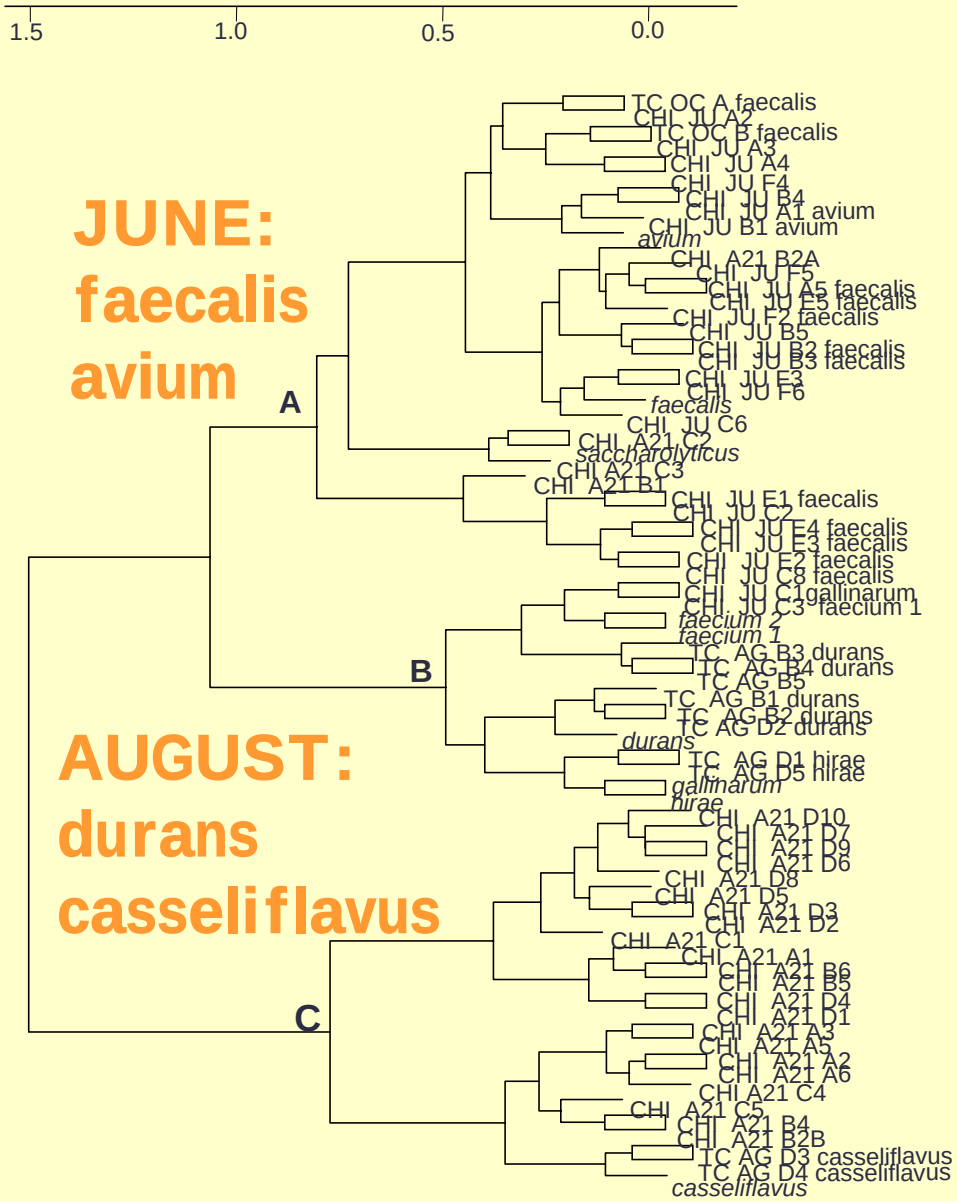
E. coli in Gull Feces

Different populations of *E. coli* in June and August

- rep-PCR DNA Fingerprints
- Vitek® Phenotype

Vitek by Mark Wolcott,USGS, WI

Enterococci in Gull Feces



Different populations of enterococci in June and August

- API Rapid ID 32

Bacterial Ecology in Hosts

- Ecology of even well-studied bacteria largely unknown
- Must be understood to reliably
  - Determine source
  - Develop predictability

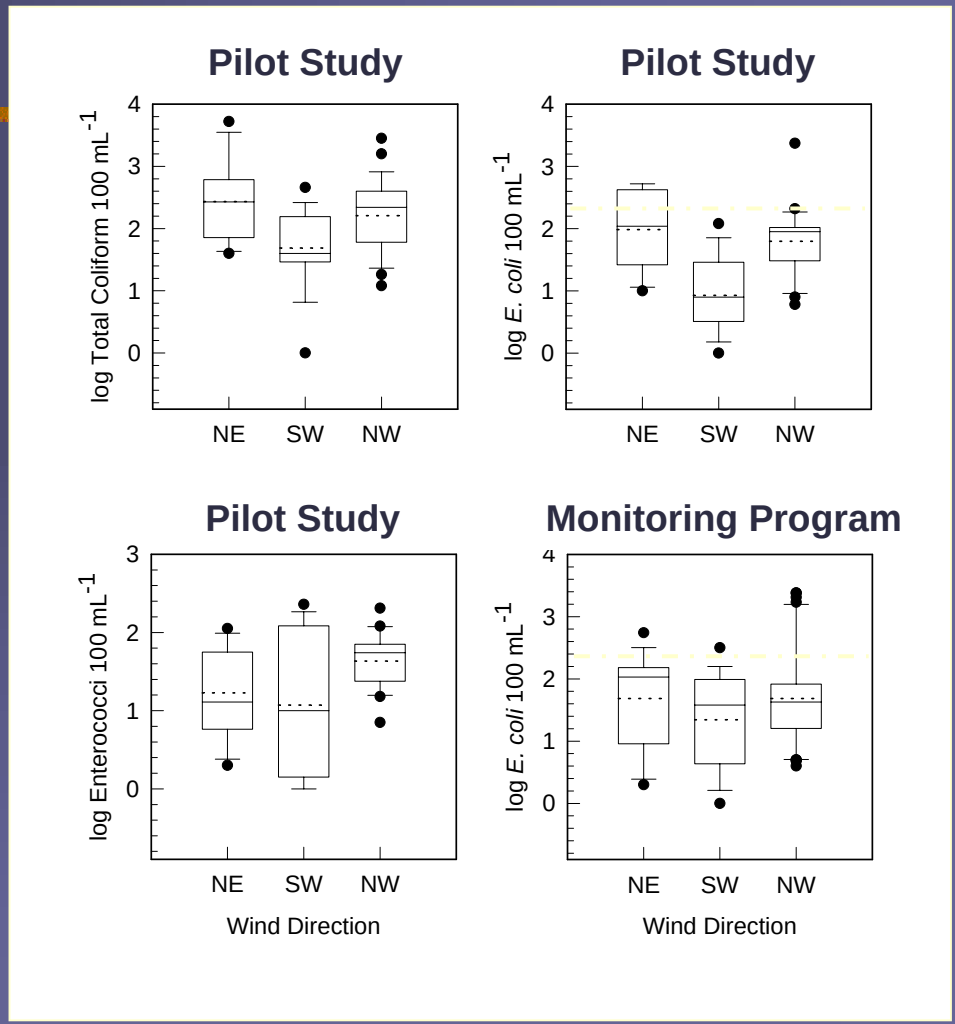
Beaches in Grand Traverse Bay, Michigan

- Pilot Study
- *E. coli* and enterococci
  - Sources
  - Patterns with respect to ambient conditions
  - *E. coli* DNA fingerprints
  - Enterococci biochemical profiles and antibiotic resistance
- Trial Monitoring Program

Clinch Beach, Traverse City, MI

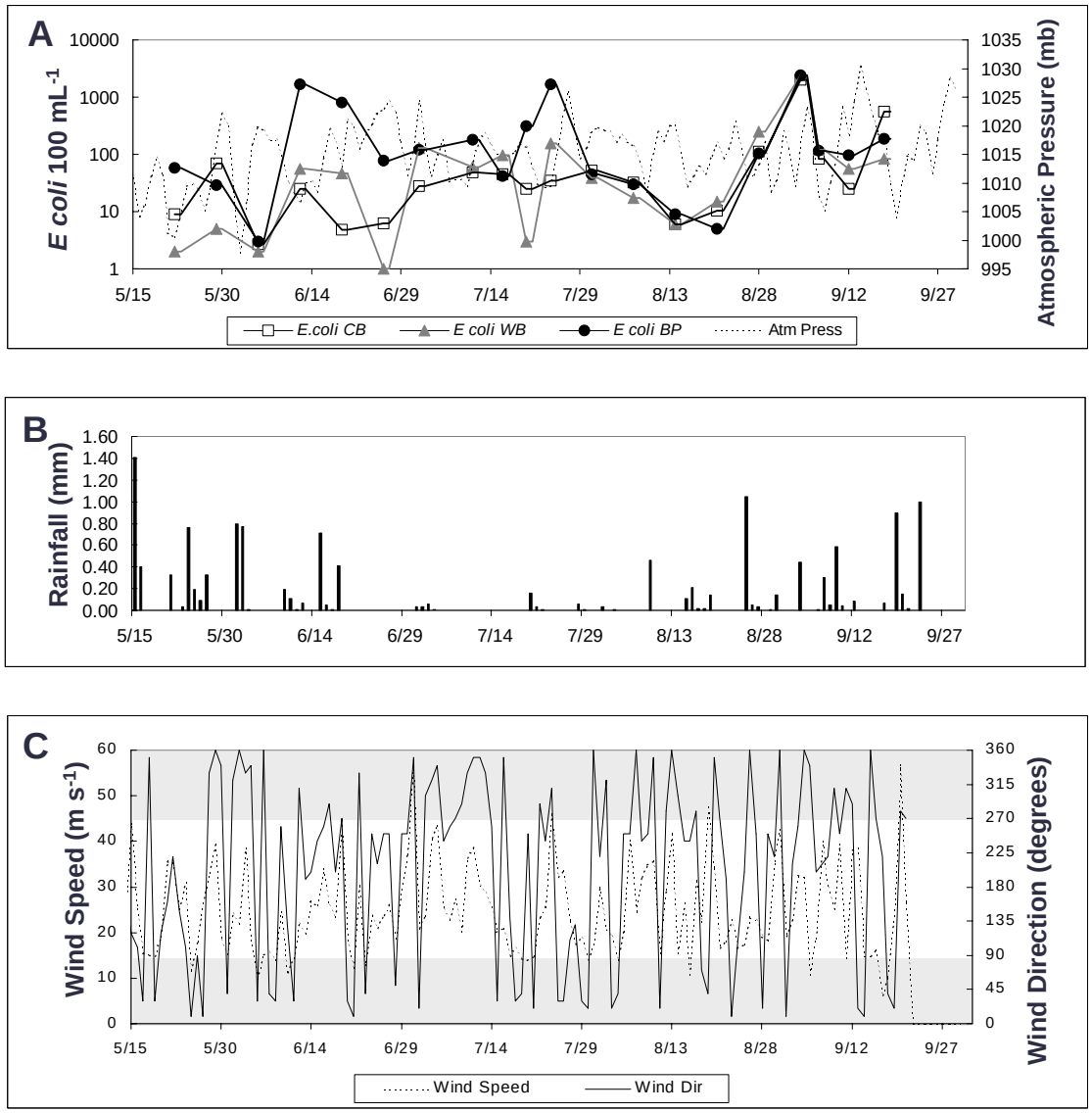
Pilot Study

- Both indicators present in most sources
  - Bird feces
  - Storm drain/river runoff
- More enterococci exceedances than for *E. coli*
- Enterococci and *E. coli* respond similarly to environmental variables
  - AM/PM, Wind, TSS



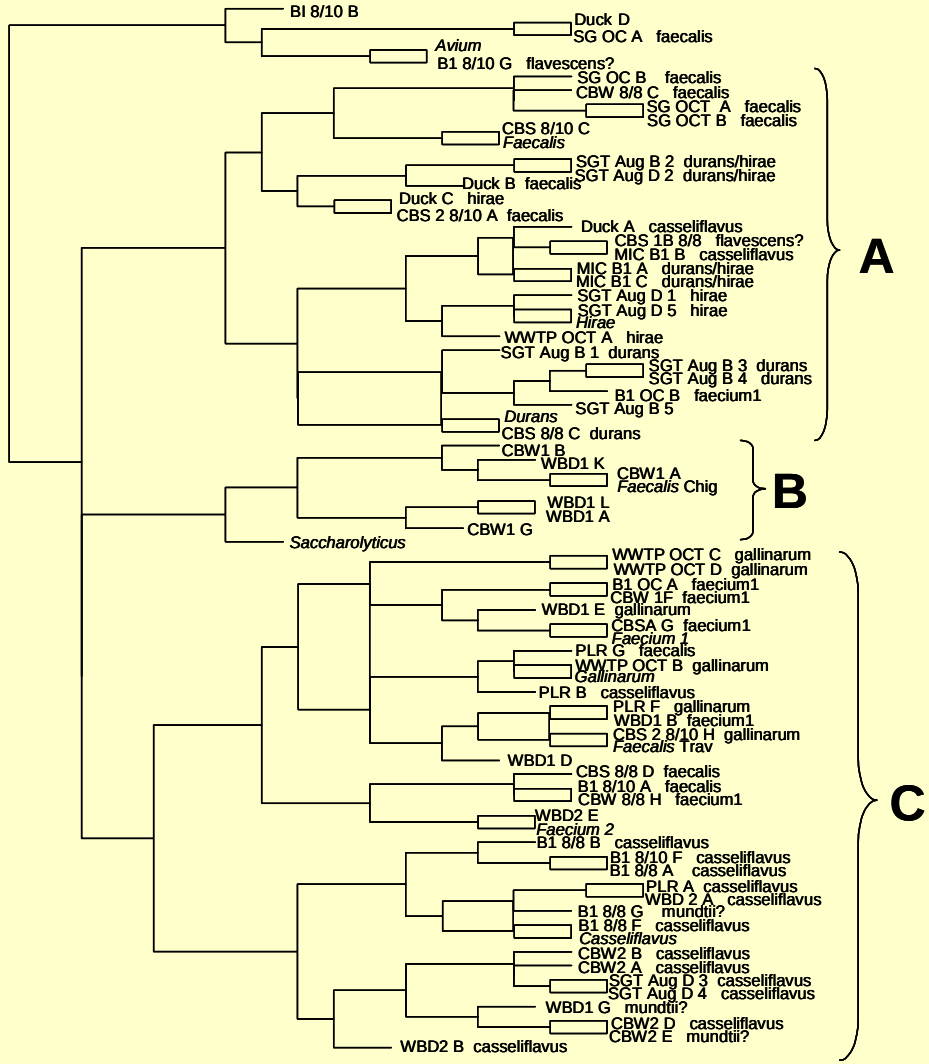
Monitoring Program

- *E. coli* (and probably enterococci) are related to weather variables in a complex way
- Beach-specific



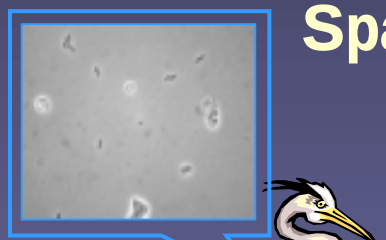
Enterococci

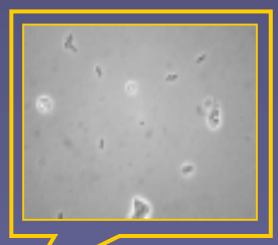
- Different *Enterococcus* species in different sources
- Sediment and bird species: *faecalis, durans, hirae*
  - More frequently resistant to streptomycin and tetracycline – genes could be tracked
- Water, river and runoff species: *faecium, casseliflavus*



Environmental Sources, Fate & Transport

Factors Influencing Beach Bacteria May Occur At Large or Small Spatial Scales

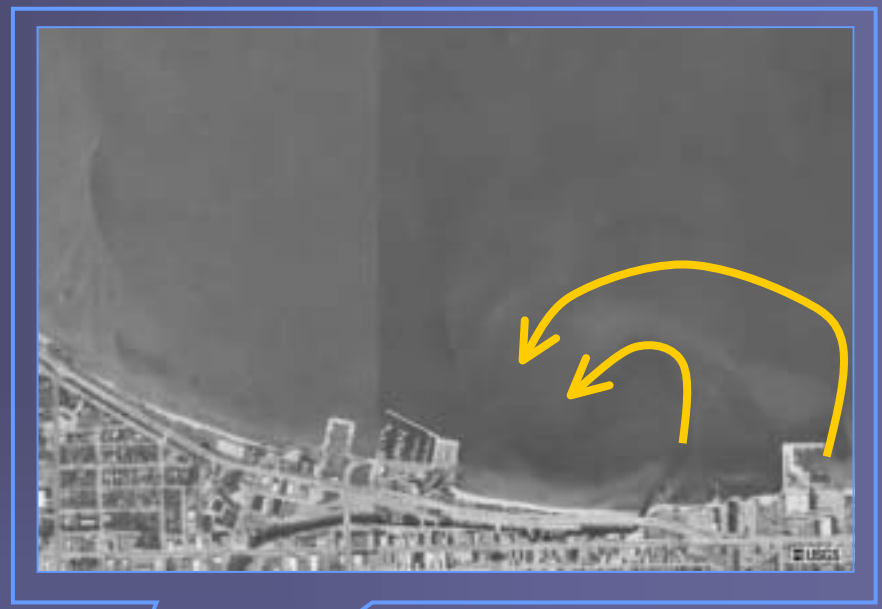




Sand

Ground Water Through Flow

Wave Action Long Shore Drift Currents



Factors Influencing Beach Bacteria May Change With Date

Sources, Fate & Transport

- Multiple sources for most surface waters
- Different sources may be dominant at different times
- Must understand the watershed, ecosystem and hydrogeological setting to
  - Collect appropriate samples
  - Interpret the samples collected
  - Make best use of new technologies

Overview

- New methods are tools that allow us to ask better questions
- Better knowledge allows us to better assess risk
  - Much better than indicators only
- Every DNA-based method could be standardized, electronically detected
- However…

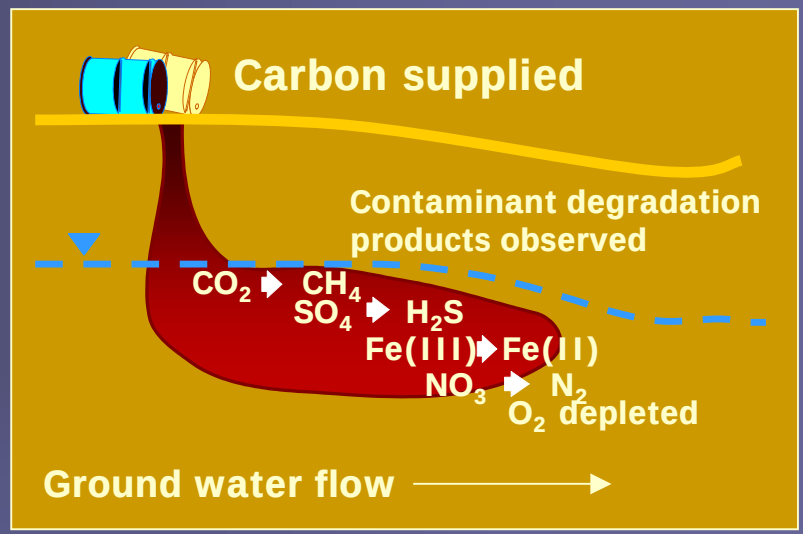
Overview

- **Fundamental information on microbial sources, fate, transport and ecology is needed for:**
  - Effective environmental modelling and decision making
  - Effective development and application of new technologies
- **Studies must be conducted in an ecosystem, watershed and/or hydrogeologic context**

Bioremediation "Footprints"

- Footprints indicate processes
- Interpretation of processes
  - Affects conceptual and numerical models
  - Affects management decisions

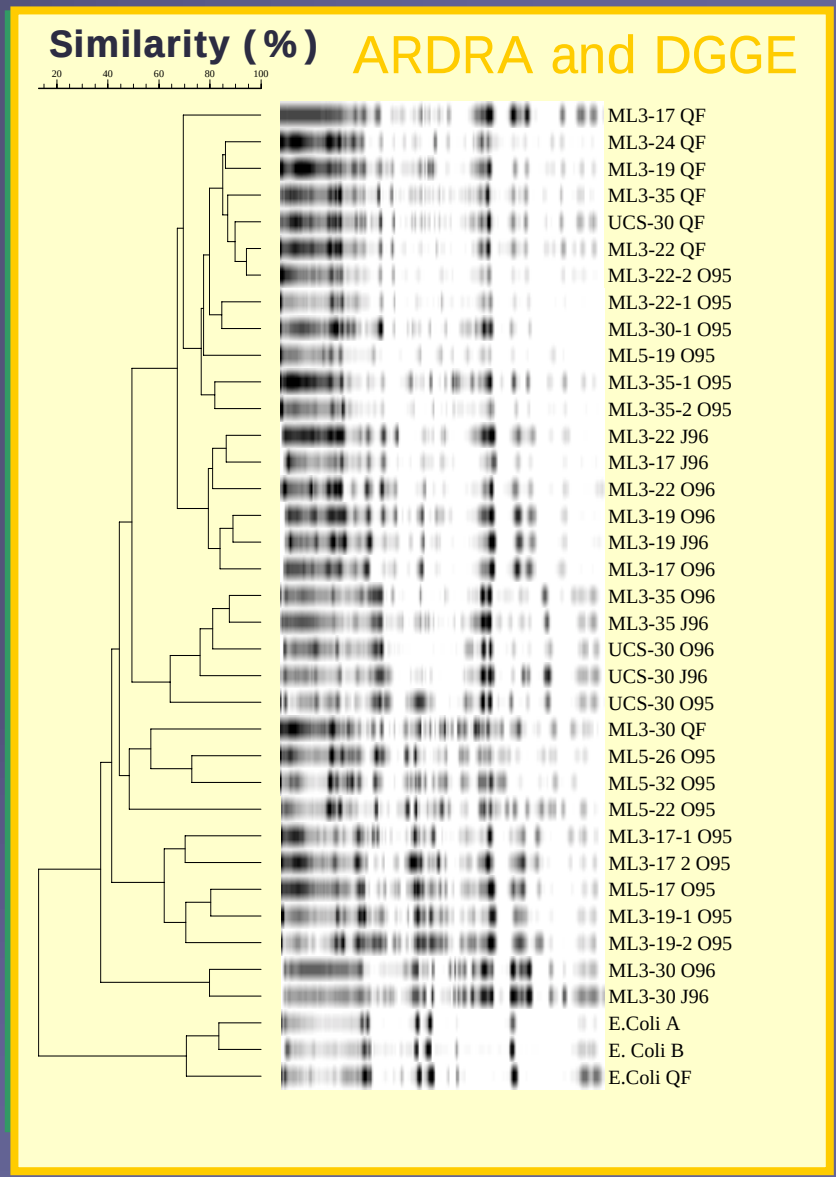
"Footprints" of Natural Attenuation



- Indicate attenuation has taken place
- Support conceptual and numerical models of plume processes
  e.g., BIOMOC – plume evolution

Community DNA Patterns

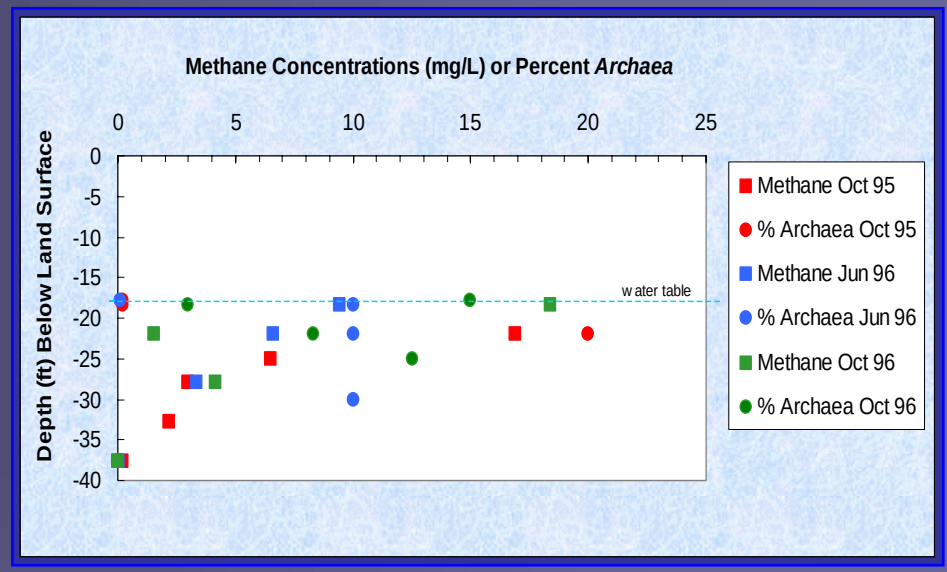
- Spatial/temporal variability
- Not correlated with “footprints” or redox zones defined by dissolved H_2 gas concentrations
- Correlated with pH, BTEX concentrations

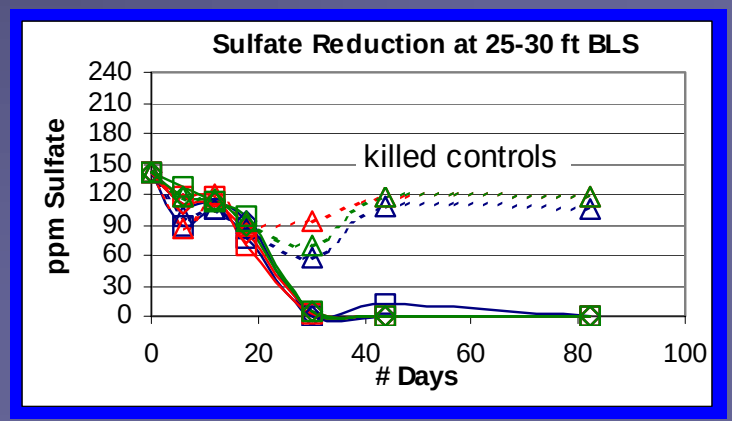


Wurtsmith Air Force Base, Oscoda, MI

Footprints are Variably Accurate

- Methanogenic populations are present and active
  - Cloning (16S rDNA-Dojka & Pace, UCB) & Hybridization (16S rRNA)
- Sulfate-reducers at very low abundance at all depths (<0.2%)
  - Hybridization (16S rRNA)
  - PCR of *dsr* gene
  - West and Alm, CMU
- Sulfate reduction can be stimulated; similar results for iron





Value of New Methods

- Can study *in situ* microorganisms
  - No isolation
  - No artificial growth
- Can approach the same question with several different techniques