The PhyloChip:
A complete microbial community approach to water quality research

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Immense microbial diversity

1,000+ species in human, animal guts
5,000+ species per liter seawater, gram soil

Most bacteria (~99%) resist culturing
Our goal: Exploit this diversity for definitive water quality diagnosis and source tracking

**Immense microbial diversity**
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We can now characterize microbial diversity quickly and cheaply with new molecular methods

**Our goal: Exploit this diversity for definitive water quality diagnosis and source tracking**
Microbial community analysis: limited by our (lack of) power to observe

Culture methods
Restricted detection, must grow in captivity

Culture-independent methods
Restricted detection (Quantitative PCR)

Low resolution (RFLP, DGGE, PLFA)

Cost/labor prohibitive (DNA sequencing)
PhyloChip analysis of microbial communities

Detects 50,000 different bacteria and archaea in a single test

Comprehensive census of whole microbial community

Rapid, repeatable and standardized method
PhyloChip
comprehensive microbial census

- High-density oligonucleotide gene microarray
- 1.1 million DNA probes for most known bacteria and archaea
- Based on entire 16S rRNA gene database (greengenes.lbl.gov)
- Analysis of entire pool of community DNA allows detection of very low abundance taxa, microbial community dynamics
PhyloChip detects 50,443 bacteria and archaea
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- 643 Archaea
- 2,105 Coliforms
- 2,272 Bacteroidales
- 12,098 Clostridia
- 146 Enterococci
- 353 Cyanobacteria
- 184 diatoms/dinoflagellates
Sample preparation

1. Extract DNA/RNA from filtered sample
2. Amplify 16S rRNA gene with PCR
3. Profile of entire community
4. Analyze composition amplified DNA/RNA
PhyloChip analysis

PCR amplification of community 16S rRNA genes

Fragment and biotin label

Wash, stain and scan

Hybridize to array

www.affymetrix.com
Reveals occurrence and relative abundance 16S rDNA for 50,000 different bacteria and archaea.

Location and intensity of fluorescence determines test results.
Clean Beaches Initiative

Goals

Better understand the fate of pollutant microbial communities in coastal waters

Identify key indicators of different sources of fecal contamination

Create more accurate, sensitive tests for routine testing and source tracking
PhyloChip water quality projects

- Fate of pollutant microbes: What happens to pollutant communities once they enter receiving waters?

- Source characterization: Which bacteria and archaea distinguish human and animal fecal sources?

- Variation in time and space: How do background microbial communities respond to changes in the coastal environment?

- Epidemiology: Which organisms are associated with human illness?
Field test:
Tracking a 764,000 gallon sewage spill in Richardson Bay
February 2009 spill at Sausalito treatment plant

Sampled 24, 48 and 72 hours after beginning of the spill

Smaller spill 10 days later

8 onshore/offshore locations

DNA analysis with PhyloChip, qPCR

Fecal indicator tests (Enterococci, E. coli, total coliform)

11-day diffusion chamber experiment
PhyloChip Results
Community analysis of 24,748 detected taxa

Nonmetric Multidimensional Scaling ordination

Axis 1 (98% variance)

Axis 2 (1% variance)

Bay samples
- Low FIB
- High FIB

Sewage source
- High Coliform (>1000)
- High Enterococcus (>35)
- High E.Coli (>126)

Meets FIB criteria
Exceeds FIB criteria
PhyloChip Results

Microbial community composition strongly correlated with fecal indicator tests

- Enterococcus
- E. coli
- Total coliform

[Graphs showing correlations with R² values 0.93, 0.83, and 0.79]
Potential molecular indicators

Clostridia

Abundances of 4,625 different taxa found in sewage were strongly correlated ($r>0.9$) with fecal indicators.

Most correlated taxa were Bacteroidales and Clostridia.
Polymicrobial Source Tracking

Water samples with high fecal indicators fall along a vector toward the known source community.

Illustrates the power of community analysis with microarray to identify the cause of exceedences when the source is unknown.
Diffusion chamber experiments

A "cage" for microorganisms: holds captive anything greater than 0.2 μm in size. Permeable to water, molecules in solution

Monitor abundance and activity of all bacteria and archaea over time upon immersion into receiving waters

What is the fate of microbial communities from different fecal wastes in fresh and marine waters?

Photo credits: Janet Fang
Experiment: fate of cattle waste and septage in fresh and marine waters

- Freshwater (Walker Creek) x 3
- Saltwater (Tomales Bay) x 3

Sampled at 6 time points: 0, 1, 12, 24, 48, 96 hours after immersion

Vacuum filtration through 0.2 μm filters
Frozen on dry ice for DNA/RNA extraction
Unfiltered sample for direct counts
Comparison of diverse communities

Cluster analysis

- Receiving waters
- Septage
- Cattle
- Bay Creek
Comparison of diverse communities

Cluster analysis

- Receiving waters
- Bay Creek
- Septage
- Cattle
- 0-24 hours
- 96 hours
Comparison of diverse communities

Cluster analysis
Bacteria and archaea unique to each waste and absent from receiving waters.
Effect of time in receiving waters on waste microbial communities

Four day immersion

Distinct differences in survivability among taxonomic groups

Most proteobacteria decrease in relative abundance in both wastes

Clostridia persist

Some bacilli (Enterococcus) and coliforms in septage persist
Composition of fecal bacteria that persist in creek versus bay water

Water type has an effect on which fecal bacteria persist over time

Similar response of cattle and septage communities

Clostridia, γ-proteobacteria, coliforms favored in creek

β-proteobacteria favored in Bay
Ongoing work: characterization of microbial communities in potential fecal sources
Sites

- Campbell Cove
- Dillon Beach
- Miller Park
- Green Bridge
- Inkwells
- Muir Beach
- Horseshoe Cove
- Baker Beach

San Francisco

Tomales Bay

Sonoma

San Pablo Bay

Marin

Bodega Bay

Drakes Bay

AB411 weekly monitoring
Molecular analysis: PhyloChips, qPCR
Culturable fecal indicators
Physical and chemical properties
Summary

PhyloChip detects most known bacteria and archaea in a single test

Enables comprehensive, high resolution surveys of microbial communities

Opportunity to gather much needed information about microbial communities in pollutant sources and receiving waters

Potential to identify new sets of indicator taxa to better track coastal pollution
Thank You

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