A comprehensive microbial community approach to water quality research using the PhyloChip

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ABSTRACT

Current methods for assessing microbial water quality in recreational waters focus on only a few types of fecal indictor bacteria and have several shortcomings. Standard fecal indicator tests are not reliable, do not distinguish human from natural sources of pollution, and measure bacteria that can grow in non-fecal environments such as aquatic sediments and decaying kelp. A new method of DNA sequence analysis developed by our group presents an opportunity to vastly improve water quality testing and risk assessment through comprehensive characterization of the bacterial community. Our 16S rRNA gene microarray (PhyloChip) detects 50,000 types of bacteria and archaea in a single test, and exploits the entire microbiome as an index of water quality. We are using this breakthrough technology to address a number of pressing questions in water and public health research: Which groups of bacteria reliably distinguish different sources of fecal contamination? What are the fate of those fecal microorganisms once they enter various types of receiving waters (fresh versus marine)? What microbial communities are present during pollution events in coastal waters? We are using a combination of field experiments and monitoring to address these questions at important recreational beaches in California.

OBJECTIVES

Use comprehensive microbial community analysis to distinguish different sources of fecal pollution

Identify unique combinations of bacterial taxa that can be used for reliable source tracking

Identify the source(s) of water quality problems during field tests

Determine the fate of microbial communities from different fecal wastes in fresh and marine waters

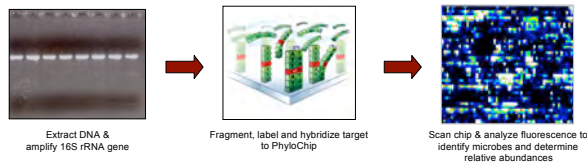## MATERIALS & METHODS

Berkeley Lab PhyloChip

Wall Street Journal Technology Innovation Award (2008)
R&D 100 Award for technology advancement (2008)

High-density oligonucleotide microarray enables rapid, high-resolution profiling of entire communities of bacteria and archaea

1.1 million 16S rRNA gene probes for 50,443 different bacterial and archaeal taxa

Probes designed from database of most known 16S rRNA gene sequences (greengenes.lbl.gov)

Analysis of entire pool of community DNA allows detection of low abundance taxa, microbial community dynamics

Extract DNA & amplify 16S rRNA gene → Fragment, label and hybridize target to PhyloChip → Scan chip & analyze fluorescence to identify microbes and determine relative abundances

Characterization of Source Bacterial Communities

Sampled feces from animal (gulls, geese, cows, horses, elk, seals/sea lions) and human sources (septic tanks, holding tanks, sewage) along the California coast

Four distinct populations sampled for each animal

PhyloChip analysis of composite sample from at least five individuals per population

Source Tracking Field Test

Photo credit: Kurt Rogers
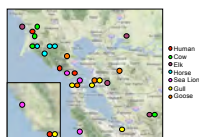
Broken pipe discharged 764,000 gallons of partially treated sewage into San Francisco Bay near Sausalito

Bay water samples (1 liter) collected for analysis 24, 48 and 72 hours after the spill at 8 locations within 1 km of the spill

Bacterial community analysis compared to standard fecal indicator tests (culturable Enterococci, E. coli, total coliform) that were used to determine beach closings and subsequent regulatory action

Fate of fecal microbial communities in fresh and marine waters

Monitored fecal microbial communities under field conditions using in situ diffusion chambers. Chambers are permeable to water and solutes but retain microorganisms.

Chambers filled with 100 mL fecal waste (cattle or septic) and immersed in fresh and marine waters (Walker Creek, Tomales Bay). Samples withdrawn and analyzed at 0, 24 and 96 hours.
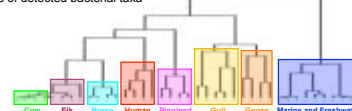

Photo credit: Janet Fang

ACKNOWLEDGEMENTS

RESULTS: Source Microbial Communities

Cluster analysis of detected bacterial taxa
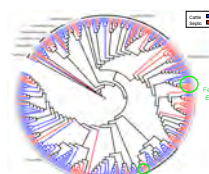

Cow | Elk | Horse | Human | Pinniped | Gull | Goose | Marine and Freshwater

Detected 3513 different bacterial subfamilies in fecal samples

Strong clustering by type of animal. Bacterial communities in human sources are distinct.

Strong potential for source identification based on bacterial community differences

Significant differences in the relative abundances of bacterial taxa between fecal and unpolluted water samples

Thousands of potential bacterial indicators for each animal

This community-based approach to source tracking offers specificity and redundancy that is lacking with single indicator tests

Bacterial taxa unique to cattle and septic wastes

RESULTS: Source Tracking Field Test

Sewage spill in San Francisco Bay: Was the spill responsible for high numbers of culturable fecal indicator bacteria (FIB) measured in Bay samples during monitoring?

Bacterial community composition of Bay samples compared by ordination (nonmetric multidimensional scaling)

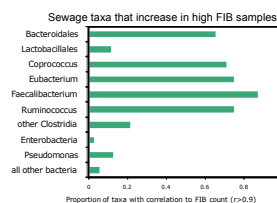Composition of waters with high FIB counts distinct from waters with low FIB counts

A few samples with high E. Coli were not similar to sewage communities
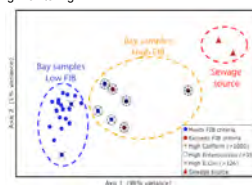
Bacteria with increased abundance in high FIB samples closely matched composition of taxa that are characteristic of human sources

Exceeds FIB criteria
Elevated FIB (> 30-day criteria)
Elevated E. coli in low FIB cluster
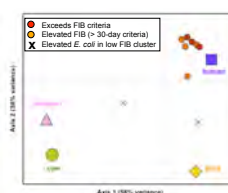
Clostridia and Bacteroidales characteristic of sewage were more abundant in high FIB samples

Sewage taxa that increase in high FIB samples

Bacteroidales
Lactobacillales
Coprococcus
Eubacterium
Faecalibacterium
Ruminococcus
other Clostridia
Enterobacteria
Pseudomonas
all other bacteria

Proportion of taxa with correlation to FIB count (r>0.9)

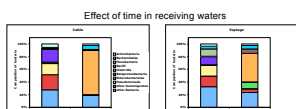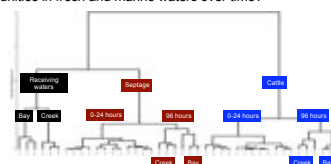## RESULTS: Fate of Source Microbial Communities

What is the fate of fecal microbial communities in fresh and marine waters over time?

Photo credit: Janet Fang

Strong effect of time in water on fecal communities

Communities shift in response to water type

Effect of time in receiving waters

Significant differences in persistence among taxonomic groups

Similar shifts in both cattle and septic communities

Different sets of bacterial taxa might be useful for tracking depending on water type, monitoring objectives.

Effect of receiving water type (fresh vs. marine)
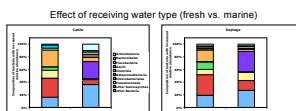
CONCLUSION

PhyloChip enables comprehensive, high resolution surveys of microbial communities

Thousands of bacterial taxa distinguish different animal and human sources of fecal pollution and are promising targets for robust source tracking

Sources of fecal pollution in coastal waters can be identified with confidence using community analysis with PhyloChip data