Exploring the Microbiome for New Indicators of Waterborne Disease Threats

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Sanitation and Waterborne Disease

Developing countries: 20% of the world's population does not have clean drinking water

1.5 million die annually, nearly half children under 5 years old

United States: recreational waters and drinking water - Outbreaks/illness (under-reported) Cryptosporidium outbreak – largest in US history, 400,000 exposed

Newton et al. 2011 Chronic sewage contamination in the Great Lakes
E. coli and enterococci - indicators of fecal pollution

EPA recommended indicators- since 1986
*Present in almost all animals and easily detectable*

**PROBLEMS**
Does not differentiate the source,
present in all animals
Human sources = human pathogens

Animal sources may also be a concern:

**E. coli/enterococci** can survive/grow in the environment
Todays talk
Microbial ecology and the data explosion
Making sense of microbiome data

Short Stories
Animals and humans

The microbiome
US and global

Sewage footprints in the Great Lakes
MICROBES PAST, PRESENT AND FUTURE - Dawn of modern microbiology

~100 years

1849 1876 1885 1929 1953

John Snow  Robert Koch  Alexander Fleming  James Watson

Francis Crick  Theodor Escherich
MICROBES PAST, PRESENT AND FUTURE - molecular revolution

105 years

Carl Wose

Norman Pace

Craig Venter

30 years

1977
1986
1997
2005

Ronald Blatner
explosion of data

2005 Eckberg et al. sequenced 13,000 clones in human gut

Diversity of the Human Intestinal Microbial Flora

Paul B. Eckburg, 1* Elisabeth M. Bik, 2 Charles N. Bernstein, 3
Elizabeth Purdom, 4 Les Dethlefsen, 2 Michael Sargent, 3
Steven R. Gill, 5 Karen E. Nelson, 5 David A. Relman 1,2,6*

2006 Sogin et al. obtained 1,000,000 sequences in ocean

Microbial diversity in the deep sea and the underexplored “rare biosphere”

Jesus M. Arrieta§, and Gerhard J. Herndl‡

*Josephine Bay Paul Center, Marine Biological Laboratory at Woods Hole, 7 MBL Street, Woods Hole, MA 02543; and †Royal Netherlands Institute for Sea Research, P.O. Box 59, 1790 AB, Den Burg, Texel, The Netherlands

Communicated by M. S. Meselson, Harvard University, Cambridge, MA, June 20, 2006 (received for review May 5, 2006)

The evolution of marine microbes over billions of years predicts Gene sequences, most commonly those encoding rRNA
explosion of data........

Cloning - 96 clones 1 sequence/sample
Next Gen - 96 samples with 100,000 sequences each
100,000X more sequence information!
How do we make sense of the data?

Sequence

Identify the members

Community analysis

Raw reads
Quality trimming
Chimeras
Barcoded samples

V2, V4V5, V6
Assign taxonomy
Cluster into OTUs
Oligotyping
Minimal Entropy
Decomposition

What region to use?
Silva, Greengenes
3% dissimilarity
New methods
Why do we want high resolution data?

Functional differences likely at the strain or ecotype level

Phylum or Class: Betaproteobacteria - water
               Acidobacteria - soil
Why do we want high resolution data?
Functional differences likely at the strain or ecotype level

Phylum or Class:
- Betaproteobacteria - water
- Acidobacteria - soil

Family:
- Flavobacteriaceae - freshwater
- Ruminococcaceae - warm-blooded gut
Why do we want high resolution data?
Functional differences likely at the strain or ecotype level

Phylum or Class: Betaproteobacteria- water
Acidobacteria-soil

Family: Comamonadaceae-water
Ruminococcaceae-warm-blooded gut

Genus: Blautia – humans
Blautia – cows
Blautia – pigs

Genus Blautia found in all gut
Core genes make up genus
Specialized genes account for the unique ecotype or strain
Tracking ecotypes by the 16S rRNA gene

OTU by clustering (3% dissimilarity)-problems

Mixed phylogenetic units, hard to detect closely related organisms that might be in two ecological niches

OTU 1

AACG TCTGCTG
AACG TCTGCTG
AACCTCTGCTG
AACCTCTGCTG

OTU 2

TTCG ACTGCGG
TTCG TCTGCGG
TTCG TCTGCGG
TTCG TCTGCGG

TTCG ACTGCGG
TTCG TCTGCGG
TTCG TCTGCGG
Looking for **ecological patterns** in millions of data points

Oligotyping

Eren et al. (2011) PLoS ONE
Oligotyping: 101

Plot entropy to identify most informative positions

String together 10-24 positions to create oligotype
Oligotyping: 101

Plot entropy to identify most informative positions

String together 10-24 positions to create oligotype
Oligotyping creates OTUs based on entropy

Resolves closely related organisms that might be in two ecological niches

Oligotype “OTU” 1a and 1b

Oligotype “OUT” 2
**Oligotyping:** 101

Plot entropy and chose most informative positions

Bin sequences according to the oligotype - Relative abundance
Sequencing Sewage
V6 rRNA gene

80% environmental origin

>50,000 reads per sample  n=49
800 different taxa
20,000 different sequences

20% faecal origin

Acinetobacter
Aeromonas
Pseudomonas

Arcobacter
Betaproteobacteria

Actinobacteria
Bacteroides

Trichococcus

Lachnospiraceae
Ruminococcaceae

VandeWalle et al. (2013) Environmental Microbiology 14(9), 2538–2552
Example of high resolution: sequencing sewage

Environmental - only one or two dominant sequences
faecal origin – very diverse

Acinetobacter
Acinetobacter Tag1 and Tag2 fluctuate with season

VandeWalle et al. (2013) Environmental Microbiology 14(9), 2538–2552
Acinetobacter Tag1 and Tag2 fluctuate with season

VandeWalle et al. (2013) Environmental Microbiology 14(9), 2538–2552
OTU clustering (3% dissimilarity)-problems
hard to detect closely related organisms that might be in two ecological niches

Oligotyping resolved-abundance is considered

OTUs by clustering 1

VandeWalle et al. (2013) Environmental Microbiology 14(9), 2538–2552
Interested in Oligotyping and Minimal Entropy Decomposition (MED)? Go to http://merenlab.org/projects/oligotyping/

FROM THE HUMAN ORAL CAVITY TO PRISTINE SOILS, WE USE OUR EXPERTISE IN MICROBIOLOGY AND COMPUTATION TO STUDY MICROBIAL COMMUNITIES AND THEIR ECOLOGY. WE STRIVE TO CREATE ADVANCED SOFTWARE PLATFORMS, AND DESIGN APPROPRIATE EXPERIMENTS TO CONTRIBUTE A WIDE RANGE OF CONCURRENT RESEARCH QUESTIONS IN THE DOMAIN OF MICROBIAL ECOLOGY. WE ARE CURRENTLY LOCATED AT THE MARINE BIOLOGICAL LABORATORY, THE JOSEPHINE BAY PAUL CENTER FOR COMPARATIVE MOLECULAR BIOLOGY AND EVOLUTION, AND CONSTANTLY LOOKING FOR COLLABORATIONS THAT WOULD CHALLENGE US WITH NOVEL AND INTRIGUING QUESTIONS.
Short Story 1
Animals and humans
Sequencing Sewage
V6 rRNA gene

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- Acinetobacter
- Aeromonas
- Pseudomonas
- Arcobacter
- Betaproteobacteria
- Actinobacteria
- Bacteroides
- Trichococcus
- Lachnospiraceae
- Ruminococcaceae

VandeWalle et al. (2013) Environmental Microbiology 14(9), 2538–2552
Genera of fecal anaerobes common to hosts


- unclassified Lachnospiraceae
- Roseburia
- Dorea
- Coprococcus
- Blautia
- Faecalibacterium
- Alistipes
- Prevotella
- Parabacteroides
- Bacteroides
What is shared and what is unique?
Compare humans, sewage, chickens and cows

McLellan et al. (2013) *Environmental Microbiology*
Lachnospiraceae as a qPCR target for alternative indicators

Clone near-full length sequences
Align and design primers for qPCR

Developed assay for qPCR
Lachno2, Lachno3, and Lachno12
Sequence profiles of *Blautia* act as a fingerprint

Heatmap of sequence abundance shows humans (sewage) and various animals have unique sequence profiles

Sequence profiles of *Blautia* act as a fingerprint

**Next Steps:** build a searchable database so sequencing data can be interpreted

**Cost:** $40-$100 for sequencing

Short Story 2
Microbiome of a city

Sequenced microbial communities in untreated sewage from 71 cities in January, April and August

80% environmental
Acinetobacter
Arcobacter

20% faecal origin
Lachnospiraceae
Ruminococcaceae
Characterizing the human microbiome

PROBLEM

The gut microbiome in individual humans is highly variable

Does the microbiome vary by geography? Health state?

Sewage can be an integrative, representative sample of million of people

Newton et al. 2015
Environmental organisms Acinetobacter and Arcobacter showed geographic patterns *temperature related

Human fecal organisms showed no geographic patterns
Cities with higher obesity rates were more similar to each other than “lean” cities—many bacteria shifted.

Newton et al. (2015) Sewage reflects the microbiomes of human populations. mBio 6(2):e02574-14
Ronald E. Blanton
Professor of International Health and Epidemiology and Biostatistics

Reservoirs of Infectious disease: Brazil biomarkers
Global applications for sewage indicators

Jenipapo
San Salvado

Schistosoma
Why identify additional human fecal indicators?

Global applications

Bacteroides very low in Brazil human population

Koskey et al. (2014) Environmental Microbiology Reports
E. coli and enterococci not informative

Human *Blautia* and *Prevotella* shows where highest sanitation concerns are

Koskey et al. (2014) Environmental Microbiology Reports
McLellan and Eren (2014) Trends in Microbiology
Short Story 3
Sewage footprints in the Great Lakes
Tracking sewage contamination in the Great Lakes

University of Michigan
Contamination enters from the watershed

850 square miles drain to Milwaukee Harbor

Sewage, Agricultural runoff, Stormwater
Contamination enters from the watershed

850 square miles drain to Milwaukee Harbor
Sewage, Agricultural runoff, Stormwater
Urban stormwater contains sewage

Urban stormwater is collected from streets and roof tops and is released directly into rivers

- **Contaminated with sewage** from failing infrastructure

- Very high pathogen levels-similar to sewage

- 250 outfalls n=2000 samples, **30% of stormwater outfalls show some level of untreated sewage**
Automated sampling
Three rivers
Estuary
Continuous virus sampling over the hydrograph
Discrete hourly sampling ~24 samples
**Concentration**
(Copies / 100 ml)

**Load** = Concentration x Flow (Copies)

**Flux** = Load / Time (Copies/day)

**Yield** = Load / Area (Copies/km²)
Conclusions

Next generation sequencing has increased data 100,000X

With new computation methods (oligotyping) we can use track populations with 16S rRNA marker genes that are ecologically relevant

Humans and animals have unique ecotypes within almost all genera
Conclusions

Humans microbiome differ global based on diet, but still consistent signature (within Blautia, etc.)

Urban areas have sewage leaking to stormwater- quantify the sewage pulse from an urban area
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WI DNR
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VandeWalle et al. (2013) *Environmental Microbiology* 14(9), 2538–2552