

Bacterial and Viral Metagenomic Approaches For studying the Built Environment Scott T. Kelley Department of Biology San Diego State University



"[H]umans move through a sea of microbial life that is seldom perceived except in the context of potential disease and decay."

Feazel LM, Baumgartner LK, Peterson KL, Frank DN, Harris JK, et al. (2009) Opportunistic pathogens enriched in showerhead biofilms. Proc Natl Acad Sci USA 106: 16393-16399.

Reasons to study indoor microbiology

- Spend 90% of our time in it.
- Disease transmission: Importance of inert surfaces in spread of microbes
- Opportunistic infections: 100,000 deaths/year (USA) Hospital-acquired infections!
- "Sick Building Syndrome"; Allergies (fungi)
- Fascinating environment in its own right

Previous studies

Low-throughput Sequencing: 16S rRNA Clone Libraries

1.<u>Shower Curtain Biofilms</u>: *Sphingomonas* & *Methlylobacteria*

2.<u>Childcare Center</u>: Surfaces dominated by *Pseudomonas* & Gut bacteria

3.<u>Airplane surfaces</u>: Lavatories – microbes from every human orifice

4. Hospital Therapy Pool





Phylogenetic distribution of pool environment bacterial rRNA genes

Angenent L. T. et.al. PNAS 2005;102:4860-4865



High-Throughput Studies

- Multiplexed pyrosequencing of barcoded 16S rRNA amplicons (Sogin, Fierer)
- Study 1: Office Buildings (NE, SF, Tucson)
 - Typical diversity of office microbiome?
 - Nested Sampling Design
 - 3 buildings/city
 - 30 offices/building
 - 5 surfaces/office (60:40 Women:Men)

Office Study

Viable Bacteria

Heterotrophic Growth Count Assay

General estimate Of abundance

Spread Plate Method (RA2 Media)



16S Seq Study **Chair and Phone** Most contaminated 54 surfaces 18/city Chair, phone **Barcoded primers** Pooled 454 Seq

Office Study

Data Analyzed using QIIME package

- ~140,000 sequences >200bp
- ~3800 distinct OTUs at the 97%
- ~400 bacterial genera (20 divisions)







High-Throughput Studies

- Multiplexed pyrosequencing of barcoded 16S rRNA amplicons
- Study 2: Newborn Intensive Care Units
 - Potential role of Inanimate Hospital Environments as reservoirs for opportunistic infections?
 - NICUs: High rates of infection, morbidity/mortality
 - 65% ELBW at least one infection, 27% motality
 - Two San Diego NICUs
 - Frequently touched surfaces

NICU Study

IHEs rampant with human commensals known to be opportunistic pathogens: Neisseria, Pseudomonas, Streptococcus,

Streptococcus, Staphylococcus, Acinetobacter, Clostridium, Flavimonas, Flavobacterium, Fusobacterium, Gemella, LeClercia, Legionella, Pasturella, Propionibacterium, Stenotrophomona

Dominated by Enterobacteriaceae



Talking Points

- Useful to establish a baseline of "typical" contamination – detect departures
- Clear evidence for robust IHE contamination
- Inspired model of Built Environment Microbiology:



SourceTracke

"Where did that schmutz come from?" Bayesian Approach for estimating sources of microbial contamination

Also potentially useful for natural environments:

- Freshwater streams?
- Insect guts?



Figure 2 | SourceTracker proportion estimates for a subset of sink samples. (**a**-**c**) Source environment proportions for three sink samples estimated using SourceTracker and 45 training samples from each source environment: mean proportions for 100 draws from Gibbs sampling (**a**), data for the same samples, including s.d. of the proportion estimates (**b**), and visualization of the 100 Gibbs draws; each column shows the mixture from one draw, with columns ordered to keep similar mixtures together (**c**).

Studies of Built Environment – Feedback to natural systems?

- SourceTracker complex natural communities
- EMP and HMP: Cleaning up the databases?
- Artificial Environments: Just how much do microbes move around?

Restroom Virome Project

- Virtually NOTHING known about indoor viral diversity
- Viral/bacterial diversity on surfaces in male and female restrooms over time
- Study Design:
 - Same three surfaces (Toilet seat, Floor, Soap Dispenser pump)
 - Sterilize early morning, collect end of day
 - Repeat every week for two months
- Data collection:
 - 16S bacterial diversity
 - Viral shotgun sequence libraries

Flores et al. (2011) **Microbial Biogeography of Public Restroom** Surfaces, PLoS ONE 6: e28132



Microscopy: Bacteria and VLPs

- Plenty of both on all surfaces
- 20 minutes of soaking in 10% bleach necessary to clean surfaces fully! 2 minutes <u>never enough</u>.



Women's restroom floor Uncleaned



2 Mintues of 10% Bleach treatment



After 20 minutes of 10% Bleach



DNA isolation (Formamide/CTAB) (OR MoBio Soil Kit) Verify Viral Particles after every spin

Current Status and Future Directions

- Four samples awaiting sequencing (ahem!)
- All ready for both bacteria/viruses (entire study) – MiMarks compatible
- Sequence 16S and DNA viral libraries on Illumina Platform (Follow EMP protocols)
- RNA viruses? Metagenomics/Virochip
- Data Analysis: MG-RAST, ShotGun Unifrac

SHOTGUN UNIFRAC

BLAST reads against full genomes to assign

Per-sample metagenomic reads

viral OTUs and generate Sample by OTU >Sample1 r1 count mapping (i.e., the "Viral OTU table"). ACCGAATTGAACCCGGAA... >Sample1 r2 Samplel Sample2 Sample3 TGGTACCAGACCAGG... otu1 0 6 9 >Sample2 r3 2 otu2 0 0 GCCGTAATAAAGTTTCCAAAC... otu3 9 0 4 2 2 otu4 ... PC2 (12%) PC1 (23%) Calculate UniFrac distances from Viral OTU Full phage genomes and Phage table and Phage Proteomic Tree. Apply Proteomic Tree Principal Coordinates Analysis.

Figure 1. Schematic of the Shotgun UniFrac analysis pipeline. doi:10.1371/journal.pone.0016900.g001

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