NEMC

ADVANCES IN METAGENOMICS FOR CHARACTERIZING MICROBES IN THE ENVIRONMENT

EMSL

Jason Dobranic, Ph.D.
VP Microbiology & Life Sciences
EMSL Analytical, Inc.

AMBIENT WATER TESTING

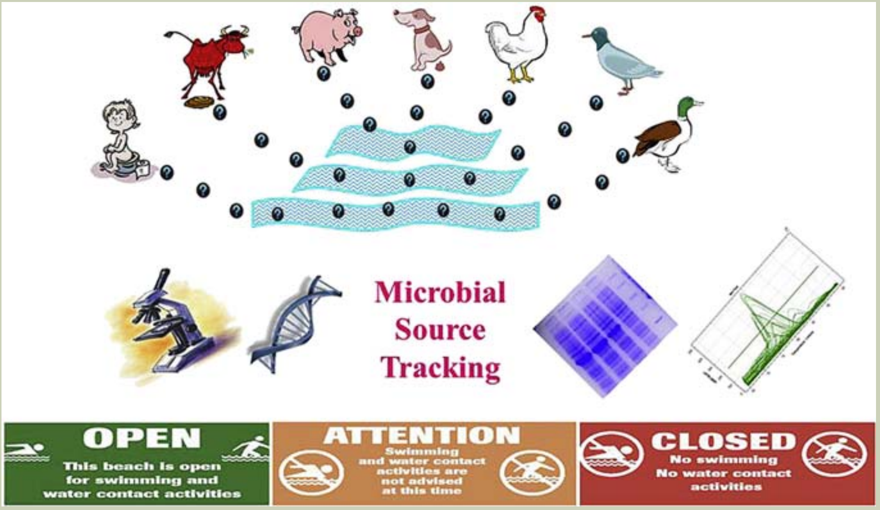
- Swimming in contaminated beach waters
  - 120 million cases of gastrointestinal (GI) disease
  - 50 million cases of acute respiratory diseases world-wide
- Shifts in rainfall and temperatures driven by climate change
  - exaggerate microbial contamination issues
  - increase risk of water-borne disease in coastal and inland regions

WATER QUALITY INDICATORS:
CULTURE-BASED BIAS

- EPA established indicators
  - Rely on culturing fecal indicator bacteria to predict risk
  - Underestimate the densities and diversity of microbes in environmental samples
- Non-culturable microbes
  - May be more important as etiological agents
  - Better indicators of fecal contamination
  - Useful in source tracking

U.S. RECREATIONAL WATER QUALITY CRITERIA

- Evaluation of the 2012 RWQC
  - other possible fecal indicator such as *C. perfringens*, *Bacteroides*, and human enteric viruses
  - or new genomic approaches (e.g., metagenomics) should also be evaluated in developing new RWQC.
- 2017, 5 YR review
  - majority of experts (>90%) favor a PCR-based methodology for MST
  - focused only on qPCR methods
  - identification of top human-associated fecal markers
    - HF183/BacR287
    - HumM2



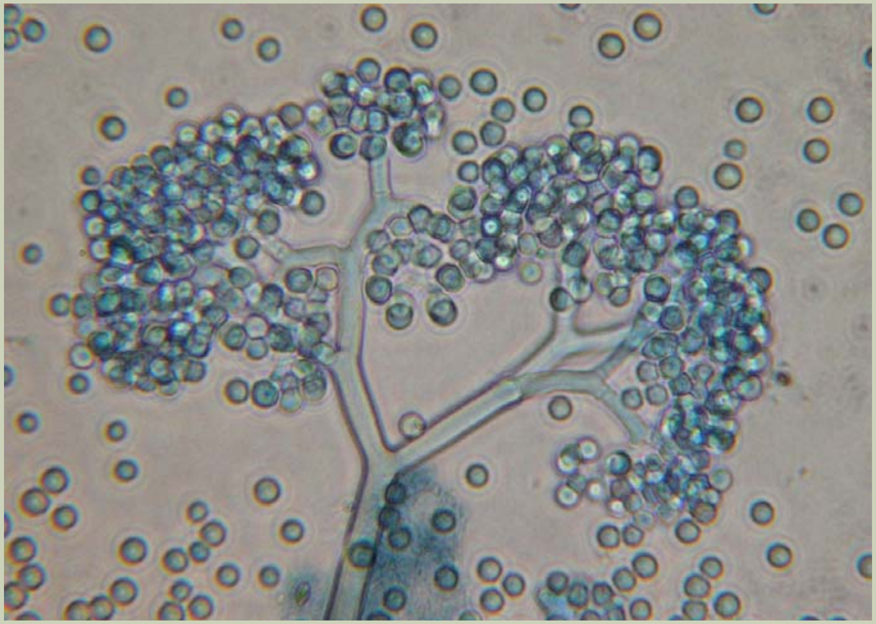
Stewart JR, et al, 2013. Recommendations following a multi-laboratory comparison of microbial source tracking methods. Water Research Vol47

MOLECULAR METHODS

- Molecular-based methods overcome culture limitations
- gut microbiota are now being considered as alternate indicators for human and animal fecal contamination
- characterize and map the different microbial community populations associated with human sewage and various animal feces
- monitor water quality using a single, comprehensive suite of microorganisms by analyzing patterns of relative abundance

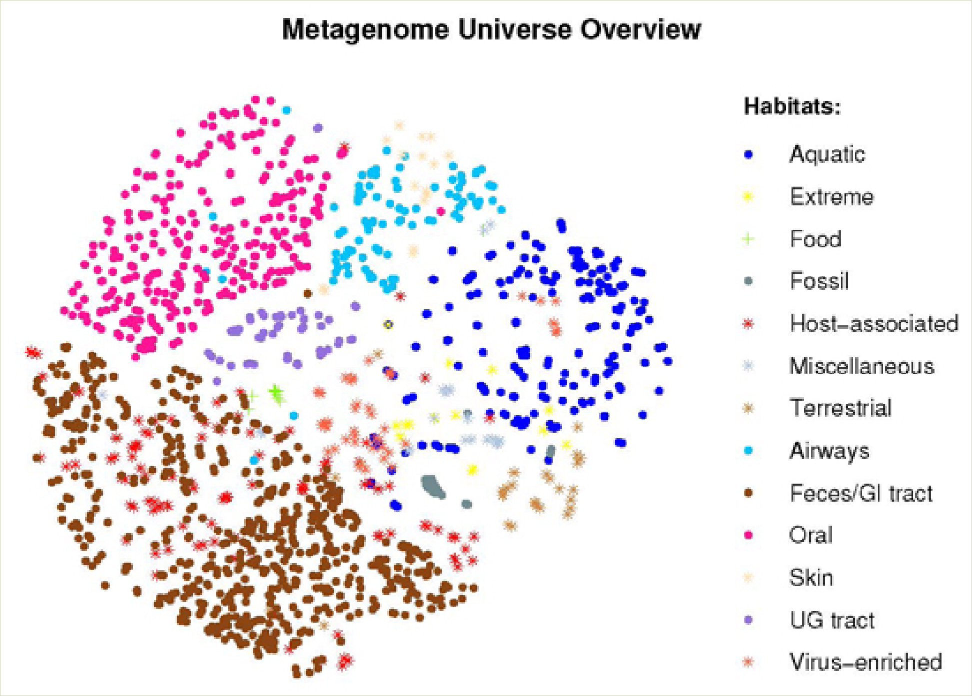
GLOBAL FUNGAL SPECIES RICHNESS

- ~100,000 species described
- ~600,000 from conservative estimates
- ~1 billion from optimistic estimates
- Newer molecular methods are increasing our knowledge of microbial diversity



METAGENOMICS

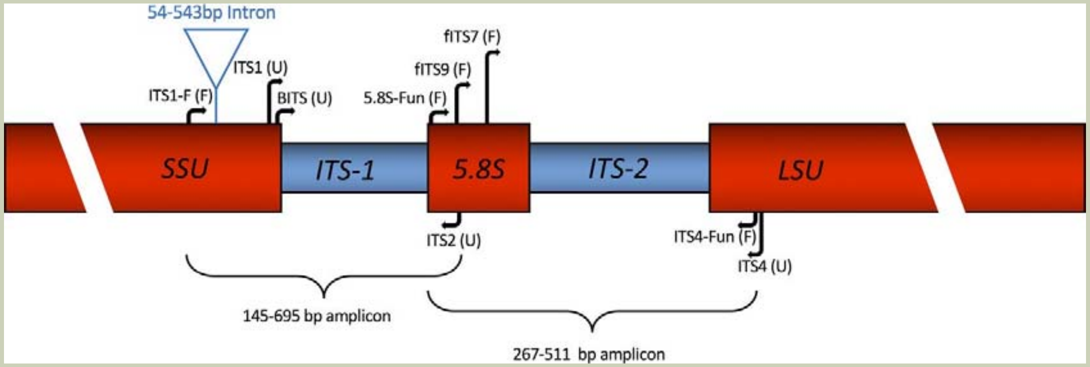
- Direct genetic analysis of genomes contained in environmental samples
- Targeted vs. shotgun approach
- Most data sets related to prokaryotic microbial communities
- Increased awareness and studies on fungi



Aßhauer, KP., et al., *Int. J. Mol. Sci.* **2014**, *15*(7), 12364-12378

USING NEXT-GEN SEQUENCING

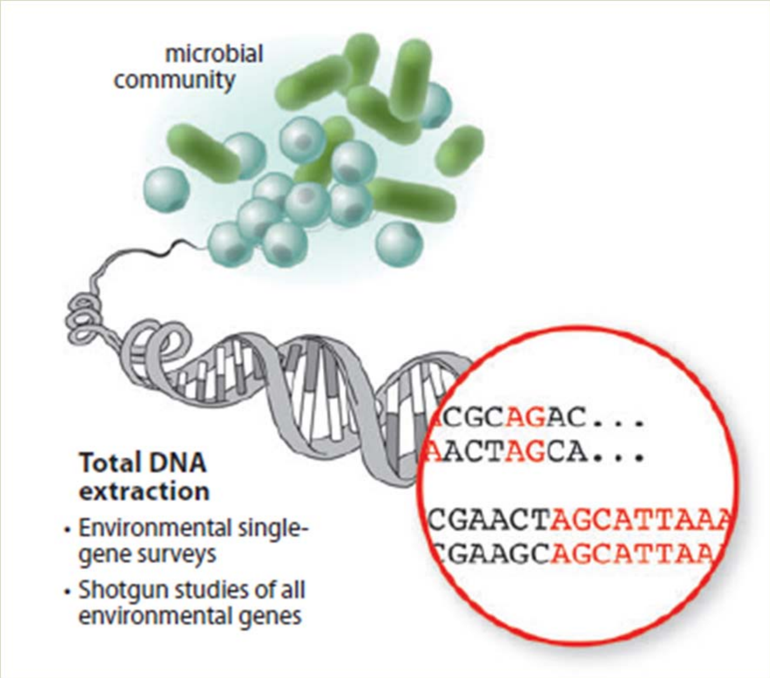
- Culture-free method (only small amount culturable in environmental samples)
- Entire microbial community analyzed in a sample
- 16s rRNA gene used for bacteria (universal marker)
- 18s rRNA gene or ITS regions for fungi
- Bioinformatics is key (computational tools chosen for sequence analysis)



MICROBIOME ANALYSIS

Sample Processing Pipeline:

1. Sampling (larger volumes increases chances of retrieving rare groups)
2. Filtration
3. Cell lysis/DNA extraction
4. Gene amplification
5. Sequencing library construction
6. Next-gen sequencing (MiSeq)
7. Bioinformatic data analysis
8. Results interpretation



Adapted from:
http://hulab.ucf.edu/research/projects/metagenomics/introduction.html

CONSIDERATIONS

- Efficiency of extraction is very important
- Amount and type of non-biological material in a sample may interfere with extraction, quantification, and amplification
- PCR inhibitors must be removed in clean-up step
  - e.g., cations, humics, pollutants can interfere

AMBIENT WATER STRATEGY

- Characterize the predominant microbes in the gut of humans and predominant microbes in the gut of various other animals
- Community "fingerprint" can be established to determine if the source of contamination is from human or other animal feces

DATA NEEDED

- More studies to determine if metagenomics methods are feasible for general use and if the generated data can be used to improve future RWQC
- As an example, McLellan *et al.*, determined that members within *Clostridales* and *Lachnospiraceae* were among the most dominant bacterial groups in Milwaukee's wastewaters, and these bacteria were found in an estuary and bathing beaches of Lake Michigan following storm events.

APPLICATIONS

- Biodiversity studies & community composition
- Study metabolic genes from microbial communities
  - Biased towards previously known genes
- Discovering novel organisms and genes in the environment
- Bioremediation studies

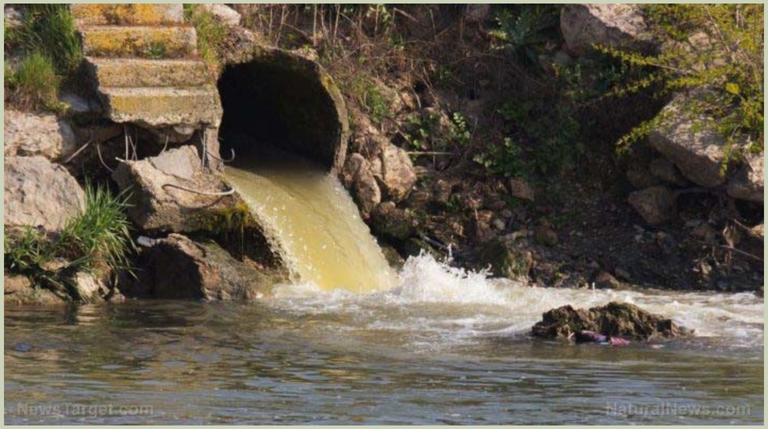
GLOBAL OCEAN SAMPLING EXPEDITION (GOS)

- Started in 2003 by Craig Venter
- Goal to assess the genetic diversity of marine microbial communities around the Earth
- Starting in Halifax, Canada, samples were collected at sites along the U.S. east coast, Gulf of Mexico, Galapagos Islands, central and south Pacific Oceans, Australia, Indian Ocean, South Africa, and across the Atlantic back to the U.S.
- A total of 41 different samples were taken from a variety of aquatic habitats
- yielding 6.4 million contiguous sequences
- Goal of understanding microbial diversity and function

COMPARATIVE METAGENOME OF A STREAM IMPACTED BY THE URBANIZATION PHENOMENON

Medeiros, JD, *et al*., Brazilian Journal of microbiology 47 (2016) 835–845

- Upstream preserved and non-urbanized area vs. a polluted urbanized area with discharged sewage
- Non-urbanized area was overrepresented by genera of ubiquitous microbes that act in the maintenance of environments
- Urbanized metagenome was rich in genera pathogenic to humans
- Antibiotic resistance, metal-resistance, and stress response-related genes were disseminated in the urbanized environment



PHYLOGENETIC PROFILES

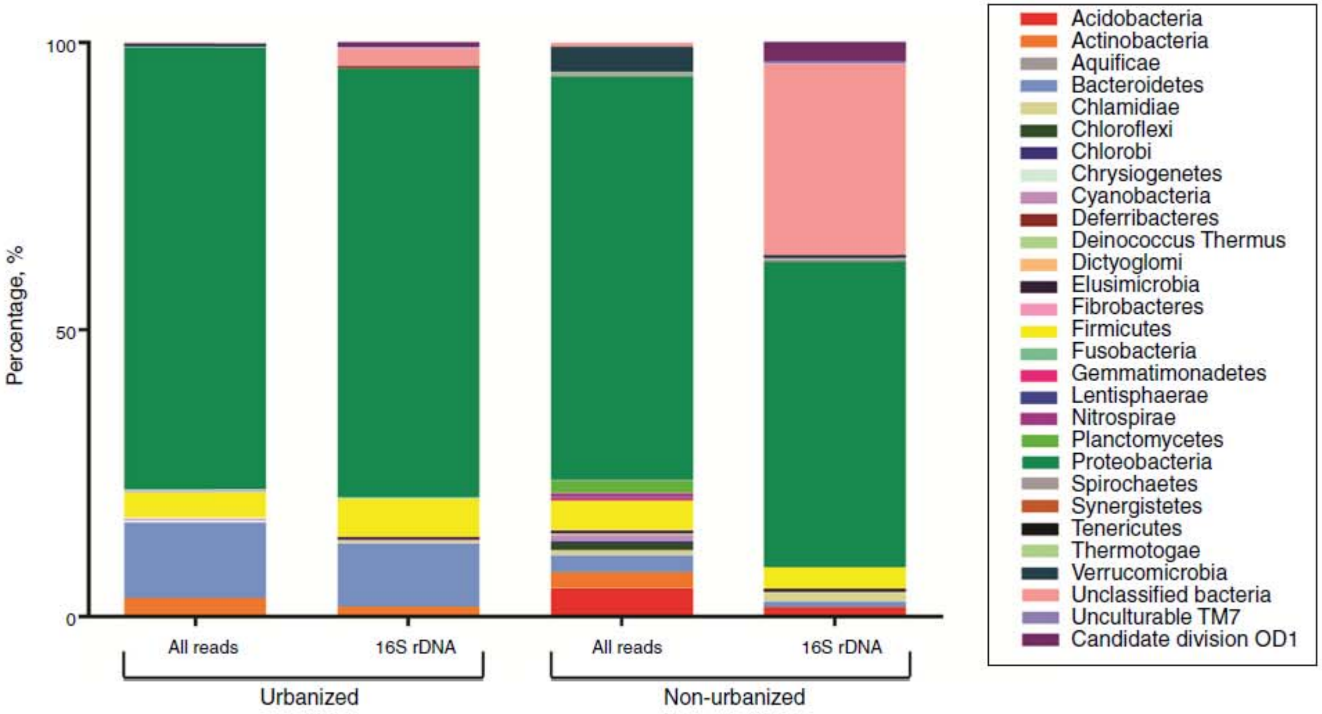


Fig. 1 – Phylogenetic profile of the freshwater urbanized and non-urbanized metagenome using the 16S rDNA sequences and all the shotgun reads.

GENE FUNCTIONS

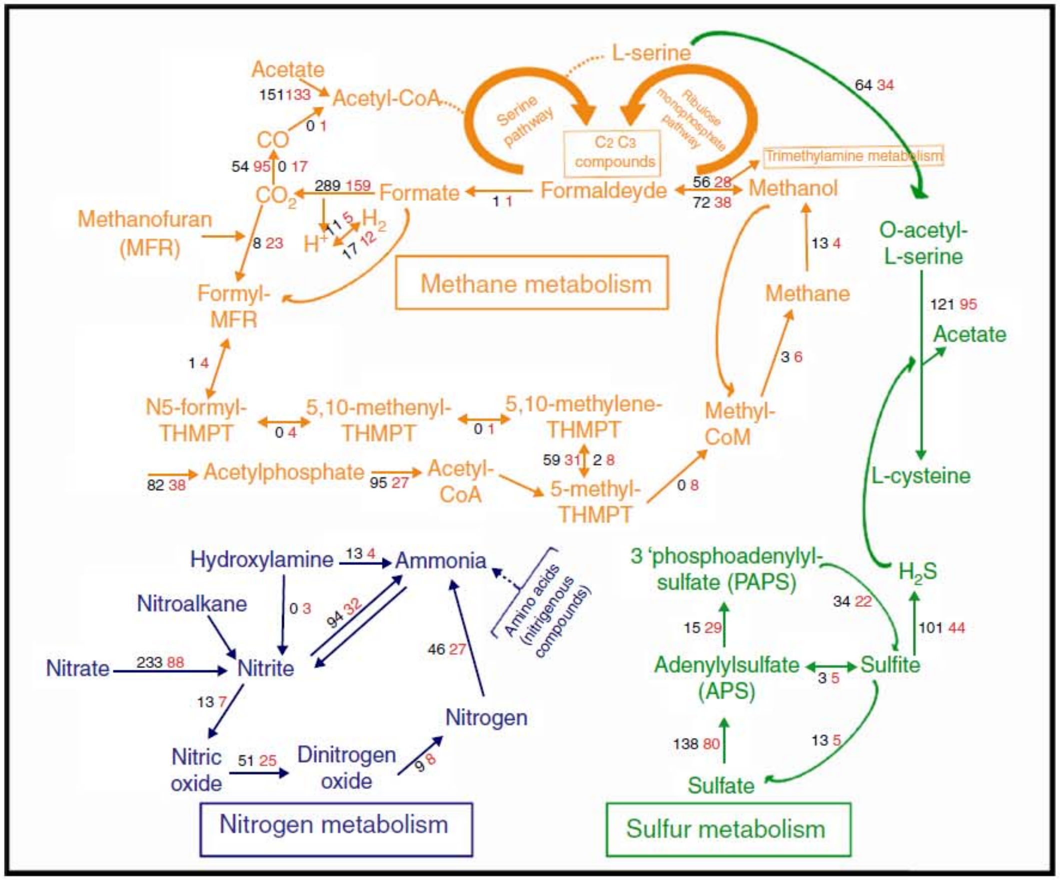
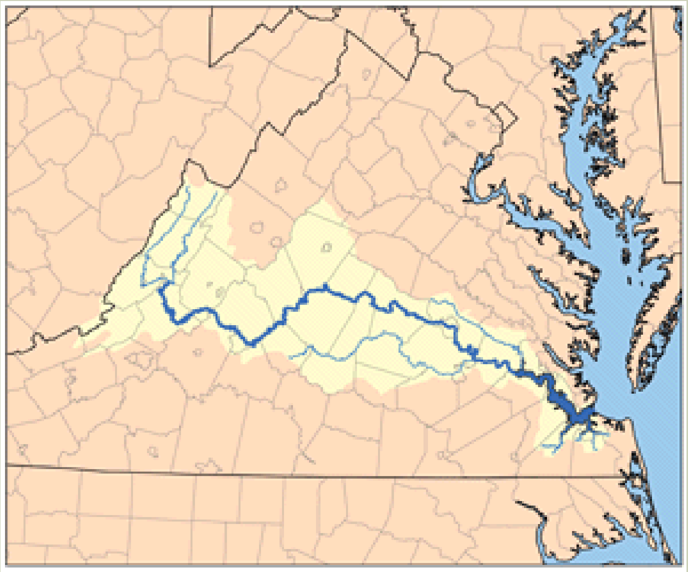


Fig. 3 – Metabolic pathways of methane, nitrogen and sulfur from the freshwater metagenomes. The numbers label in black indicate the number of sequences from urbanized metagenome affiliated with the KEGG function and the number label in red indicate the non-urbanized metagenome. Data obtained through MEGAN.

JAMES RIVER METAGENOME PROJECT

Brown *et al*. Standards in Genomic Sciences (2015) 10:65

- one of the largest tributaries of the Chesapeake Bay
- home to more than 25 million Virginians
- >1500 point sources permitted to discharge pollutants
- nonpoint source pollutants from urban, agricultural, wildlife, and transportation runoff
- heavily accessed for recreation



JAMES RIVER METAGENOME PROJECT

- Current recreational water monitoring practices (e.g., *E. coli* and coliform testing)
  - only as coarse indictors of potential contamination
  - provide little information on the diversity, source, ecology, or evolution of organisms that cause WBDOs

PUTATIVE ROLES OF THE MOST ABUNDANT BACTERIAL AND EUKARYOTIC OTUs

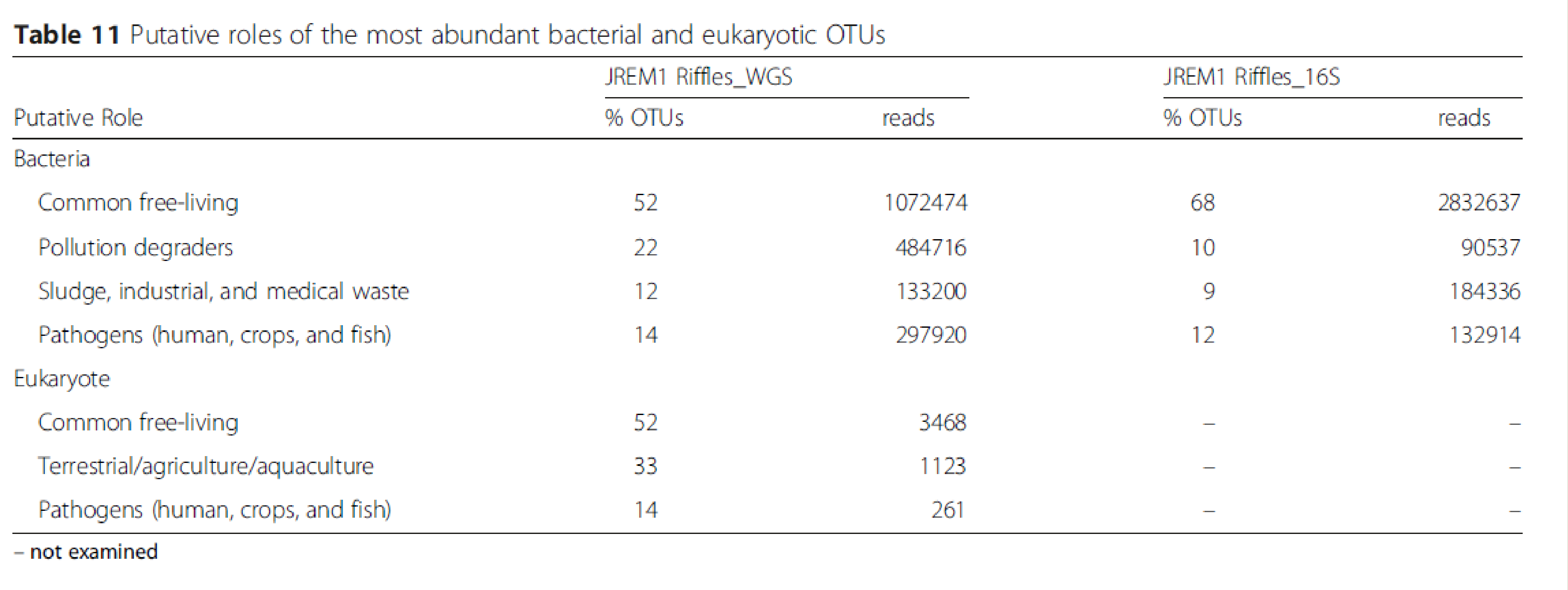


Table 11 Putative roles of the most abundant bacterial and eukaryotic OTUs

	JREM1 Riffles_WGS		JREM1 Riffles_16S	
Putative Role	% OTUs	reads	% OTUs	reads
Bacteria				
Common free-living	52	1072474	68	2832637
Pollution degraders	22	484716	10	90537
Sludge, industrial, and medical waste	12	133200	9	184336
Pathogens (human, crops, and fish)	14	297920	12	132914
Eukaryote				
Common free-living	52	3468	–	–
Terrestrial/agriculture/aquaculture	33	1123	–	–
Pathogens (human, crops, and fish)	14	261	–	–

– not examined

STUDY CONCLUSIONS

- Microbial community closely mirrors the upper Mississippi River
- A river microbial response exists to anthropogenic pollution
- Limited sampling, generalizations cannot be made regarding spatio-temporal distributions
- Further studies should allow health agencies to better identify organism specific health risks and to enhance waterborne disease prevention efforts

DEVELOPMENT, CALIBRATION, AND VALIDATION OF A SIMPLE TOOL FOR GUIDING MOLD INSPECTION AND REMEDIATION IN U.S. HOMES

- Dr. Peccia (Yale University), Dr. Shaughnessy (U. of Tulsa), Jason Dobranic (EMSL)
- Mold assessment in homes using NextGen Sequencing
- Water damaged vs. non-water damaged
- Nationwide HUD funded research

CONCLUSIONS

- Instrumentation and technical knowledge achieved
- Improvements needed on bioinformatics tools (ITS databases), fungal genomics, & overall knowledge of fungi in the environment
- Momentum is there to use metagenomic data for microbial characterization of ambient water
- Metagenomic approach will be common place in the future

CONTACT INFO

Jason Dobranic, Ph.D.
Vice-President of Microbiology & Life Sciences
EMSL Analytical, Inc.
jdobranic@emsl.com
www.emsl.com