

Microbial Community Structure, Diversity, and Heterotrophic Activity Within The Saturated Zone of The 300 Area, Hanford Site

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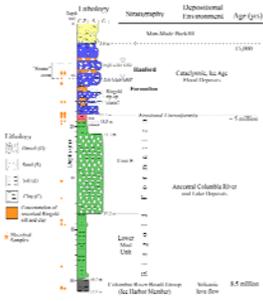
Introduction and Objective

Relatively little is known about the structure and function of subsurface microbial communities at DOE's Hanford Site, particularly in the sensitive Columbia River near-shore environments. Characterization of microbial biomass and activity, potential functionality, and phylogenetic diversity is an essential element to develop conceptual and mechanistic models of how the microbial community might impact the fate and transport of mobile contaminants such as U(VI) and Tc(VII). We hypothesize important roles for sediment physical (e.g., pore size distribution, permeability), and chemical (e.g., organic matter content, electron acceptor type and availability) properties on structuring microbial community composition.

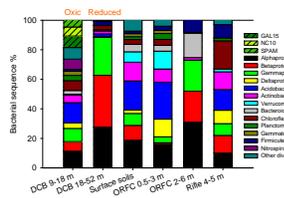
Approach

- Hanford IFRC project provided 12 m of core over 52 m Deep Characterization Borehole (399-2-25, well C6209), drilled down to the basalt flow-top
- JGI conducted Sanger sequencing (~500 seq/sample) of 21 Bacterial and 14 Archaeal 16S rRNA gene clone libraries
- Approximately 8000 Bacterial and Archaeal near-full length 16S rRNA gene sequences analyzed across multiple geological strata
- Bacterial heterotrophic activity assessed by 3H-Leucine incorporation
- Limiting factors assayed via 3H-leucine uptake in samples amended with combinations of ammonia, phosphate, and organic carbon

Hydrogeologic Profile of Deep Characterization Borehole (399-2-25, well C6209)

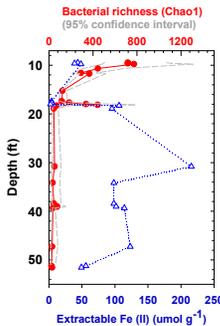


The oxic sediment within the saturated zone of the Hanford Site 300 Area was rich in uncharacterized candidate divisions (e.g. GAL15, NC10, SPAM), compared with surface soils and other uranium-contaminated subsurface soils (Oak Ridge Field Center-ORFC and Rifle field sites).



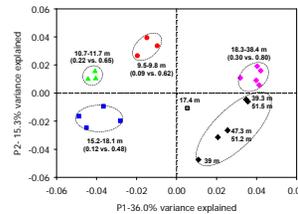
Surface soils: Janssen PH (2006) Appl. Environ. Microbiol. 72:1719-1728
 ORFC 0.5-3m: Hansel et al. (2008) Appl. Environ. Microbiol. 74:1620-1633
 ORFC 2-6m: Akob DM, et al. (2008) Appl. Environ. Microbiol. 74:3159-3170
 Rifle 4-5m: Vosses HA, (2005) Appl. Environ. Microbiol. 71:5308-5318

Bacterial "species" richness and cross-sample community similarity (Operational Taxonomic Units (OTUs) specified at 97% 16S rRNA gene sequence similarity)



Vertical profiles of bacterial richness
 1) Primary peak in the "smear" zone
 2) Secondary peak just above oxic-reducing interface in Ringold Formation (indicated by sharp change in extractable Fe(II)).
 (See Lee et al. poster for Fe reduction)

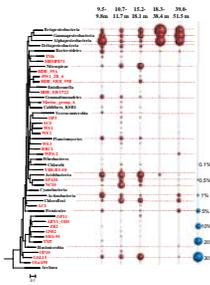
Principal coordinate display of bacterial community similarity (UniFrac analysis) indicating stratified distribution of microbial community composition.



* Numbers in parenthesis: fraction of shared OTUs vs. weighted UniFrac similarity (average of pairwise comparisons within each cluster).

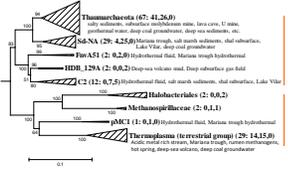
Bacterial and Archaeal community composition

Bacterial phyla revealed at the different strata (1233 unique OTUs were distributed among 42 bacterial divisions.)



Distinct archaeal OTUs resided in different strata

- Archaea were most diverse in the oxic Ringold Formation;
- Dominance of Thaumarchaeota in Hanford formation and oxic Ringold;
- Some OTUs were related to ammonia oxidizing archaea Nitrososphaera sp. and Nitrosopumilus sp. in both the Hanford formation and Ringold Formations;
- Halobacteria and Methanospirillum were found only in the Ringold Formation;
- (See H₂ & CH₄ data in Fredrickson et al. IFRC poster)



* Numbers in parenthesis are total OTUs in the specific group, OTUs in the Hanford formation, OTUs in oxic Ringold, OTUs in reduced Ringold, respectively. Habitat affiliations were indicated at each clade.

Summary and Conclusions

- Microbial community structure and richness varied substantially across the different geological strata.
- "Transition zones" at the top of the saturated zone and near the Ringold oxic-reducing interface had the highest Bacterial diversity.
- Microbial activity was primarily limited by organic carbon in the saturated zone. ³H-Leucine incorporation rate was 3X higher in the oxic Ringold sediment than in the transmissive Hanford formation.
- The bacterial community above the Ringold oxic-reducing interface contained members of 9 major well-recognized phyla as well as unusually high proportions of 3 poorly-characterized candidate divisions (GAL15, NC10, and SPAM). The reduced zone had ca. 90% Proteobacteria, with substantive differences in specific OTUs between samples.
- In addition to the high relative abundance of some candidate divisions, novel orders of Bacteria were detected in comparison to other well-characterized subsurface strata.
- Identifying populations were prevalent in the saturated zone and were dominated by *Ochrobactrum anthropi* and *Actinobacter xylosoxidans*.

Future Research

- Pyrosequencing data analysis to reveal horizontal and vertical heterogeneity of bacterial community composition in the IFRC well field, and relate to geological / geochemical / geophysical properties of the sediments;
- In-situ downwell microcosms to characterize microbial populations colonizing different surrogate solid phases (natural sands vs Bio-Sep beads baited with various electron donors and acceptors); (see preliminary results in Fredrickson et al. poster)
- Use novel staining techniques (e.g. Redox Sensor Green) to identify and sort metabolically active microbes (via flow cytometry) for further isolation and characterization
- A high-frequency groundwater sampling campaign is ongoing to understand temporal dynamics of bacterial community composition and its driving forces (environmental or biotic factors) in the saturated zone
- Quantify the activity and rates of target microbial populations and processes with fresh sediment samples from wells to be drilled in April.

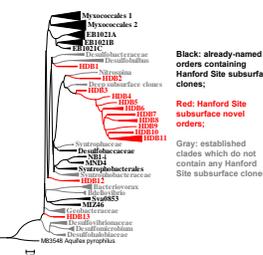
Acknowledgment

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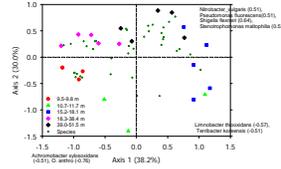
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Parimony tree of δ -proteobacteria showing a large number of novel δ -proteobacteria orders identified in the Hanford Site subsurface, as well as their distance to established "touchstone" clades (see Lee et al. poster for sulfate reducing bacteria)



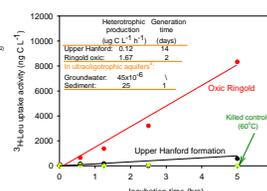
Black: already-named orders containing Hanford Site subsurface clades;
 Red: Hanford Site subsurface novel orders;
 Gray: established clades which do not contain any Hanford Site subsurface clades.

High representation of denitrifiers in ordination space using OTUs affiliated with characterized bacterial species is consistent with our previous quantification of nosZ gene copy number by real time PCR



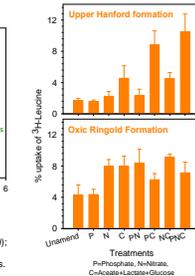
- Only 6% of 1233 unique Bacterial OTUs were closely related to cultivated bacteria;
- There was high representation of denitrifiers (e.g. *Ochrobactrum anthropi* and *Actinobacter xylosoxidans*) in the "smear" zone and upper reduced Ringold sediments. (See Lee et al. poster for denitrifying activity)

Higher microbial ³H-leucine assimilation activity in the oxic Ringold sediment than in the upper Hanford formation



a. Wilhartz et al. 2009, FEMS Microbiol Ecol 68: 287-299
 Assumption: 1.55 kg C per mol leucine (Simon & Azam 1989);
 20 fg C per cell
 Subsurface sediment biomass estimated by DAPI cell counts.

Do nutrient amendments stimulate microbial activity (³H-Leucine incorporation)?



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