ENIGMA: Novel Bio-Signatures and Activity in Fractionated Groundwater from Uncontaminated and Contaminated Sites

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Project Goals: ENIGMA -Ecosystems and Networks Integrated with Genes and Molecular Assemblies use a systems biology approach to understand the interaction between microbial communities and the ecosystems that they inhabit. To link genetic, ecological, and environmental factors to the structure and function of microbial communities, ENIGMA integrates and develops laboratory, field, and computational methods.

Recent work has shown the existence of ultra-small bacteria (100-300 nm) in groundwater but no work has confirmed *in situ* activity, and the ultra-small bacteria could display very different mass transport and activity distributions in porous media flow. Therefore, field observation and characterization are needed to determine the functional role and activity of ultra-small bacteria that could specialize in specific activity and distributions as well as potential metabolic interactions. Moreover, microorganisms that can alter size in response to nutrient levels need to be differentiated from microorganisms that remain small. Total cell numbers, translationally-active cell numbers with bioorthogonal non-canonical amino acid tagging (BONCAT), and microbial activity (^{3}H-Leucine incorporation) were investigated for both low biomass uncontaminated and contaminated groundwater (both 0.2 and 0.1 μm fractions). In addition, metagenomics characterization was performed for the 0.2 and 0.1 μm fractions of both uncontaminated and contaminated groundwater.

In a recent injection experiment of emulsified vegetable oil, the phyla *AC1*, *Gemmatimonadetes*, *OP8*, *WS2*, *WS3*, and *WWE1* displayed notable increases in the 0.1 μm fraction from five shallow wells downgradient of the injection well. These results suggested that both novel and previously observed OTUs could be in the 0.1 μm fraction. However, few cultivars exist for these bacterial phyla.

We then assessed microbial numbers and activities for unstimulated field samples (uncontaminated and contaminated). Total cell numbers (0.2 μm filter) for uncontaminated groundwater (GW271) were 1.3×10^6 +/- 4.4×10^5 cells/ml, whereas, for contaminated groundwater the total cell abundances were 7.3×10^5 +/- 3.4×10^4 and 4.6×10^5 +/- 8.4×10^4 cells/ml for FW115-24 and FW106, respectively. Abundances of smaller cells (0.1 μm filter) were highest for the uncontaminated groundwater 6.40×10^4 +/- 2.1×10^4 cells/ml, while abundances for the contaminated wells were 6.7×10^2 +/- 1.1×10^1 and 6.3×10^2 +/- 6.5×10^1 cells/ml for FW115-24 and FW106, respectively. The results demonstrated that cell numbers for the 0.2 μm fraction were approximately an order of magnitude higher for the uncontaminated

compared to the contaminated groundwater (10^6 v. 10^5). Cell numbers for the small fraction (0.1 μm fraction) were also at least an order of magnitude higher for the GW271 compared to the contaminated groundwater (10^4 v. 10^2).

For uncontaminated groundwater activity, the small cell fraction (0.1 μm) made up almost 20% of total BONCAT activity (per ml), and the small cell fraction had roughly 3-fold greater activity on a per cell basis. When uncontaminated groundwater was compared to the contaminated, there was a drastic reduction in the BONCAT activities and the contaminated groundwater was between 172-769-fold less. Additionally, the rate of leucine incorporation (^{3}H-leucine) on a per cell basis for the 0.2 μm fraction in pristine groundwater was 172 and 8,000 times greater than the contaminated groundwater (FW115 and FW106, respectively). While the overall activity for contaminated wells was low (0.5-2.0 ng C/ml/d), between 25 and 57% of the total activity was from the 0.1 μm fraction. Moreover, for the tested groundwater (uncontaminated and contaminated), the 0.1 μm fraction had higher activity on a per cell basis than the 0.2 μm fraction. Overall, for both size fractions, activity was lower (both per volume and per cell) in contaminated groundwater compared to uncontaminated groundwater. From 21 separate metagenomic assemblies (GW271, FW106, FW115-24), we currently have over 40 genomes with >98% completeness and <2% contamination. We are comparing the presence of these genomes across our samples and previous metagenomics data from the ORR test site. In addition, we are analyzing the potential functional roles of these novel microorganisms. Known species that have been classified as ultramicrobacteria are enriched in the 0.1μm fraction and predictions for potential biochemical capacity are in progress.

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