Culturing Bacteria from Sediment Macroaggregates from the 4850' Level of the Sanford Underground Research Facility, Lead, South Dakota

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Introduction

Macroaggregates are tightly bound clusters of minerals and organic material in soil or sediment and are about 0.1 to 0.5 mm in size (1). Each microaggregate is often home to dozens of species of microbes, which may interact closely in close associations known as consortia (1, 2). Studying soil and sediments on a microscopic level allows us to fully understand the interaction of microbes with their microbial neighbors and substrate particles. The Sanford Underground Research Facility (SURF). SURF is a particularly interesting habitat for microbial growth as it is an incredibly deep (over 1478 m) artificial cave system with no light, little organic material, and a constant temperature (3). In This study, we attempt to culture bacteria from SURF sediment macroaggregates and characterize them by next generation sequencing of 16S rRNA genes.

Materials and Methods

Samples were initially collected from the "pipe drift" adjacent to the Yates shaft on the 4850 ft. level of SURF using a sterile tools. About 30 uL of sediment (Fig. 1) was added to 20 mL of sterile PBS buffer gently stirred with a pipette tip, and diluted 200 times in fresh PBS, Using a dissecting microscope 37 individual macro aggregates were gently micro-pipetted in 2 uL of buffer into separate wells of a 96 microwell plate with 200 ul of primary media (0.1X R2B (4) in 10% SURF sediment extract with fungicides cycloheximide and Nystatin). These macroaggregate primary cultures were left at room temperature for 5 days and were then viewed with an inverted microscope to see initial growth. After a week, primary cultures in each well were stirred and transferred to a new microwell plate with secondary media (1X R2B, 0.1% ATCC vitamin and minerals, and fungicides) and incubated a week as before. We did this in an attempt to rid our samples of existing cells from the sediment that may have been present but were not actually growing.

2 uL of each secondary culture (Fig. 2) were lysed and directly used for direct PCR using the Invitrogen Platinum Direct PCR Mix and primers 27F and 1492R to amplify the 16s rDNA gene. The PCR products were cleaned and the V4-V5 regions of the 16S rRNA genes sequenced using the Nextera kit and Illumina MiSeq DNA sequencer at CCBR at Black Hills State University. Data was analyzed using the Microbial Genomics module by CLC Bio (Qiagen Inc.).

Results

There was a significant difference in the abundance of bacterial species between the DNA extracted and sequenced directly from the SURF sediment (Fig.3) and the macroaggregate (MA) cultures grown in the liquid R2B media (Fig. 4). Although there were fewer species cultured in the R2B media it was found that there may have been some consortia because several of the microwells had multiple species residing in them (Fig. 5). A comparison between the two sunburst charts shows that the sediment and MA samples shows that both shared several species of bacteria including, species of *Lysobacter, Alcanivorax, Polycyclovorans* and I*diomarina*. Interestingly, there were large numbers of bacteria of the phylum Firmicutes from MA cultures. Firmicutes, which may form endospores, were hardly present in the original sediment sample indicating that the R2B media provided a favorable growing environment for these species. From the 484 species found in the sediment 8.5% were culturable from MAs on the R2B media. While this is substantial, we did also find that many of the species present in the sediment were missing from our cultures. Incidentally, several of the genera we cultured have been found previously only from ocean or saline habitats.

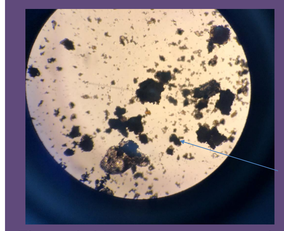

Figure 1. Macroaggregates under 400x magnification. All of the macroaggregates used in our primary cultures before transfer ranged from approximately 15 micrometers across to 500 micrometers across.

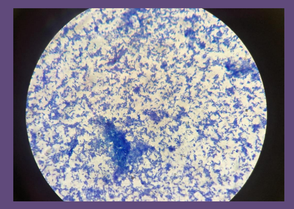

Figure 2. Cells from a secondary macroaggregate culture stained with methylene Blue (1000X).

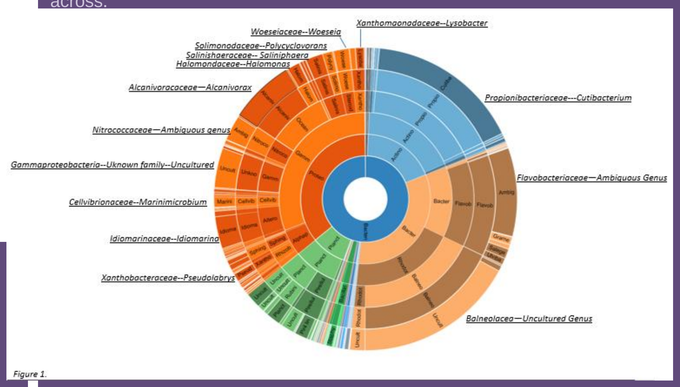

Figure 1.

Figure 3. This sunburst chart shows the relative abundance of bacterial *taxa in the sediment sample from the "Pipe Drift" at the Yates shaft of the 4850 ft.*

-Figure 4. A sunburst chart for the relative abundance of bacterial groups from the combined 37 macroaggregate secondary cultures.

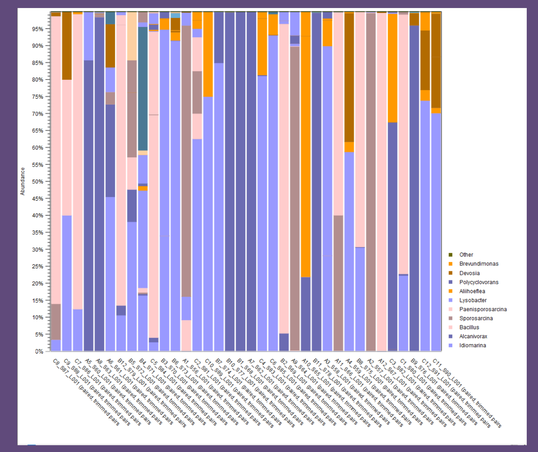

Figure 5. Chart showing the relative abundance of different bacterial species (OTUs) in each of the 37 macroaggregate cultures.

Conclusions

The composition of our individual macroaggregate (MA) cultures varied a lot, reflecting the known variability of MA microbial communities. Because of the initial PCR step we performed on MA cultures prior to DNA sequencing, we may have introduced some biases in the abundance of some bacterial 16S rRNA genes. Nonetheless, we still did see a considerable amount of *Idiomarina* , *Alcanivorax* , *Polycyclovorax* and *Lysobacter* present in both the SURF sediment and secondary MA cultures. Strangely, *Idiomarina* and *Alcanivorax* are only known from marine habitats (5). Some of the MA culture species, like *Sporosarcina, Bacillus,* and *Sphaerosporosarcina*, are spore formers which were scarce in the SURF sediment, indicating that the R2B media used provided favorable growing conditions to bacterial spores.

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References

(3) Ethan Thompson, Molly Erickson, Naveen Malik, Raeann Mettler, Bethany Reman, Yi Ren, David Bergmann 2020 *"Culture Independent Characterization of "Cave Silver" Biofilms from the 1470 M Leve of the Sanford Underground Research Facility, Lead SD".* Proceedings of the South Dakota Academy of Science, Vol. 99

(1) Regina L. Wilpiszeski, Jayde A. Aufrecht,a Scott T. Retterer,a Matthew B. Sullivan, David E. Graham,a Eric M. Pierce, Olivier D. Zablocki,Anthony V. Palumbo, Dwayne A. Elsass 2019 *"Soil Aggregate Microbial Communities: Towards Understanding Microbiome Interactions at Biologically Relevant Scales"* American Society for Microbiology Vol 85

(2) Sonia R. Vartoukian, Richard M. Palmer & William G. Wade 2010 *"Strategies for culture of 'unculturable' bacteria"* Federation of European Microbiology Studies.

(4) Reasoner DJ, EE Geldreich EE (Jan 1985). "A new medium for the enumeration and subculture of bacteria from potable water". *Appl. Environ. Microbiol.* **49** (1): 1–7. PMC 238333 PMID 3883894

(5) Wen Dar Jean, Wung Yang Shieh, Hsiu-Hui Chiu 2006 *"Pseudidiomarina taiwanensis gen. nov., sp. nov.,a marine bacterium isolated from shallow coastal water of An-Ping Harbour, Taiwan, and emended description of the family Idiomarinaceae"* International Journal of Systematic and Evolutionary Microbiology