The Sub-Seabottom Biosphere

Life looks extremely different in the deep ocean. Here on the earth's surface, we have light, heat, oxygen and other resources not present on the ocean floor. Living organisms need different strategies to survive in this deep ocean environment. It is cold and dark with different geochemical compositions. In addition, it is difficult to study this environment because it is hard to physically access the living organisms and conditions. Scientists in the C-DEBI (Center for Dark Energy Biosphere Investigations), however, have been successfully exploring this deep biosphere. Following are three abstracts that address new findings about life under these conditions.

Before reading the three abstracts, watch this introduction video to C-DEBI:
https://www.youtube.com/watch?v=6Hv_JF7_ECQ#t=168

For all three abstracts, read the questions first. Then read the abstract and answer the questions. Be prepared to discuss your answers with the class.

1. Microbial Communities and Geochemistry in the Artic

Read the following abstract and answer the following questions:
1. What specific question does this research address?
2. What were the principal methods used?
   - What does pyrosequencing mean?
3. What is the principal conclusion?

Title: Correlating microbial community profiles with geochemical data in highly stratified sediments from the arctic mid-ocean ridge
Authors: Steffen Leth Jorgensen et. al.
Publication source: Proceedings of the National Academy of Sciences of the United States of America (2012) vol. 109 no. 42

Microbial communities and their associated metabolic activity in marine sediments have a profound impact on global biogeochemical cycles. Their composition and structure are attributed to geochemical and physical factors, but finding direct correlations has remained a challenge. Here we show a significant statistical relationship between variation in geochemical composition and prokaryotic community structure within deep-sea sediments. We obtained comprehensive geochemical data from two gravity cores near the hydrothermal vent field Loki's Castle at the Arctic Mid-Ocean Ridge, in the Norwegian-Greenland Sea. Geochemical properties in the rift valley sediments exhibited strong centimeter-scale stratigraphic variability. Microbial populations were profiled by pyrosequencing from 15 sediment horizons (59,364 16S rRNA gene tags), quantitatively assessed by qPCR, and phylogenetically analyzed. Although the same taxa were generally present in all samples, their relative abundances varied substantially among horizons and fluctuated between Bacteria- and Archaea-dominated communities. By independently summarizing covariance structures of the relative abundance data and geochemical data, using principal components analysis, we found a significant correlation between changes in geochemical composition and changes in community structure. Differences in organic carbon and mineralogy shaped the relative abundance of microbial taxa. We used correlations to build hypotheses about energy metabolisms, particularly of the Deep Sea Archaeal Group, specific Deltaproteobacteria, and sediment lineages of potentially anaerobic Marine Group I Archaea. We demonstrate that total prokaryotic community structure can be directly correlated to geochemistry within these sediments, thus enhancing our understanding of biogeochemical cycling and our ability to predict metabolisms of uncultured microbes in deep-sea sediments.

2. Microbial communities of deep sea cores

Read the following abstract and answer the following questions:
1. What specific question does this research address?
2. What were the principal methods used?
   - What does "cultivation-dependent and cultivation-independent (molecular) analyses" mean
3. What is the principal conclusion?
   - What does 'meters beneath/below the seafloor' imply chronologically?
   - Why are 16s rRNA sequences significant – what do they reveal?

Title: Microbial Communities Associated with Geological Horizons in Coastal Subseafloor Sediments from the Sea of Okhotsk
Authors: Inagaki et. al.
Publication Source: Applied Environmental Microbiology (2003) **69**, 7224-7235

Microbial communities from a subseafloor sediment core from the southwestern Sea of Okhotsk were evaluated by performing both cultivation-dependent and cultivation-independent (molecular) analyses. The core, which extended 58.1 m below the seafloor, was composed of pelagic clays with several volcanic ash layers containing fine pumice grains. Direct cell counting and quantitative PCR analysis of archaeal and bacterial 16S rRNA gene fragments indicated that the bacterial populations in the ash layers were approximately 2 to 10 times larger than those in the clays. Partial sequences of 1,210 rRNA gene clones revealed that there were qualitative differences in the microbial communities from the two different types of layers. Two phylogenetically distinct archaeal assemblages in the *Crenarchaeota*, the miscellaneous crenarchaeotic group and the deep-sea archaeal group, were the most predominant archaeal 16S rRNA gene components in the ash layers and the pelagic clays, respectively. Clones of 16S rRNA gene sequences from members of the gamma subclass of the class *Proteobacteria* dominated the ash layers, whereas sequences from members of the candidate division OP9 and the green nonsulfur bacteria dominated the pelagic clay environments. Molecular (16S rRNA gene sequence) analysis of 181 isolated colonies revealed that there was regional proliferation of viable heterotrophic mesophiles in the volcanic ash layers, along with some gram-positive bacteria and actinobacteria. The porous ash layers, which ranged in age from tens of thousands of years to hundreds of thousands of years, thus appear to be discrete microbial habitats within the coastal subseafloor clay sediment, which are capable of harboring microbial communities that are very distinct from the communities in the more abundant pelagic clays.

3. Using DNA analysis to understand deep biosphere activity

Read the following abstract and answer the following questions:
1. What specific question does this research address?
2. What is the principal method used?
   - What is a metatranscriptome?
   - What is DNA sequencing?
3. What is the principal conclusion?
   - What are the three domains of life?
   - Why is the increase in the transcripts involved in cell division as a function of microbial cell concentration significant?

Title: Gene expression in the deep biosphere
Authors: William D. Orsi, Virginia P. Edgcomb, Glenn D. Christman and Jennifer F. Biddle
Publication source: Nature (2013), **499**, 205–208

Scientific ocean drilling has revealed a deep biosphere of widespread microbial life in sub-seafloor sediment. Microbial metabolism in the marine subsurface probably has an important role in global biogeochemical cycles, but deep biosphere activities are not well understood. Here we describe and analyse the first sub-seafloor metatranscriptomes from anaerobic Peru Margin sediment up to 159 metres below the sea floor, represented by over 1 billion complementary DNA (cDNA) sequence reads. Anaerobic metabolism of amino acids, carbohydrates and lipids seem to be the dominant metabolic processes, and profiles of dissimilatory sulfite reductase (dsr) transcripts are consistent with pore-water sulphate concentration profiles. Moreover, transcripts involved in cell division increase as a function of microbial cell concentration, indicating that increases in sub-seafloor microbial abundance are a function of cell division across all three domains of life. These data support calculations and models of sub-seafloor microbial metabolism and represent the first holistic picture of deep biosphere activities.

TOOLKIT CREDITS:
Developed by Esat Atikkan, (Montgomery College, MD) with support by the rest of the C-DEBI Collaborative Toolkit Team.

WEBSITE:
http://www.coexploration.org/C-DEBI/toolkits_biology.html