DOEI: Investigation of Protists in the Deep Marine Subsurface Biosphere

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The primary questions asked in this project were: 1) Are microbial eukaryotes present in the deep subsurface? and 2) In light of the fact that archaeal communities in the deep subsurface were shown previously to be heterotrophic, if protists are present, will they also represent heterotrophic taxa?

DNA- and RNA-based clone library analyses were used to examine the microbial eukaryotic diversity and to identify the potentially active members in deep-sea sediment cores of the Peru Margin and the Peru Trench. We compared surface communities to those much deeper in the same cores, and compared cores from different sites. Fungal sequences were the dominant sequences recovered from both DNA- and RNA-based clone libraries (see Figure 1). Some fungal sequences represented potentially novel organisms and some represented ones with a cosmopolitan distribution in terrestrial, fresh and salt-water environments. Our results indicate that fungi appear to be the most consistently detected eukaryotes in the marine sedimentary subsurface; and further, that some species may be specifically adapted to the deep subsurface and may play important roles in the utilization and recycling of nutrients. The results of this project contribute valuable methodological groundwork for working with RNA-based methods for analysis of marine deep subsurface core samples. It also provides an initial diversity assessment of microbial eukaryotes and proof that some of these are metabolically active. Much less is known about the presence of microbial eukaryotes than about Archaea or Bacteria in marine sediments deeper than a few centimeters.

Through consumption of dissolved and particulate organic matter, and by grazing in subsurface horizons where bacterial and/or archaeal numbers are high, protists and fungi may significantly impact carbon cycling in the marine subsurface. Through their grazing activities protists may alter bacterial and archaeal community composition, having impacts on microbial net production and nutrient cycling in the extensive marine subsurface biosphere. The most unusual result from this study was not just that there are indeed active eukaryotes (in particular fungi) in the deep marine subsurface, but that some of those presumably active fungi are affiliated with known terrestrial forms. This presents a next avenue of research which will examine the degree to which active subsurface fungi are distinct from terrestrial forms, their degree of activity, and consequent influence on nutrient cycling.

The greatest challenges were to refine molecular tools (RNA extraction protocols, PCR primers) for use with deep subsurface sediments in order to conduct this preliminary assessment of the presence and diversity of eukaryotes in subsurface sediments from 4 deep subsurface samples collected during the Ocean Drilling Project Leg 201 cruise. The four selected cores represent a range of sediment organic content and water depth. By targeting RNA and not just DNA, our data show the portion of the community that are ***active*** eukaryotes and not simply detrital organisms that are inactive. Working with RNA in humic- and hydrocarbon-rich sediments is notoriously problematic.

This research was conducted at Woods Hole Oceanographic Institution. No new field research was required, as sediment core samples were made available to us from Andreas Teske at University of North Carolina, Chapel Hill, originating from his participation in the Ocean Drilling Project leg 201 cruise. We used RNA-based molecular methods starting with the extraction of total RNA, production of cDNA and amplification of target genes from this cDNA. This research is becoming part of a larger program in my lab focusing on understanding the role

of deep marine subsurface fungi. As noted above, the discovery of active fungi, some of which affiliate with known terrestrial forms presents a next avenue of research which will examine the degree to which active subsurface fungi are distinct from terrestrial forms and their degree of activity and consequent influence on nutrient cycling. A proposal to NSF Biological Oceanography will be submitted this coming February to examine these questions. Publications resulting from this funding are noted below.

Edgcomb, V.P., Beaudoin, D., Gast, R., Biddle, J., and Teske, A. 2010, Marine subsurface eukaryotes: the fungal majority. Environ. Microbiol. doi:10.1111/j.1462-2920.2010.02318.x.

Edgcomb, V.P., and Biddle, J. Submitted. Microbial eukaryotes in the marine subsurface? In: J. Seckbach (ed.) *Anoxia: Paleontological Strategies and Evidence for Eukaryote Survival* Springer-Verag.

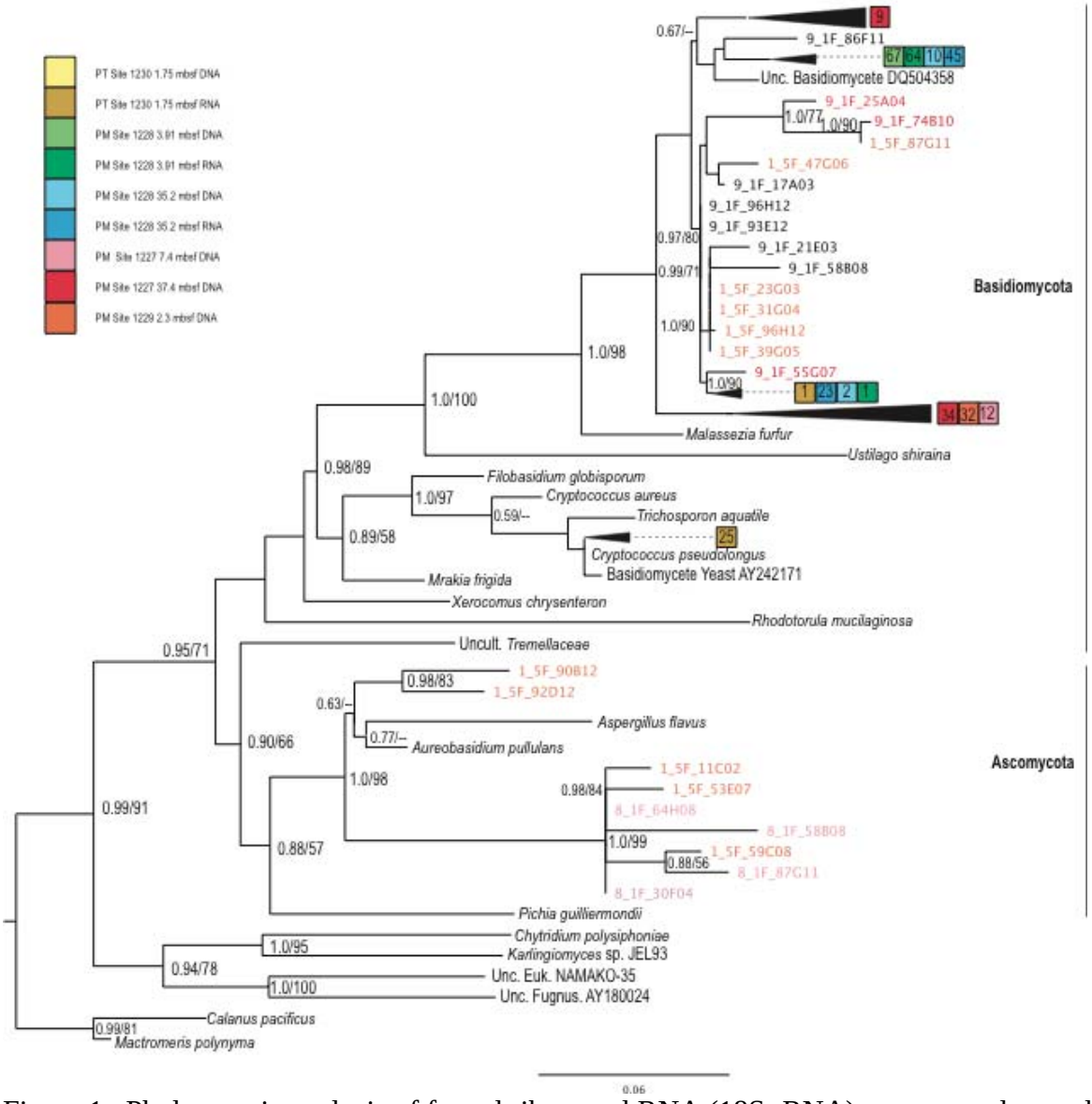

Figure 1. Phylogenetic analysis of fungal ribosomal RNA (18S rRNA) sequences detected in five marine subsurface cores: Peru Trench Site 1230 core 1H2 (1.75 mbsf) in yellow tones, Peru Margin Site 1228 core 1H3 (3.91 mbsf) in green tones, and Peru Margin site 1228 core 5H2 (35.1 mbsf) in blue tones. For these first three cores the darker color shade for each of the three color groups corresponds to RNA-based clone library and lighter shade the DNA-based clone library for the same core sample. In addition we present sequences from DNA-based clone libraries from Peru Margin Site 1227 cores 2H2 (7.4 mbsf) and 5H3 (37.4 mbsf), and Peru Margin Site 1229 core 1H2 (2.3 mbsf). Numbers in boxes represent numbers of clones in that cluster retrieved from a given library. Tree is based on an alignment of 1208 aligned nucleotide positions. Bootstrapping and determination of the best estimate of the ML tree topology for these datasets were conducted with the Rapid Bootstrapping algorithm of RAxML version 7.0 under the GTR+I model running on the CIPRES portal (Stamatakis, 2006)(www.phylo.org). Bayesian posterior probabilities greater than 0.50 (calculated using MrBayes, also running on the CIPRES portal) and bootstrap (RAxML) values greater than 50% are shown at nodes in the order PP/ML.