Protist Stimulation of Biodegradation of Hydrocarbons by *in situ* Deep Sea Bacterial Communities

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Introduction. Recent events in the Gulf of Mexico have reignited interest in understanding the long-term consequences of contamination on the marine food web. Unfortunately, not enough is known about the long-term process of biodegradation of hydrocarbons, particularly in deep-sea sediments. Without this knowledge it is impossible to model the long-term consequences of a major spill on the marine environment. Protists are unicellular eukaryotes that are an essential component of microbial food webs, and play key roles in global biogeochemical cycles. Despite their ecological importance in aquatic and terrestrial ecosystems, relatively little is known about their role in hydrocarbon degradation compared with that reported for bacteria. A more detailed knowledge of the role of protists in microbial interactions with hydrocarbons is essential to trace and model the fate of hydrocarbon contaminants in terrestrial and aquatic ecosystems. It is also now believed that hydrocarbon degradation is a multi-step procedure where each step is performed via distinct processes carried out by different functional groups of microbial organisms (Dalby et al. 2008). Selective grazing by protists can alter the structure of bacterial communities and effectively eliminate some types of bacteria while enabling others to flourish. It is well known that hydrocarbon degradation is typically limited by the bioavailability of nutrients such as nitrogen and phosphorous (Head et al. 2006). If grazing impacts are greatest upon populations of prokaryotes most involved in hydrocarbon degradation then their presence could suppress hydrocarbon degradation. On the other hand, in environments where growth is nutrient limited, protists may stimulate biodegradation by prokaryotes via re-mineralization of these growth-liminting nutrients (e.g., Sherr et al. 2002). Furthermore, there is some evidence for the direct hydrocarbon-degrading abilities of protists. *The purpose of this investigation was to determine whether the presence of protists in naturally hydrocarbon-rich sediments of Gulf of Mexico enhanced or suppressed hydrocarbon degradation.*

Experimental Design. Surface sediment samples were obtained from Andreas Teske from the Rudyville site at the Mississippi Canyon Federal Lease Block 118 long-term observatory in the Gulf of Mexico. This is an active gas-hydrate and oil seep located on the continental shelf. Microcosm experiments were conducted whereby whole sediment slurries were incubated under anoxic and oxic conditions with and without the addition of a mixture of protist inhibitors (cyclohexamide and colchicine), and with and without the addition of nutrients (NH_4 Cl and K_3 PO_4 to final concentrations of 5 and 0.9 mmoll^{-1} respectively). A spike of 100% fossil diesel fuel oil (WP 681 USEPA Standard Oil) was added to each microcosm, and all microcosm treatments were conducted in triplicate. The diesel spike was added to make it easier to detect biodegradation of hydrocarbons since this oil contained known quantities of labile hydrocarbons.

Aerobic microcosms were run for 30 days, and anaerobic microcosms were run for 90 days. Profiles of hydrocarbons were measured using comprehensive two-dimensional gas chromatography (GCxGC-FID) at T_0 and T_F after adding methyl tributyl ether to stop all activity. Profiles of hydrocarbons were compared to profiles in autoclaved control sediment slurries run in parallel. Clone libraries for bacteria and eukaryotes (small subunit ribosomal RNA genes) were generated for selected replicates to observe changes in community profiles over the duration of the experiment.

Findings. Hydrocarbon degradation was assessed by measuring changes in pristane /*n*-C_{17} and phytane/*n*-C_{18} ratios within microcosms (Figure 1). Changes were not detectable under anaerobic conditions over a 90-day period, consistent with previous data suggesting that the fastest degradation of hydrocarbons occurs under aerobic conditions. Oxic treatments receiving nutrient addition generally

did not exhibit higher rates of degradation. This was surprising, however this may be a function of the sediments used for this experiment. More nutrient-poor sediments may have produced a different result. Aerobic microcosms receiving inhibitors of protists generally displayed slightly elevated levels of degradation in relation to treatments where protists were not inhibited, suggesting that for these sediments, protist grazing negatively impacted hydrocarbon degradation. At the start of the experiment, bacterial populations included known hydrocarbon degraders, including members of the Oceanospirillaceae and Piscirickettsiaceae. Among gamma-proteobacteria (~33% of bacterial clone libraries at the start), members of Alteromonadales, a metabolically diverse group previously detected in oil-fouled marine environments (Redmond and Valentine, 2011) were the most abundant. Nutrient supplementation of aerobic treatments without addition of protist inhibitors resulted in higher total numbers of gamma-proteobacterial sequences after 30 days, but less overall diversity relative to T_0.

When nutrients and protist inhibitors were added, an increase in gamma-proteobacterial signatures was also observed, and taxonomic composition of bacterial groups shifted in relation to both T_0 and treatments without inhibitors (Figure 2). The greatest degree of hydrocarbon degradation, as determined by increased pristane /*n*-C_{17} and phytane/*n*-C_{18} ratios, was observed in oxic treatments that received protist inhibitors. This suggests that presence of protist grazers in these Gulf of Mexico sediments influences bacterial community composition and/or activity either by relieving grazing pressure on hydrocarbon degraders, or by allowing hydrocarbon degraders who otherwise maintain low activity rates as a strategy to escape predation, to increase activity. Alternatively, addition of protist inhibitors may have released limiting nutrients from dead protists, which may have stimulated activity. Longer-term incubations that allow any newly released nutrients from dead protists to be consumed may help tease apart these alternative explanations. Following addition of the diesel oil spike, taxonomic composition of protists in oxic microcosms shifted as well, with a reduction in ciliates and dinoflagellates, and an increased representation of signatures affiliating with Bacillariophyta (Coscinodiscophyceae).

The greatest challenge in this study was detection of biodegradation of the hydrocarbons. We underestimated the starting concentration of *in situ* hydrocarbons, and so less costly mass spectrometry approaches were not possible due to inadequate sensitivity. This resulted in the fact that we were not able to gather mass spectrometry data for all replicates in both anoxic and control groups within the scope of this project. We also may have obtained a different result had we used marine sediments from a site not previously exposed to hydrocarbons *in situ*. A microbial community that is not pre-adapted to hydrocarbon exposure may respond differently. This study suggests protists may suppress hydrocarbon degradation by bacterial communities, and demonstrates proof of concept of an experimental design that is appropriate for examining the role/impact of protists on these processes. The effects of protist grazers on hydrocarbon degrading bacterial communities should be examined using a wider range of sediment types with and without previous hydrocarbon exposure before generalizing results to all marine sediments. Pairing microcosm experiments with specific examinations of expressed genes associated with hydrocarbon degradation are a logical next step. A paper presenting our results is in preparation for submission to *Deep Sea Research Part II.*

References

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Figure 1. GCxGC chromatogram showing the loss of *n*-C_{17} and *n*-C_{18} peaks due to microbial activity at T_0 (top) versus T_F after 30 days (bottom) in an oxic microcosm with added nutrients and protist inhibitors.

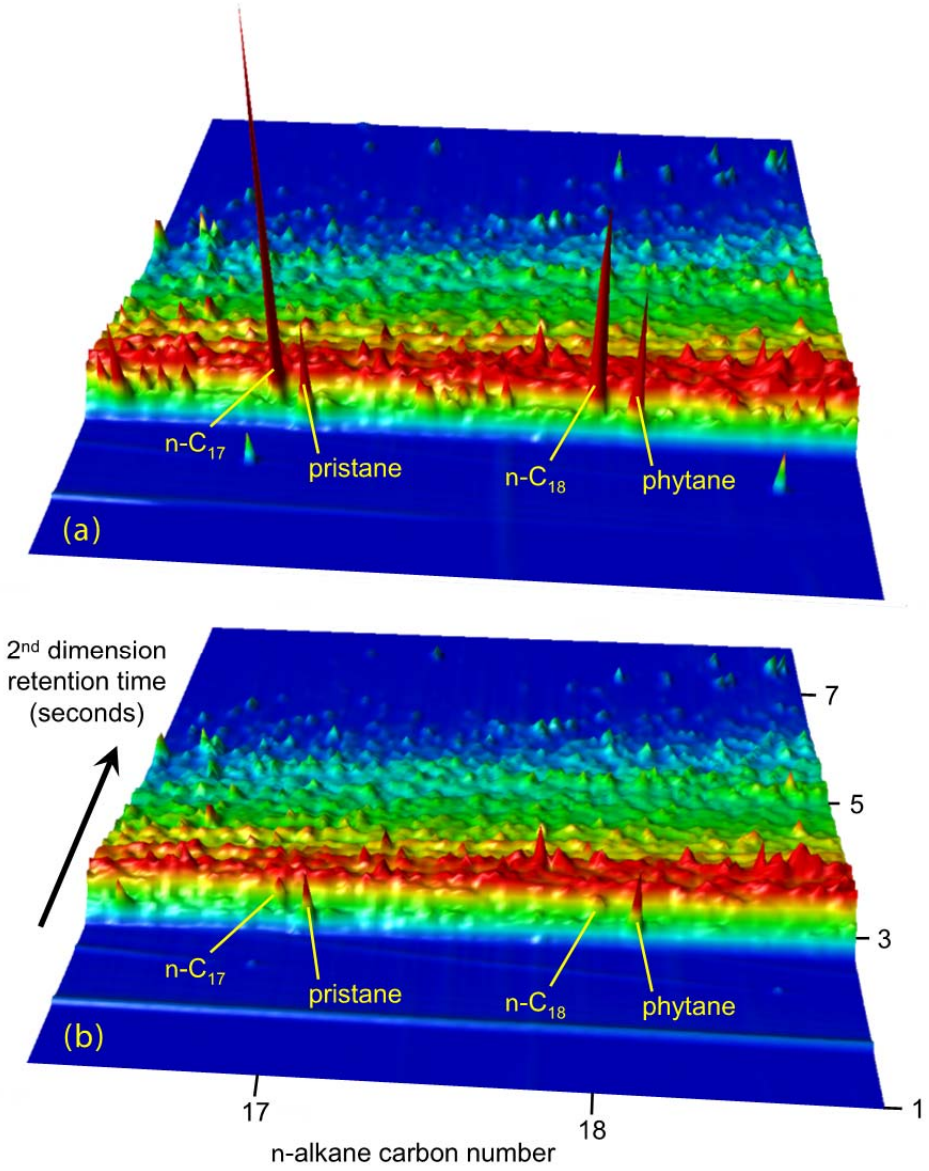

Figure 2. Taxonomic diversity of (a) Bacteria and (b) proteobacteria recovered from selected microcosm clone libraries (operational taxonomic units clustered at 97% sequence similarity).