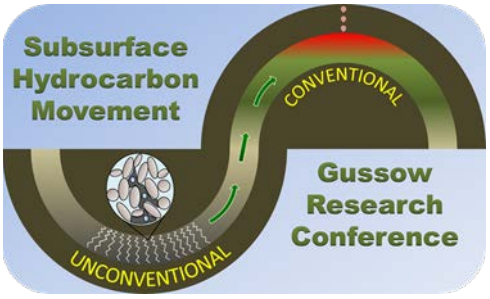

Distributions of thermophilic, endospore-forming bacteria in hydrocarbon seep prospective sediments in the Eastern Gulf of Mexico

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Introduction

Dormant endospores of thermophilic bacteria (thermospores) can be routinely detected in permanently cold marine sediments. These hardy and low-abundance endospores, belonging to the phylum *Firmicutes*, remain undetected in typical nucleic-acid-based community surveys, but proliferate when sediments are experimentally heated. Thermospores are close phylogenetic relatives of microorganisms indigenous to subseafloor petroleum reservoirs; if they originate from these deep habitats, hydrocarbon seepage from reservoir to seabed could explain their occurrence in the cold ocean. As such, thermospore distributions and physiological features around seabed hydrocarbon seeps might have utility in locating and characterising working petroleum systems.

Theory and/or Method

Marine sediments from 111 locations in the Eastern Gulf of Mexico (100 to 3300 m water depth; 6 to 600 km apart) were sampled during an industry-sponsored piston coring survey. Geochemical analyses of three segments per core allowed oil-positive and oil-negative classifications for each location. To test for thermospores, sediments were thawed and amended with 20 mM sulfate and a mixture of organic substrates, pasteurized at 80°C, and incubated at 50-55°C for 14 days.

Examples

Thermophilic sulfate reduction occurred to a greater extent in oil-positive sediments (n=59/71) relative to the oil-negative (n=25/40) sediments. 16S rRNA gene amplicon libraries (V3-V4 region; Illumina MiSeq) before and after 14 days of high temperature incubation revealed enrichment of species-level thermospore OTUs in all 110 locations except one. Using abundance cut-offs, 115 significantly enriched thermospore OTUs were chosen to investigate thermospore biogeography in the study area. Most of these OTUs were considered to be endemic, with only 16 OTUs present in >20 locations. Among these, the most ‘cosmopolitan’ OTUs, detected in up to >70% of the 111 sediment locations, included those affiliated with thermophilic sulphate-reducing *Desulfotomaculum* spp. detected in similar sediment surveys elsewhere in the world’s oceans. On the other hand, 26 OTUs appeared only in a single location, with 16 of these instances being oil-positive sediments. The site with the strongest geochemical signals for hydrocarbon seepage harbored 4 of these strictly endemic OTUs, including

Conclusions

The ability of thermospores to survive in cold sediments where they can be routinely detected in high temperature activity assays despite low in situ abundance points towards potential utility for these organisms as biosensors for hydrocarbon seep prospection in offshore oil and gas exploration. The biogeography of thermospores may therefore complement nucleic acid surveys of in situ sediment microbial communities better adapted to these cold environments, as well as seabed geochemistry, for mapping interesting areas of the sea floor.