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SCIENCE CAREER

Identifying polycyclic aromatic hydrocarbon-degrading bacteria in oil-contaminated surface waters at *Deepwater Horizon* by cultivation, stable isotope probing and pyrosequencing

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Abstract Polycyclic aromatic hydrocarbons (PAHs) are an important class of chemical pollutants that constitute a major component of total hydrocarbons in crude oils. Based on their poor water solubility, toxicity, persistence and potential to bioaccumulate, these compounds are recognized as high-priority pollutants in the environment and are of significant concern for human health. At oil-contaminated sites, PAH-degrading bacteria perform a critical role in the degradation and ultimate removal of these compounds. In April 2010, enormous quantities of PAHs entered the Gulf of Mexico from the thousands of tons of oil that were released from the ill-fated drilling rig *Deepwater Horizon*. In the ensuing months after the spill, intense research efforts were devoted to characterizing the microorganisms responsible for degrading the oil, particularly in deep waters where a large oil plume, enriched with aliphatic and low molecular-weight aromatic hydrocarbons, was found in the range of 1,000–1,300 m. PAHs, however, were found mainly confined to surface waters. This paper discusses efforts utilizing DNA-based stable isotope probing, cultivation-based techniques and metagenomics to characterize the bacterial guild associated with PAH degradation in oil-contaminated surface waters at *Deepwater Horizon*.

Keywords Polycyclic aromatic hydrocarbons (PAHs) · *Deepwater Horizon* · DNA-based stable isotope probing (DNA-SIP) · Bioremediation · Crude oil

1 Introduction

The recent *Deepwater Horizon* blowout of April 2010 is heralded as one of the worst maritime oil spills on record. An estimated 4.4 million barrels (0.7 million tons) of oil entered the Gulf over a period of 84 days (Crone and Tolstoy 2010). A comparable catastrophe occurred in 1979 when the Ixtoc I platform in the Bay of Campeche—also in the Gulf of Mexico—suffered a major blowout, releasing an estimated 0.47 million tons of oil over 9 months (Jernelöv and Lindén 1981). Oil spills at sea are not infrequent events, with one occurring almost every year, often from oil tankers, since detailed records began in the 1970s (Oil Tanker Spill Statistics 2009). Crude oils are composed of several thousand chemicals, of which polycyclic aromatic hydrocarbons (PAHs) generally comprise 25–35% of total hydrocarbons (Head et al. 2006). Based on their toxicity, persistence, and ability to accumulate in animals and plants, PAHs are

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recognized as priority pollutants to the environment (Agency for Toxic Substances and Disease Registry 2007). Studies investigating the distribution and concentration of PAHs in the wake of the Gulf spill demonstrated a spatial and temporal distribution of dissolved and dispersed aromatic hydrocarbons in the water column. Low molecular-weight PAHs predominated at discrete depths in deep waters (1,000–1,400 m), whereas higher molecular-weight PAHs were predominant in surface waters (Diercks et al. 2010; Hazen et al. 2010; Camilli et al. 2010). Although various factors will influence the fate of these hydrocarbon chemicals in the Gulf, their ultimate removal will depend on the presence and activities of hydrocarbon-degrading bacteria (Head et al. 2006; Yakimov et al. 2007).

Our understanding of the microbial systems that play key roles in the bioremediation of priority substances and other toxic pollutants in the marine environment has progressed, but remains far from complete. Since these microbial processes are the foundation of natural and anthropogenic oil-spill remediation, a thorough and integrated microbiological investigation in parallel with field and laboratory experiments is necessary. Ultimately, a critical understanding of these processes is at the heart of designing successful bioremediation methods that may be applied either immediately or after the onset of an oil spill. The *Deepwater Horizon* spill represented a unique opportunity to investigate these microbial processes in the degradation of the oil. In the ensuing weeks after the spill, metagenomic studies performed on samples taken from the deepwater plume (1,000–1,300 m) had identified certain bacterial species that were enriched as a result of the oil spill—the most dominant (>90% of all sequences in metagenomic libraries) was represented by a single operational taxonomic unit (3% distance cutoff) that comprised sequences most closely related to *Oceanospirillales* (Hazen et al. 2010). The role of these organisms in the degradation of the oil is unknown, although their relative abundance in the plume suggests it was significant.

Metagenomic approaches have revolutionized our ability to explore the microbial world, revealing at higher resolution the structure of complex microbial communities which conventional cloning and sequencing methods have not been able to achieve. However, even such techniques preclude our ability to accurately infer on an organism's metabolic capacities. A powerful technique that circumvents the necessity to isolate an organism and link its identity to function is stable isotope probing (Dumont and Murrell 2005). The aim of the current MARPAH project was to apply DNA-based stable isotope probing (DNA-SIP) to identify new oil-degrading bacteria in the ocean, including those that are not amenable to cultivation. In the second year of this project, the *Deepwater Horizon* spill occurred and provided a unique opportunity to apply DNA-SIP, together with cultivation-based techniques and metagenomics to gain a more complete understanding of the bacterial communities that contributed to the degradation of PAHs in oil-contaminated waters at the *Deepwater Horizon* site.

2 Working principle

The sequencing of taxonomic marker genes (*e.g.* 16S rRNA) in environmental samples provides useful information on the identity and abundance of the microorganisms that constitute that environment. However, one cannot draw conclusions to their function(s) purely from sequence libraries. Considering that the majority of microorganisms in the biosphere are not amenable to cultivation in the laboratory (Head et al. 1998), one of the greatest challenges in microbial ecology has been to link function with phylogeny. In recent decades we have experienced an emergence of new and exciting experimental tools that have helped unravel complex microbial systems and widened our lens for capturing information about *'who's doing what'*. One method that has successfully been applied to identify a target group of microorganisms in the environment based on a specific function that they perform is DNA-stable isotope probing (DNA-SIP) (Dumont and Murrell 2005). This utilizes a substrate that is labeled with a stable isotopic element, often ^{13}C or ^{15}N, and incubated with the sample to be interrogated (*e.g.* oil-contaminated seawater). During the incubation, microorganisms that are able to utilize the labeled substrate as a food source will incorporate the heavy isotope into the formation of cellular components within the cells (*e.g.* membrane lipids, protein, RNA, DNA, etc.). After allowing the sample to incubate with the labeled substrate, the DNA is extracted and processed, and the taxonomic identity of the organism(s) that metabolized the labeled substrate can be determined. SIP experiments cannot infer on the relative abundance of specific taxa that are

identified to have consumed the labeled substrate. However, when this information is coupled with clone library data that is derived from the original sample used in the SIP experiment, the identity and function of specific taxa (identified from SIP) can now be linked to their abundance against the whole microbial community.

The Department of Environmental Sciences and Engineering at the University of North Carolina at Chapel Hill (UNC-CH) has the unique in-house capability to synthesize uniformly ^{13}C-labeled PAHs, which are not commercially available. Since these chemicals are uniformly-labeled, during a SIP experiment their use increases the labeling of the DNA in cells of organisms that are capable of mineralizing and incorporating the carbon from these labeled substrates. For subsequent separation and downstream molecular analysis, it is expected that this greatly improves the chances of identifying all the target microbes that can degrade and incorporate the labeled substrate. Other than naphthalene and phenanthrene, the group at UNC-CH is the only one in the world that has exclusively used ^{13}C-labeled PAHs in DNA-based SIP experiments (Singleton et al. 2007). UNC-CH also has an on-campus High-Throughput Sequencing Facility that utilizes the 454 Life Sciences Titanium platform of Roche Diagnostics (Branford, CT, USA) which can be used for barcoded 16S rRNA gene pyrosequencing. The *Deepwater Horizon* oil spill of April 2010 was a unique opportunity to apply these techniques and investigate the microbial response to the oil. Figure 1 illustrates a combinatorial SIP-cultivation-metagenomics approach that was used to investigate PAH-degrading bacteria in oil-contaminated surface waters from the Gulf of Mexico that were taken in the immediate aftermath of the *Deepwater Horizon* blowout.

3 Applications

The impact of the *Deepwater Horizon* spill in the Gulf of Mexico is testament to how the health of the marine ecosystem is inextricably linked to the livelihood of its coastal inhabitants. Tourism, fishing and other economies in the Gulf were seriously affected and have only recently started to bounce back. Oil clean-up operations at sea have often used large amounts of dispersants to help break up and disperse the oil. At the vicinity of the *Deepwater Horizon* spill site, several tons of the dispersant Corexit 9500 were released. Little is known of what effect this had and will have on the degradation of the oil and impact to the environment, despite initial reports suggesting that it may be potentially toxic (Judson et al. 2010). Of the estimated 0.7 million tons of oil that entered the Gulf waters from the *Deepwater Horizon* spill, ca. 10% was either skimmed from surface waters or burned (Lubchenco et al. 2010). The remainder of the oil will ultimately be degraded and mineralized by indigenous communities of oil-degrading bacteria in the water column and sediment—a process that can take many years and even decades. Coastal waters all over the world will differ in many respects, including the types of indigenous microbial communities present in the water column and sediment, and by how they would respond to an oil spill. Since these organisms are at the foundation of oil-spill remediation, a thorough and integrated microbiological investigation in parallel with field and laboratory experiments is necessary to guide the design of effective response operations. Such investigations would be deemed particularly necessary at coastal environments considered to be in a high-risk zone for potentially becoming impacted by oil. The Gulf of Mexico and parts of the North Sea would be deemed to fit this category, based on the prevalence there of offshore oil-drilling operations. A critical understanding of these microbiological processes is at the heart of designing successful site-specific bioremedial strategies.

4 Training of young researchers

The Marie Curie Outgoing International Fellowship (OIF) program provides a European researcher with an excellent opportunity to pursue a research project initially for a 2-year period at an outgoing host institution (in my case, the University of North Carolina at Chapel Hill, USA), and then for a final year at the Fellow's home institute (in my case, at the University of Lancaster, UK). During the initial phase at the outgoing host institution, the fellow gains valuable experience and learns new skills that they may otherwise not have access to in their home country. As part of the OIF program, these experiences and skills would then be brought back by the Fellow and implemented into their return home institution. My Marie Curie OIF allowed me the opportunity to conduct my project with a research group in the USA

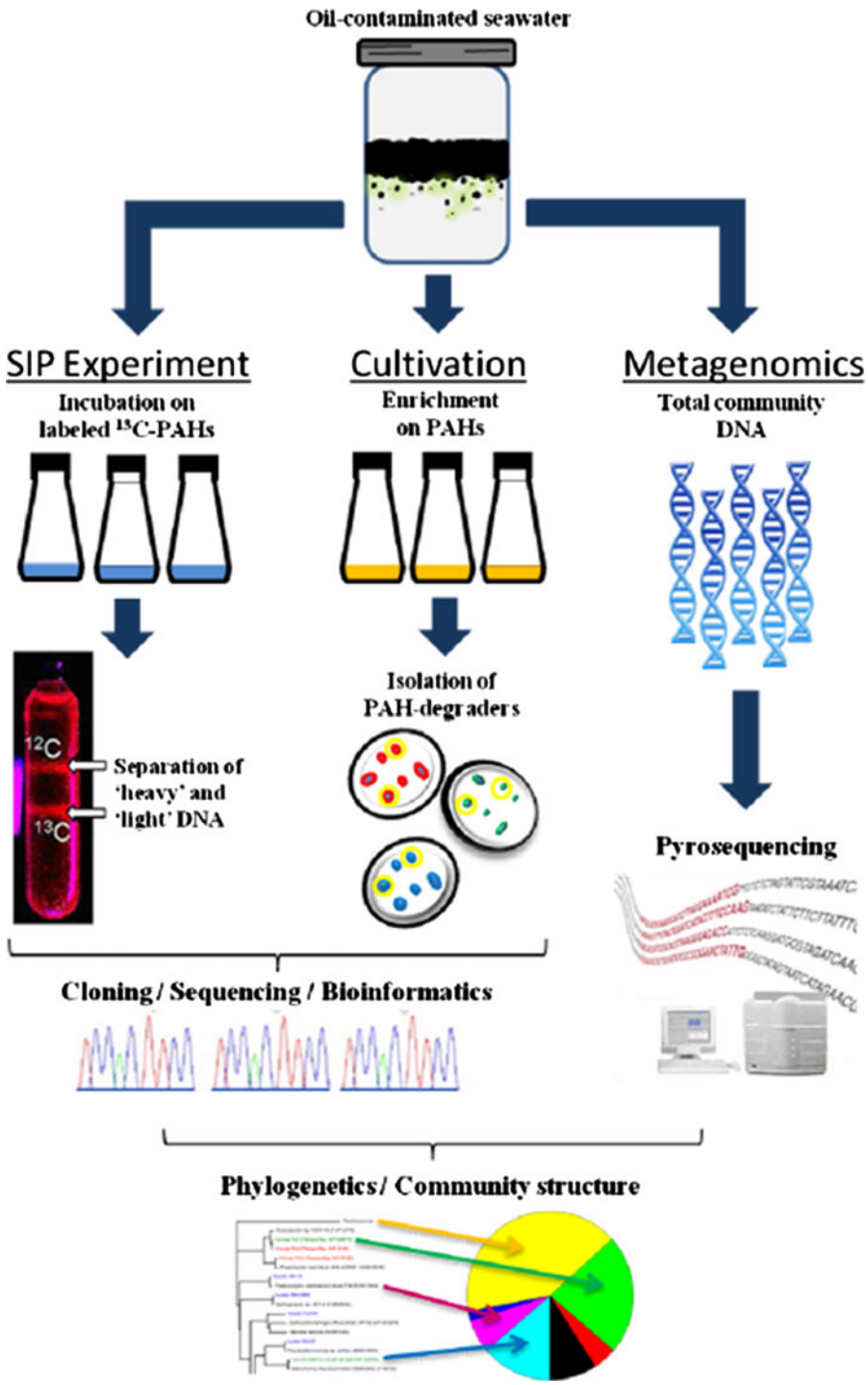

Fig. 1 Schematic representation showing the combined application of SIP, microbiological cultivation, and metagenomics analysis to identify the microorganisms responsible for contributing to the degradation of PAH compounds during the *Deepwater Horizon* oil spill

who are at the forefront in applying DNA-SIP to investigate PAH-degrading bacteria in the environment. This offered me an excellent opportunity to acquire skills and training in conducting SIP experiments, and in its application to investigate PAH-degrading bacteria in the ocean. One unique opportunity that emerged during the second year of my Fellowship in the USA was to investigate the microbial

response to the *Deepwater Horizon* oil spill. For this, I applied DNA-SIP, cultivation-based microbiological techniques and pyrosequencing to determine the microbial communities contributing to the degradation of PAHs in oil-contaminated surface waters collected near the spill site (unpublished results). Overall, this Marie Curie OIF allowed me to learn new skills which I applied to my Fellowship project on the occurrence of PAH-degrading bacteria in the ocean, as well as to investigate the enrichment of these organisms at the *Deepwater Horizon* oil spill.

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