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Initial community and environment determine the response of bacterial communities to dispersant and oil contamination

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ABSTRACT

Bioremediation of seawater by natural bacterial communities is one potential response to coastal oil spills, but the success of the approach may vary, depending on geographical location, oil composition and the timing of spill. The short term response of coastal bacteria to dispersant, oil and dispersed oil was characterized using 16S rRNA gene tags in two mesocosm experiments conducted two months apart. Despite differences in the amount of oil-derived alkanes across the treatments and experiments, increases in the contributions of hydrocarbon degrading taxa and decreases in common estuarine bacteria were observed in response to dispersant and/or oil. Between the two experiments, the direction and rates of changes in particulate alkane concentrations differed, as did the magnitude of the bacterial response to oil and/or dispersant. Together, our data underscore large variability in bacterial responses to hydrocarbon pollutants, implying that bioremediation success varies with starting biological and environmental conditions.

1. Introduction

Following the release of oil from the Deepwater Horizon in 2010, strong responses in the microbial community were detected in the deep-water bacterial community in the northern Gulf of Mexico. The community diversity changed rapidly following the input of oil from the leaking well and the addition of dispersants (Hazen et al., 2010; Valentine et al., 2010; Kessler et al., 2011; Redmond and Valentine, 2011). The rapid response was likely due to a long history of natural seeps in the region (MacDonald et al., 2002), which likely led to the development of a bacterial community with high hydrocarbon-oxidizing potential (Haritash and Kaushik, 2009). The deep-water plume was dominated by a few types of Bacteria, with some samples dominated by a single operational taxonomic unit (OTU) representing up to 90% of the total sequences. Outside the plume, this sequence contributed only 5% of the total sequences (Hazen et al., 2010; Mason et al., 2012). This OTU was identified as a member of the Oceanospirillales, and sequencing of single cells indicated the potential for these organisms to degrade *n*-alkanes and cycloalkanes. Several inorganic nutrient transporters were also identified, suggesting a requirement for ammonium and phosphate (Mason et al., 2012). Other members of the deep-water plume included *Cycloclasticus* spp., which are known to degrade aromatic hydrocarbons (Head et al., 2006; Teira et al., 2007), and *Colwellia* spp., which were likely metabolizing ethane and propane (Redmond and Valentine, 2011). Both *Cycloclasticus* spp. and *Colwellia* spp. appeared to increase as the distance from the wellhead increased, following the bloom of the Oceanospirillales. In the older plume, furthest from the wellhead, an increase in methanotrophs was detected, suggesting a slower response of this community to the inputs of methane gas (Kessler et al., 2011).

In surface waters near the well site, a rapid increase in bacterial respiration was observed, but no increase in biomass or cell number occurred (Edwards et al., 2011). The addition of inorganic nutrients to incubations stimulated cell division, suggesting the microbial community in the surface waters was nutrient limited. Observations of phosphate stress supported this conclusion, and several previous studies have indicated the importance of inorganic nutrients in the ability of some Bacteria to respond to oil spills (Jimenez et al., 2007; Jean et al., 2008). The bacterial community in surface waters contaminated with oil and dispersants was determined to be more diverse than the community at depth. Where oil was present as a thin sheen, Gammaproteobacteria, such as

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Alteromonadales and Oceanospirillales, composed about 15% of the community (Redmond and Valentine, 2011). Heavy oiling increased the contribution of Gammaproteobacteria to almost 100%, but *Colwellia* spp. and *Cycloclasticus* spp. represented <5% of the surface community and the Oceanospirillales sequences detected in surface waters were not from the same species that dominated the deep plume.

These surface studies were carried out in oligotrophic waters where the oil reaching the surface had undergone weathering and modification. In coastal waters of the northern Gulf of Mexico, there are a large numbers of oil rigs and heavy ship traffic with the potential for a spill to impact highly productive surface waters with less weathered oil (Carassou et al., 2011). Mesocosm experiments across the globe have determined that the bacterial community response depends on the location of oil exposure (Gertler et al., 2012), the composition of the oil (Jurelevicius et al., 2013; Viggor et al., 2013) and the type of ecosystem exposed (Jurelevicius et al., 2013). While location may be important, it is not clear if the bacterial community will respond similarly in the same location over time. Furthermore, none of these studies compared the effects of oil, dispersant and dispersed oil on the bacterial community structure.

In the present study, two mesocosm experiments carried out in the summer of 2011 measured the effects of oil and dispersants on the coastal pelagic microbial community. During the experiments, the addition of dispersant (alone or as dispersed oil) led to a significant decrease in the abundance of primary producers and microzooplankton, with an increase in the abundance of prokaryotes (Ortmann et al., 2012). From these same mesocosms, samples were collected to determine the impact of dispersant and/or oil on the structure of the bacterial community. Using 16S rRNA gene tags, we tested the hypothesis that the bacterial community structure changed in response to the addition of dispersant, oil or dispersed oil compared to a control treatment. We also predicted that the community response would be similar across two experiments carried out two months apart under similar conditions.

2. Materials and methods

Two independent mesocosm experiments were carried out in June and August of 2011 at the Dauphin Island Sea Lab as previously described (Ortmann et al., 2012). Briefly, 200 L barrels were lined with Teflon bags and placed in 2000 L tanks with circulating water. Water was pumped into each mesocosm through an intake tube with a 6.35 mm screen from south of Dauphin Island 6 h (June) or 24 h (August) before the start of the experiments to coincide with high tide. Treatments were randomly assigned to each mesocosm, with each treatment present in each of the five 2000 L tanks.

In each experiment, there were 4 treatments, with 5 replicates each (20 barrels in total). A pipe with an air bubbler at the end was extended to the mid-water depth in the middle of each barrel, thereby preventing stratification and hypoxia within the barrel. In order to avoid disturbing surface layers, sampling was conducted using a peristaltic pump and Teflon tubing inserted into the center pipe. The treatments included a control (no addition), dispersant treatment (5 ml Corexit 9500A, Nalco Holding Company), oil treatment (100 ml Macondo 252 oil) and dispersed oil treatment (100 ml oil and 5 ml Corexit). The ratio of oil to Corexit (20:1) was chosen based on the recommendations of the ExxonMobil Research and Engineering Company (2008). Initial samples were collected from the 5 control barrels (t_0), immediately before the addition of the oil and/or dispersant. Over 5 d, samples were collected to monitor the temperature, salinity, dissolved oxygen, inorganic nitrogen and phosphate, and biomass of different microbial groups (Ortmann et al., 2012).

2.1. DNA collection and extraction

At t_0, 24 h (t_{24}) and 72 h (t_{72}) samples were collected to characterize the diversity of the Bacteria. At t_0 samples were collected only from the control treatments ($n = 5$ for each experiment) to characterize the structure of the starting community. For t_{24} and t_{72}, samples were collected from each of the 20 (June) or 19 (August, one dispersed oil replicate was lost due to a tear in the Teflon bag, which allowed mixing with the surrounding water) barrels from the middle of the water column. 50 ml of water was filtered through a 25 mm, 0.22 μm Durapore (Millipore) filter in an ethanol washed filter holder using a 60 ml syringe. Filters were immediately folded inward, placed in a sterile 1.5 ml tube and flash frozen in liquid N_2. Tubes were stored at -86 °C until DNA was extracted.

Lysis buffer (40 mM EDTA, 50 mM Tris–HCl, 0.75 M sucrose) was added to the filter along with 1 mg ml^{-1} lysozyme (Massana et al., 1997). The filters were incubated at 37 °C for 45 min and then 1% SDS and 0.2 mg ml^{-1} proteinase K were added followed by 1 h incubation at 55 °C. The DNA was extracted once with buffered phenol followed by an extraction with 25:24:1 phenol:chloroform:isoamyl alcohol. DNA was precipitated overnight in 2.5 M ammonium acetate and isopropanol, pelleted and washed with a 70% ethanol solution. The clean DNA was resuspended in TE (10 mM Tris–Cl, pH 8.0, 1 mM EDTA). DNA quality was confirmed by running subsamples on agarose gels and staining with ethidium bromide.

2.2. PCR amplification and sequencing

The V6 hypervariable region of the bacterial 16S rRNA gene was amplified using a mix of 5 forward and 4 reverse primers at equal ratios (Sogin et al., 2006; Huse et al., 2008). Primers were modified to include a 5 bp barcode (forward primers) and adapter sequences for the Ion Torrent PGM sequencer (Life Technologies, CA). Three separate 20 μl reactions were carried out for each sample using Phusion Master Mix (Thermo Fisher Scientific, MA). Thirty-five cycles of 98 °C for 10 s, 63 °C for 15 s and 72 °C for 15 s were followed by 5 min at 72 °C. The three reactions were pooled and enzymes and dNTPs were removed from the PCR reactions using the MinElute PCR Clean-up kit (Qiagen, CA).

Ten samples were pooled based on the estimated concentrations from NanoDrop (Thermo Fisher Scientific, MA) measurements to obtain equimolar quantities of each sample. Pooled samples were quantified using the High Sensitivity DNA kit on a Qubit fluorometer (Life Technologies, CA) and diluted to obtain the desired number of molecules for template preparation following the One Touch/ES system protocols. Sequencing was carried out using an Ion Torrent PGM, the 100 bp Sequencing Kit V2.0 and 314 chips.

2.3. Sequence analysis

The FASTQ files were downloaded from the Torrent Server and split into fasta and qual files using Mothur (Schloss et al., 2009). These files were then processed using QIIME (Caporaso et al., 2010). Individual sequences were matched to their sample according to the barcodes and filtered to remove sequences that failed to (a) be longer than 70 bases, (b) have quality scores >20, (c) have no ambiguous bases and (d) have homopolymer runs with >4 bases. No tolerance was allowed for mismatches between barcode and forward primers, and reverse primers were removed along with the barcode and forward primer. All sequences from the two experiments were combined into one fasta file for further processing. Sequences were processed using the *pick_open_reference_otus.py* workflow with the default values and uclust (Edgar, 2010).