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Microbial community and metagenome dynamics during biodegradation of dispersed oil reveals potential key-players in cold Norwegian seawater

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ABSTRACT

Oil biodegradation as a weathering process has been extensively investigated over the years, especially after the Deepwater Horizon blowout. In this study, we performed microcosm experiments at 5 °C with chemically dispersed oil in non-amended seawater. We link biodegradation processes with microbial community and metagenome dynamics and explain the succession based on substrate specialization. Reconstructed genomes and 16S rRNA gene analysis revealed that *Bermanella* and *Zhongshania* were the main contributors to initial *n*-alkane breakdown, while subsequent abundances of *Colwellia* and microorganisms closely related to *Porticoccaceae* were involved in secondary *n*-alkane breakdown and beta-oxidation. *Cycloclasticus*, *Porticoccaceae* and *Spongiiabcteraceae* were associated with degradation of mono- and poly-cyclic aromatics. Successional pattern of genes coding for hydrocarbon degrading enzymes at metagenome level, and reconstructed genomic content, revealed a high differentiation of bacteria involved in hydrocarbon biodegradation. A cooperation among oil degrading microorganisms is thus needed for the complete substrate transformation.

1. Introduction

The fate of oil released to the marine environment is largely dependent on environmental conditions and oil properties, being influenced by various processes like physical, chemical and biological weathering of the oil, spreading and dispersion in the water column, and even sedimentation (Brakstad et al., 2004; Haritash and Kaushik, 2009). However, biodegradation is the only process that completely mineralizes oil compounds. Oil biodegradation has been extensively studied over the years, and more frequently in a combination with oil dispersants after the Deep Water Horizon (DWH) oil spill incident. Significant microbial activity will start shortly after an oil spill, depending on the indigenous microbial community structure, oil characteristics and environmental conditions present at the time in the affected environment. Microbial concentrations close to the spill site may increase in numbers (Hazen et al., 2010), and the community compositions will temporarily shift towards bacteria able to utilize the oil compounds (Hazen et al., 2010; Dubinsky et al., 2013; King et al., 2015). While microbes generally involved in hydrocarbon biodegradation are represented within many phylogenetic groups (Prince, 2005), biodegradation of aromatic hydrocarbons in marine environments has been associated with genera like *Cycloclasticus*, *Pseudoalteromonas* and *Colwellia* (Dubinsky et al., 2013; Dyksterhouse et al., 1995; Geiselbrecht et al., 1998; Harayama et al., 2004; Mason et al., 2014a). Typical alkane-degrading bacteria include members of the genera *Alcanivorax*, *Oleiphilus*, *Oleispira* and *Thalassolituus* (Harayama et al., 2004; Hara et al., 2003; Head et al., 2006). Following the DWH spill novel molecular biology techniques were used, both to characterize the changes in microbial communities related to the deep water plume (mostly based on 16S rRNA gene analysis), and to identify essential microbial processes involved in oil biodegradation (Hazen et al., 2010; Bælum et al., 2012; Lu et al., 2012; Mason et al., 2012). These data coupled to detailed chemical analysis of targeted oil compounds has a potential for elucidating the driving mechanisms involved in oil biodegradation. Many of the oil biodegradation studies, however, focus solely on microbial community (Dubinsky et al., 2013; Valentine et al., 2010; Kostka et al., 2011) or purely on oil compound decay dynamics (Brakstad et al., 2015a; McFarlin et al., 2014; Valentine et al., 2014). Studies that combine both of the approaches, however, have not exploited the full potential of metagenome analysis (Brakstad et al.,

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2015a; Wang et al., 2016). Nevertheless, to study oil biodegradation in detail, experiments were designed using enrichment cultures as microbial source (Bælum et al., 2012; Kostka et al., 2011). This approach is rather different from real oil spill conditions and can potentially result in incomplete conclusions, since pure cultures do not have the capacity to mimic interactions between numerous groups of microorganisms found in environment. In this study our aim is to bridge the gap between processes involved in microbiological degradation of chemically dispersed oil with chemistry by performing detailed analysis of both and mimicking close-to-real oil spill conditions. For elucidating the microbial community response, we employed 16S rRNA gene and metagenome shotgun analysis in combination with binning approach. GC–MS analysis we used for identifying degradation dynamics of targeted oil compounds. The studies were conducted in a system developed for studying biodegradation of dispersed oil (Brakstad et al., 2015a; Nordtug et al., 2011) and the source of microbial community is local seawater, rather than enrichment cultures.

2. Materials and methods

2.1. Oil, seawater and dispersant

Seawater (salinity 34 PSU) was collected from a depth of 80 m (below thermocline) in a Norwegian fjord (Trondheimsfjord; 63°26′N, 10°23′E), supplied by a pipeline system to our laboratories. The seawater was incubated at 5 °C overnight before start of the experiments.

Dispersions with nominal median diameter of 10 μm droplets were prepared from premixed fresh paraffinic oil (Statfjord crude, batch 1998–0170), pre-mixed with the dispersant Slickgone NS (Dasic International Ltd., Romsey, Hampshire, UK) at dispersant to oil ratio (DOR) 1:100, as previously described (Brakstad et al., 2015a; Nordtug et al., 2011). Stock oil dispersions (200 mg/L) were diluted with seawater to a final concentration of 3 mg/L in 2-L pre-sterilized (autoclaved 120 °C, 15 min) flasks (SCHOTT), based on Coulter Counter measurements (see below). Natural seawater with oil dispersions (NSOD) were generated in unfiltered non-amended seawater, while sterilized seawater with oil dispersions (hereinafter referred to as “chemical control”) were prepared in seawater filtered through 1 μm Nalgene™ Rapid-Flow™ filters (ThermoFisher Scientific, MA USA), autoclaved (120 °C, 15 min) and poisoned with 100 mg/L (final concentration) $HgCl_2$. In addition, flasks of natural seawater without oil were included as biological controls (hereinafter referred to as “biological control”). The flasks were mounted on a carousel system with continuous slow rotation (0.75 r.p.m.) and incubated at 5 °C for up to 64 days. Flasks with dispersions (NSOD and chemical controls) and biological controls were sacrificed for analyses after 0, 3, 6, 9, 13, 16, 32 and 64 days. At each sampling date flasks with NSOD (triplicate), chemical control (duplicate) and biological control (one replicate) were sampled. Each sample was analyzed for oil droplet size and semi-volatile and volatile oil compounds, while microbiological analyses (microbial enumerations, community characterization, and metagenome analyses) were performed on NSOD or control treatment from all samples. The experimental and analytical approach is described in Fig. S1.

2.2. Microbiological analyses

Microbiological analyses included fluorescence microscopy for determination of total cell counts, and most probable number (MPN) analysis of oil-degrading microorganism (ODM). 16S rRNA gene amplicon sequencing was used for bacterial community analyses and shotgun sequencing (Whole Genome Sequencing) was employed for mapping the metabolic potential of microbial communities. Detailed information on microbiological analysis can be found in supplemental material.

2.2.1. Total and viable microbial cell counts

Cells were stained with 4′,6-diamidino-2-phenylindole (DAPI) and counted using an epifluorescence microscope (1250× magnification) (Brakstad et al., 2007). Most probable number (MPN) analysis of hydrocarbonoclastic prokaryotes was conducted in accordance with Rand et al. (1976) and Brakstad and Lødeng (2005).

2.2.2. 16S rRNA library and analysis

Detailed description of 16S rRNA gene workflow analysis can be found in supplementary material 1. Briefly, raw pair-end reads were assembled with fastq-join in QIIME 1.9.1 (Caporaso et al., 2010a). Assembled sequences were demultiplexed and quality filtered to remove low quality reads (Phred score < 20; -q 19). UCHIME was employed for chimera detection on assembled quality filtered reads (Edgar et al., 2011). Operational Taxonomic Units (OTUs) were determined by clustering assembled sequences on 97% nucleotide identity using UCLUST (Edgar, 2010) with open reference clustering option. Representative sequences were aligned with PyNAST (Caporaso et al., 2010b) and taxonomy assignment was performed with RDP classifier (Wang et al., 2007) based on SILVA-123 database (Quast et al., 2013). In order to visualize differences in taxonomical composition, relative abundances for OTUs on each sampling point were calculated. For the purpose of statistical analysis of OTUs, DESeq2 (Love et al., 2014), an R package, was used to standardize the counts between samples rather than rarifying to the number of reads present in the sample with smallest number of reads.

2.2.3. Metagenome sequencing and analysis

Detailed analysis description can be found in supplementary material 1. Briefly, five samples were used for metagenome exploration; two controls (day 0 and day 64) and three NSOD (day 9, 16 and 31). Illumina MiSeq paired raw reads were subject to quality filtering using Sickle (Joshi and Fass, 2011) and assembled into contigs with MEGAHIT assembler (Li et al., 2015). PROKKA pipeline was used to find and annotate genes using the default settings (Seemann, 2014). Reads were mapped to contigs with Bowtie2 (Langmead and Salzberg, 2012) and counting was performed with HTSeq (Anders et al., 2015). Counts were standardized based on “transcripts per million” (TPM) calculation (Wagner et al., 2012). In our case, transcripts correspond to reads. Annotations containing enzyme commission number (EC number) were matched against list of gene ontology (GO) terms to produce file containing GO of hydrocarbon degrading genes and their abundances. In order to determine the contribution of microorganisms to specific biodegradation processes, binning of metagenomic reads was performed. Reads from all five samples were co-assembled with MEGAHIT assembler with default parameters. Annotation and mapping was done as described previously. Resulting co-assembled file with contigs and BAM files from five samples were used as input for Anvi'o v2.2.2, binning and analysis tool (Eren et al., 2015). Bins were further manually curated to achieve desired completeness and redundancy. Additional quality check and taxonomical assignment was done with CheckM (Parks et al., 2015). We reconstructed phylogeny additionally using Phylosift (Darling et al., 2014) and FastTree (Price et al., 2009) on bins that were taxonomically poorly resolved or not in consensus after Anvi'o and CheckM analysis.

2.2.4. Nucleotide sequence data

Raw metagenome and 16S rRNA sequences and genome assemblies were deposited in the European Nucleotide archive (ENA) under the study accession number PRJEB14899 entitled as “Oil spill dispersant strategies and bioremediation efficiency”. Raw metagenome sequences can be found from sample accession ID ERS1289858 to ERS1289862, while 16S rRNA sequences from samples accession ID ERS1265011 to ERS1265037. Under the sample accession ID ERS1867669 to ERS1867687, 19 genome assemblies were deposited.