Simulation of *Deepwater Horizon* oil plume reveals substrate specialization within a complex community of hydrocarbon degraders

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The *Deepwater Horizon* (DWH) accident released an estimated 4.1 million barrels of oil and 10^{10} mol of natural gas into the Gulf of Mexico, forming deep-sea plumes of dispersed oil droplets and dissolved gases that were largely degraded by bacteria. During the course of this 3-mo disaster a series of different bacterial taxa were enriched in succession within deep plumes, but the metabolic capabilities of the different populations that controlled degradation rates of crude oil components are poorly understood. We experimentally reproduced dispersed plumes of fine oil droplets in Gulf of Mexico seawater and successfully replicated the enrichment and succession of the principal oil-degrading bacteria observed during the DWH event. We recovered near-complete genomes, whose phylogeny matched those of the principal biodegrading taxa observed in the field, including the DWH Oceanospirillales (now identified as a *Bermanella* species), multiple species of *Colwellia*, *Cycloclasticus*, and other members of Gammaproteobacteria, Flavobacteria, and Rhodobacteria. Metabolic pathway analysis, combined with hydrocarbon compositional analysis and species abundance data, revealed substrate specialization that explained the successional pattern of oil-degrading bacteria. The fastest-growing bacteria used short-chain alkanes. The analyses also uncovered potential cooperative and competitive relationships, even among close relatives. We conclude that patterns of microbial succession following deep ocean hydrocarbon blowouts are predictable and primarily driven by the availability of liquid petroleum hydrocarbons rather than natural gases.

hydrocarbon biodegradation | Gulf of Mexico | microbial communities | Macondo oil | genome succession

During April to July 2010, 4.1 million barrels of crude oil were released into the Gulf of Mexico due to the *Deepwater Horizon* (DWH) accident. The DWH accident was the first major spill to occur in the deep ocean (1,500-m depth), and the processes that determined the fate of oil released at this depth were largely unknown. An important phenomenon observed in the deep ocean was the unexpected suspension of small oil droplets (<100 μm) that remained trapped at depth in the water column because they lacked enough buoyancy to rise to the surface. Consequently, vast plumes of oil microdroplets containing not only soluble but also insoluble fractions of oil were retained at depth, largely between 900 and 1,300 m deep (1, 2), and subject to biodegradation by the deep ocean microbial community (3–6). Furthermore, the application of dispersants at the wellhead may have enhanced oil droplet formation, oil retention, and biodegradation at depth, although potential inhibitory effects of dispersants on biodegradation have been reported (7). There continues to be considerable uncertainty and disagreement about the rates of microbial biodegradation under these conditions and the factors controlling the fate of the complex mixture of crude oil compounds that were trapped deep in the water column (8–10).

Many studies of the plume samples reported that the structure of the microbial communities shifted as time progressed (3–6, 11–16). Member(s) of the order Oceanospirillales dominated from May to mid-June, after which their numbers rapidly declined and species of *Cycloclasticus* and *Colwellia* dominated for the next several weeks (4, 5, 14). Following containment of the DWH wellhead, all of these dominant members declined in abundance. Multiple hypotheses have been put forth regarding the drivers of microbial community succession during the period of hydrocarbon contamination. Redmond and Valentine (14) proposed that the input of natural gas structured the microbial community response. A modeling study suggested it was a consequence of different metabolic growth rates combined with ocean fluid dynamics (6). Dubinsky et al. (3) found succession was primarily controlled by hydrocarbon rather than nutrient availability. A 16S rRNA gene-based study using surrogate oil suggested that dispersant might

Significance

The *Deepwater Horizon* drilling accident was the first major release of oil and natural gases in the deep ocean, and considerable uncertainty remains about the fate of vast amounts of hydrocarbons that never reached the surface. We simulated the deep-sea plumes of dispersed oil microdroplets and measured biodegradation of crude oil components. We successfully reproduced the successive blooms of diverse bacteria observed in the field and obtained near-complete genomes of all major hydrocarbon-degrading species, providing an assessment of the metabolic capabilities of the microbial community responsible for biodegradation. Our results show that rapidly degraded components of oil were consumed by bacteria with highly specialized degradation capabilities and that crude oil alone could explain the microbial dynamics observed in the field.

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Data deposition: The whole genome shotgun project and the associated draft genomes have been deposited at the DNA Data Bank of Japan (DDBJ), European Nucleotide Archive, and GenBank (accession nos. MAAA00000000–MAAU00000000 and MAAW00000000–MABF00000000). The version described in this paper is version MAAA00000000–MAAU00000000 and MAAW00000000–MABF00000000. The raw reads have been deposited at DDBJ, the European Molecular Biology Laboratory, and GenBank (accession no. SRP075617). The project description and related metadata are accessible through BioProject PRJNA320927.

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promote microbes that are able to use dispersant for growth. Therefore, it was thought that the relative abundance of the natural hydrocarbon degraders, such as *Marinobacter*, was kept low (7). No study, to date, has been able to specify the relationship between hydrocarbon substrate availability and the metabolic capacities of the diverse group of organisms responsible for hydrocarbon degradation in the DWH plume.

Our goals in this study were to mimic the conditions present in the deep-sea oil plume at the time of the DWH oil release by producing highly dispersed (~10-μm) oil droplets in natural seawater, correlate the progression of oil degradation with shifts in the endemic microbial community by analyzing changes in hydrocarbon chemistry and bacterial populations over a 64-d time course, and recover high-quality draft genomes to determine the metabolic factors that drove the microbial community shifts throughout the oil biodegradation process.

Results

Microbial Community Structural Changes Correlated with Hydrocarbon Groups. To observe the interaction between the microbial community and the consumption of hydrocarbons we simulated the deep-sea plume observed during the DWH incident in the laboratory. Natural seawater collected from Mississippi Canyon block MC-294 at 1,100- to 1,200-m depth was mixed to a final concentration of 2 ppm oil and 0.02 ppm Corexit EC9500A dispersant. Microdroplets were produced using a pressurized flow injection (PFI) droplet generator (15) to produce median-size oil droplets of 10-μm diameter using Macondo (MC252) oil (*SI Methods*). Oil droplets remained in suspension in replicate 2-L bottles for the 64 d of the experiment.

The chemical analysis in this deep-sea hydrocarbon plume simulation demonstrated that the biodegradation of linear alkane molecules began first, followed by biodegradation of one- to three-ring aromatics, followed by four- to six-ring polycyclic aromatic compounds; the half-lives were as follows: 6–13 carbon alkanes 6.22 d, 14–25 carbon alkanes 8.14 d, alkanes above 25 carbons 22.2 d, monoaromatics [benzene, toluene, ethylbenzene, and xylene (BTEX)] 17.8 d, two- to three-ring polycyclic aromatics 25.3 d, and four- to six-ring polycyclic aromatics >64 d (Fig. 1). Full results for the hydrocarbon analyses are provided in Dataset S1. The rates and sequence of hydrocarbon biodegradation are comparable to those observed in the deep-sea plume of the DWH oil release (3). Both the sequence and rates of hydrocarbon biodegradation are in overall agreement with observations of the decreases of hydrocarbon concentrations in the DWH oil plumes over time (3). Nutrient analysis showed no depletion of other nutrients (Dataset S2).

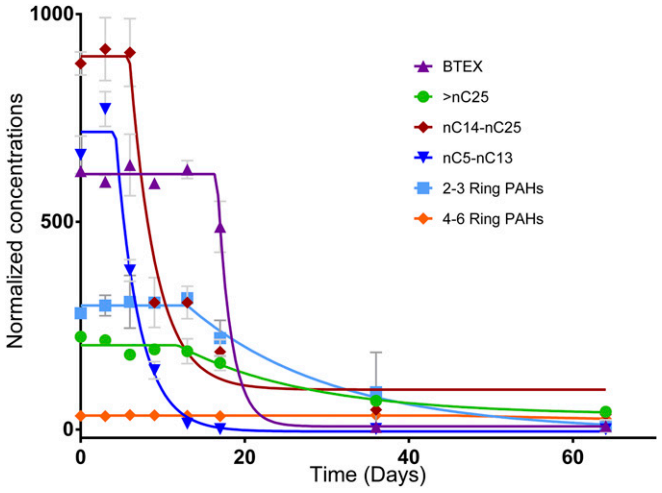

Fig. 1. Degradation of MC252 oil components under simulated deep-ocean conditions. Concentrations are normalized to the recalcitrant biomarker 30αβ-hopane (28). The degradation data are fitted to a model describing a one-phase decay from a plateau after a lag period. Error bars are SDs around the mean of three replicates.

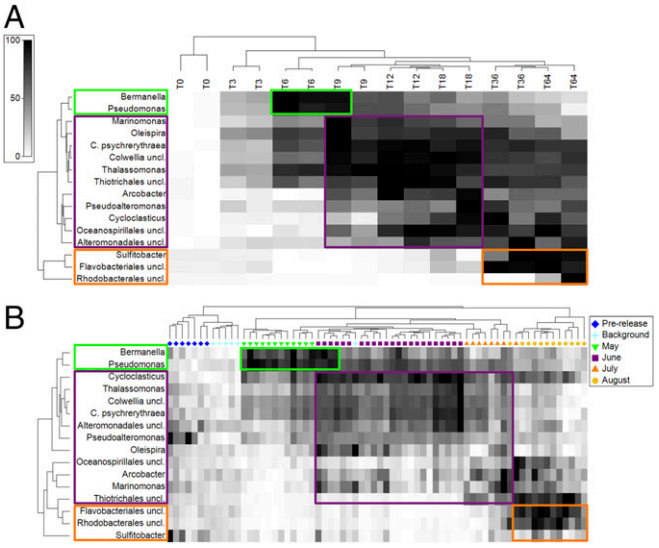

Fig. 2. Response of deep-ocean bacteria to MC252 oil. Heat maps of PhyloChip hybridization intensities for the incubation experiment (*A*) and field samples collected in 2010 during the DWH event (*B*). Hybridization intensities were standardized to the maximum value for each operational taxonomic unit (OTU). OTUs are shown that represent the 10 most enriched genera in incubation samples compared with starting abundances (t0). OTUs enriched in 2010 field samples that matched enriched genera in the incubation experiment are shown in *B*. OTUs that dominated early, intermediate, and late stages of succession in the experiment are indicated by green, purple, and orange boxes, respectively (*A*) and corresponding OTUs in field samples are indicated with identical colors (*B*).

Analysis of 16S rRNA sequence data indicates that the microbial community changed concordant with the chemical changes in the residual oil. The starting relative abundance of all hydrocarbon-degrading bacteria in the laboratory was low, similar to 2010 field samples collected from uncontaminated waters. With the input of oil, the microbial response closely resembled successional patterns observed in 2010 during the DWH event (Fig. 2). Notably, in the laboratory simulation the DWH *Oceanospirillales* initially identified by Hazen et al. (4) and *Pseudomonas* taxa reached maximum relative abundance by 16S rRNA gene analysis at day 6, whereas *Colwellia* and *Cycloclasticus* peaked between days 9–18, and the orders Alteromonadales, Flavobacteriales, and Rhodobacterales peaked between days 36–64. This successional response was nearly identical to those seen in samples collected during the DWH event. In day-6 and day-9 samples, DWH *Oceanospirillales* and *Pseudomonas* reached peak dominance, analogous to the late-May, early-June samples from the DWH event that also contained the highest concentrations of linear alkanes (3). Between days 9 and 18, *Colwellia*, other related Alteromonadales, and *Cycloclasticus* emerged as dominant taxa as DWH *Oceanospirillales* and *Pseudomonas* receded, which was nearly identical to the successional pattern observed between early to mid June 2010 (3, 14). In late stages of the experiment (days 36 and 64), Flavobacteriales and Rhodobacteriales became dominant as most Gammaproteobacteria populations declined, analogous to observations in advanced stages of degradation observed in field samples collected from the deep-sea plume during the DWH oil release (3, 5). Ordination analysis of the 16S rRNA-derived microbial community profile showed the largest shifts in microbial community structure between day 3 and day 6 and followed a sequential trajectory from day 6 to day 64 (Fig. S1). Replicated total cell counts started to increase between day 3 and day 6 and

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continued to increase until day 18, after which they remained around six times higher than initial counts until day 64 (Fig. S2). Control replicates with no added oil remained constant for the duration of the experiment, at 5×10^5 cells·mL^{-1}, with no overall changes in community composition (Figs. S1 and S2).

Based on the 16S rRNA information that identified time periods during which the microbial community underwent major shifts in composition, three samples from days 6, 18, and 64 were chosen for metagenomic sequencing and analysis. Through the reconstruction of the dominant genomes from the metagenome data we identified the same hydrocarbon-degrading organisms and successional pattern that was observed in the PhyloChip 16S rRNA analysis of both the laboratory and the 2010 DWH event samples (Figs. 2 and 3 and Fig. S3).

The Initial Stage of Low-Molecular-Weight Alkane Biodegradation Was Dominated by a Novel *Bermanella* Species. The initial stage of degradation, primarily resulting in biodegradation of low-middle-weight linear *n*-alkanes starting by day 6, was dominated by a single novel *Bermanella* species with its contigs representing almost 33.5% relative abundance of all of the assembled sequences (Fig. 3 and Fig. S3) of the day-6 sample. Other prominent taxa in the day-6 assembly included *Oleispira* (0.44%), *Pseudomonas* (<1%), *Marinomonas* (<1%), and *Pseudoaltermonas* (<1%). By subsampling (random sampling reads to achieve various coverage levels) of the day-6 dataset we recovered a near-complete draft genome of *Bermanella* sp. (2.55 Mb, all bacterial single-copy genes and 51 out of 55 ribosomal sequences were recovered). The 16S rRNA gene (1,532 bp) was 99% identical to the full-length DWH *Oceanospirillales* 16S rRNA sequences previously identified by Hazen et al. (4). The closest sequenced relative is *Bermanella marisrubri* RED65 (17) (National Center for Biotechnology Information reference sequence: NZ_AAQH00000000.1) (Fig. 3), with 16S rRNA sequence identity at 94%. Thus, we have taxonomically resolved the culture-resistant and numerically abundant organism found in the initial stage of the DWH spill to the genus level. This novel *Bermanella* genome rapidly decreased in relative abundance to 1.3% and 0.02% of the bacterial community in day-18 and day-64 samples, respectively (Fig. S3).

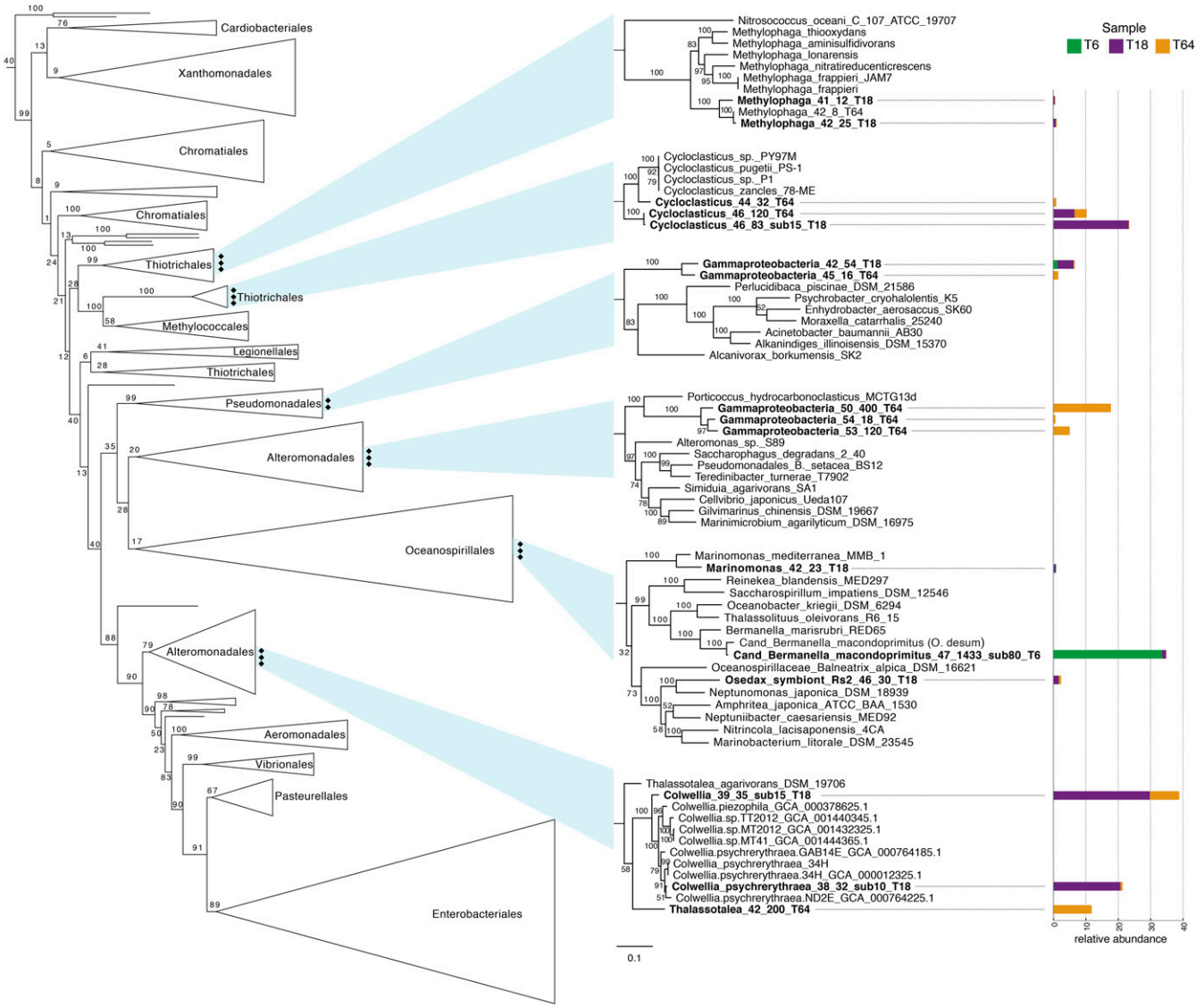

Fig. 3. Phylogeny of Gammaproteobacteria genomes reconstructed from metagenome data, based on concatenated ribosome protein sequences, using the maximum likelihood algorithm RAxML. Genome assembly of *Cycloloclsticus* sp. Phe_8 by Dombrowski et al. (37) was not included in our tree, because it was highly incomplete. Length of bar represents relative abundance of resolved genomes for each time point.

We searched for the presence of potential hydrocarbon-utilization genes in both genome-resolved contigs as well as in assembled sequences that were not binned. Genes involved in three different alkane degradation reactions were detected, including alkane monooxygenase (*alk*B) and CYP153 alkane hydroxylase (EC 1.14.15.1) that degrade medium-chain (C5-C20) alkanes and hydrocarbon monooxygenase (18) for short-chain (C2–C5) alkane oxidation. Of these, only the abundance of *alk*B changed considerably between early and later phases and closely correlated with the shifts of the *Bermanella* genomes. *Alk*B was highly abundant by day 6 and decreased significantly afterward as the concentrations of linear alkanes diminished (Fig. S4). The *alk*B gene was the only hydrocarbon degradation gene identified in the nearly complete *Bermanella* genome (Table S1). Calculating the inferred in situ replication rates with the algorithm iRep (19), we determined that the *Bermanella* was the fastest-growing organism in the early and also in the intermediate stage of the simulation experiment (Fig. S3).

***Colwellia*, *Cycloclasticus*, and Single-Ring Aromatic Hydrocarbon Degradation Are Highly Abundant in the Intermediate Stage.** The day-18 metagenome sequence sample was dominated by members of the genera *Colwellia* and *Cycloclasticus* (Fig. S3). *Bermanella* that dominated the day-6 samples was present (1.3%), as were *Oleispira* (0.74%), *Arcobacter* (2.2%), and *Marinomonas* (0.5%). We detected at least three different genomes of *Colwellia*, two of which were nearly complete (Fig. 3), and recovered one nearly complete genome of *Cycloclasticus* with the highest coverage at day 18. Another *Cycloclasticus* genome (Cycloclasticus sp. 44_32_T64) was detected, but at much lower relative abundance compared with this highly abundant one, and with an increase in relative concentration at day 64 (Fig. S3).

The abundances of aromatic degradation genes peaked at day 18, both in ratio to alkane degradation genes and in relative abundance (Fig. S4). This is consistent with the conclusion from chemical data and the community analysis based on both genomes and 16S rRNA genes and provides evidence that there was a shift from alkane to aromatic utilization by the dominant members of the microbial community (Figs. 1, 2, and 3 and Fig. S5). We resolved a number of genomes from the day-18 sample with the genetic potential to biodegrade single-ring aromatics, including those genomes with the highest relative abundance. For instance, nearly complete genome sequences for the two most abundant *Colwellia* isolates were identified in the day-18 sample. One is closely related to the sequenced genome *Colwellia psychrerythraea* 34H, whereas the other *Colwellia* is phylogenetically distinct at the species level (Fig. 3). Resolved genomes of *Marinomonas*, *Oleispira*, and an additional genome with the Osedax symbiont as its nearest neighbor contained cyclohexanone monooxygenase and mandelate racemase genes with the potential to degrade single-ring aromatic hydrocarbons.

A dominant genome recovered at day 18 is represented by Cycloclasticus_46_83_sub15_T18. This genome does not have as many varieties of hydrocarbon degradation genes (Table S1) as the known *Cycloclasticus* polycyclic aromatic hydrocarbon (PAH) degraders, PS-1 (20), PY97M (21), 78ME (22), or P1 (23), although it does have hydrocarbon monooxygenase, which can degrade low-molecular-weight hydrocarbons (18), and 2-polyprenylphenyl hydroxylase, ring-hydroxylationg dioxygenase, which involves degradation of aromatics substrates.

Genomes Capable of Degrading Polycyclic Aromatics Were Observed During Late-Stage Hydrocarbon Degradation. At day 64 we detected the increase of Rhodobacterales and Flavobacteriales (Figs. 2 and 3 and Fig. S3). There was also transition within Oceanospirillales (Fig. 3). On day 6, the dominant genome within Oceanospirillales was *Bermanella*, and then by day 18 switched to *Marinomonas*, *Oleispira antarctica* (represented by a partial genome, not included in Fig. 3), and an organism related to an Osedax symbiont bacterium Rs2 (24). Finally, only the relative to the Osedax symbiont bacterium genome was observed within Oceanospirillales on day 64.

At this late stage we detected a substantial increase in abundance for genes that are involved in the biodegradation of PAHs. These genes were found in the genomes of Rhodobacterales, Rhodospirillales, *Cycloclasticus*, and unbinned contigs (Table S1). A resolved genome (Gammaproteobacteria_45_16_T64, Fig. 3) was related to the well-known hydrocarbon degrader *Alcanivorax* (Fig. 3). This genome possessed enzymes that are involved in the degradation of short-chain alkanes, BTEX, aromatic carboxylic acid, cyclohexane, and other aromatics (Table S1). Aromatic hydrocarbon degradation genes were also found in genomes of *Bacteriovorax marinus*, *Thalassotalea* sp., *Sulfitobacter* sp., *Hydrocarboniphaga effuse*, and many other partial genomes belonging to Bacteroidetes, Alphaproteobacteria, and Gammaproteobacteria.

Discussion

This study used a laboratory-based method to recreate the conditions that were present in the deep ocean in the Gulf of Mexico and recapitulate the hydrocarbon degradation pattern and microbial community succession observed in the 2010 DWH event and partially observed during an incomplete time course in an earlier laboratory simulation (15). The combination of cold temperature, lack of light, bioavailable oil in the form of 10-μm droplets, dispersant, and the indigenous microbial community allowed us to successfully resolve high-quality genomes and their functional capacities for all key microbes implicated throughout the entire DWH plume oil degradation time course. It also allowed us to monitor the biodegradation of low-molecular-weight aliphatic and aromatic hydrocarbons including benzene, toluene, and xylene.

In this simulation we were able to enrich the dominant hydrocarbon-degrading organism that was detected in the initial stage of the DWH plume and reconstructed it as a nearly complete genome. This genome is most closely related to *Bermanella marisrubri* strain RED65 from the Red Sea (17). Given only 94% homology over the entire 16S rRNA gene sequence, we propose a new species within this genus with the name "*Candidatus* Bermanella macondoprimitus" to reflect the region from within the Gulf of Mexico where this uncultured organism was obtained.

This novel *Bermanella* species seemed to have the fastest replicating genome at both days 6 and 18 by measuring assembled sequence from high-quality draft genomes with the iRep algorithm. This highlights not only the fast acquisition of carbon by this organism but also provides evidence that the organism is very successful in the occupied niche likely defined by rapid degradation of short chain alkanes. It is interesting that the replication rate of this *Bermanella* was still high at day 18 when *Cycloclasticus* and *Colwellia* emerged as the dominant members of the microbial community.

Laboratory experimental studies by other groups have not replicated the suspension of dispersed oil droplet conditions observed in deep-water plumes, which may explain why these studies have been unable to enrich the early responding *Bermanella* and recreate the succession of bacteria observed in the field. For example, Kleindienst et al. (7) observed an initial enrichment of *Colwellia* and *Marinobacter* rather than *Bermanella* in their experiments, but they provided only water-soluble fractions of crude oil as substrate and did not include insoluble microdroplets to test the effects of dispersants on hydrocarbon-degradation rates and microbial community responses. Unlike *Marinobacter* that can efficiently degrade a variety of soluble PAHs in addition to aliphatics, the metabolic capabilities of *Bermanella* are constrained to poorly soluble aliphatics. Studies that underrepresent insoluble fractions incompletely represent the physical and chemical conditions of the crude oil that was encountered by the deep-ocean microbial community, and conclusions about the overall effectiveness of chemical dispersants that are drawn from such studies are incomplete. If chemical dispersants enhanced the formation of crude oil microdroplets that fed the large bloom of *Bermanella* then they may have had an overall positive effect on the degradation of the poorly soluble oil fractions.

In uncovering the genetic potential of the 18 draft genomes identified in this study to degrade hydrocarbons we see a more complex and dynamic community-level response to the sudden influx of oil in the deep ocean than was previously determined by 16S rRNA gene sequence analysis. The relatedness of the isolated genomes at the ribosomal protein level to hydrocarbon-degrading organisms for which there is current genetic or physiological information is, for the majority, distant. Among the diverse set of hydrocarbon degraders enriched upon the addition of the MC252 oil, it was unexpected that we would find multiple members within a genus, each with distinctive complement of hydrocarbon degradation genes. We found that several genomes from novel taxa possessed fewer hydrocarbon degradation genes and seemed to be present in equal or higher abundance relative to their better-studied counterparts. For instance, we recovered three high-quality genomes within the genus *Cycloclasticus*. Cycloclasticus_44_32_T64, with 11 identified aromatic degradation genes, was closely related to the known PAH degrader *Cycloclasticus* P1, PY97M, 78ME, or P1 (Fig. 3) (22, 23, 25, 26), which is generally used as a reference organism for studying hydrocarbon degradation in the ocean. However, although the more distantly related Cycloclasticus_46_83_sub15_T18 and Cycloclasticus_46_120_T64 each possessed only three genes capable of degrading aromatic substrates, they were present in higher relative abundance at both days 18 and 64. This trend was also noted in the novel *Bermanella*, which was the dominant organism identified early in the DWH oil spill. This fast-replicating genome possessed only a single *alk*B gene capable of hydrocarbon degradation. Although there are *alk*B genes in the genomes of other hydrocarbon degraders, considering the dominance of *Bermanella* genome in the day-6 sample we concluded the *Bermanella alk*B was the major contributor in response to linear alkane biodegradation. We speculate that specialization of hydrocarbon substrate provided a substantial advantage due to the given hydrocarbon composition and thus generalists (capable of degrading a wide range of the MC252 oil components) are likely not as responsive in the event of a large environmental release with a sudden influx of high concentrations of hydrocarbons.

One major difference between our laboratory experiment and the DWH oil plume was the lack of input of natural gas. Methane was the greatest single hydrocarbon species emitted during the oil release, whereas ethane and propane were considered to be the most microbially accessible. No study, to date, has been able to create the concentrations and pressure that would have existed in the deep plume to definitively identify any natural gas-degrading microorganism that would have been present in the spill. However, in our study the nearly identical structure and succession pattern of the microbial community observed in the DWH oil plume strongly suggests that the microbial community structure was primarily a consequence of crude oil rather than these natural gases. Modeling efforts have shown that separation of the gas and oil plumes were possible under the DWH release (27). Given this potential separation we could postulate an alternative explanation to the proposal of Redmond and Valentine (14) that ethane and propane jump-started hydrocarbon degradation by *Colwellia,* because we found a rapid enrichment of *Colwellia* with crude oil alone in the absence of natural gases. Also, we did not identify any genes in the *Colwellia* genomes from this study or others that have significant homology to known natural gas-degrading enzymes. It is possible that *Colwellia* played a role in the degradation of the abundant ethane and propane that was present in the plume. However, the increase that was observed by day 18 in the relative abundance of *Colwellia* from this laboratory simulation strongly indicates that the natural gasses that were present in the plume were not needed for its dominance in the intermediate stages of the DWH event. In addition, although *Methylophaga* were thought to be indicators of methane and natural gas utilization, we found increased *Methylophaga* abundance in the intermediate to late stages of hydrocarbon degradation without added methane. Methylotroph enrichment and the lack of observed methane monooxygenase genes (*Supporting Information*) suggests that as recalcitrant substrates were degraded concurrent C1 metabolism existed to use carbon derived from crude oil compounds.

Previous studies of the DWH incident indicated a succession of hydrocarbon-degrading microbial populations and rapid biodegradation rates of the dispersed oil in the deep water, leaving open the question of how this succession occurred. In this experimental reproduction of the biodegradation of dispersed oil we were able to show that similar successional changes appeared in the absence of natural gas, and rapid degradation of oil components in the presence of chemical dispersants. Experimental conditions that replicated oil droplet conditions of the plumes reproduced patterns of microbial succession and enabled the recovery of near-complete genomes of the principal hydrocarbon-degrading bacteria observed during the DWH oil release, which included the previously unidentified DWH *Oceanospirillales* as a novel alkane-degrading *Bermanella* species. Based on the reconstructed genomic content, many organisms were revealed to be specialists with the capacity for degrading specific hydrocarbon constituents, resulting in a diverse community for degradation of a complex hydrocarbon mixture. Consequently, we show the near-complete metabolic capability of the primary bacteria involved in the hydrocarbon degradation that occurred in the DWH oil spill. The timing of enrichment of different classes of bacteria in relation to the degradation of the multiple hydrocarbon components demonstrates the importance of resource partitioning in the evolution of this microbial community.

Methods

Chemistry Analysis. All chemistry samples for hydrocarbon characterization were sent to Battelle and processed by liquid-liquid extraction with methylene chloride for total petroleum hydrocarbons and saturated hydrocarbons by gas chromatography–flame ionization detector using a modification of SW-846 Method 8015. Parent and alkylated PAHs, decalins, and the recalcitrant biomarker 30αβ-hopane (28) were analyzed by gas chromatography/mass spectrometry in selected ion monitoring using modifications of SW-846 Method 8270. Samples for volatile organic compounds were analyzed by purge-and-trap GC/MS (Battelle SOP 5-245, a modification of SW-846 Method 8260).

Analytical methods for hydrocarbons fulfilled the requirements described in the National Oceanic and Atmospheric Administration's Mississippi Canyon 251 (*Deepwater Horizon*) Natural Resource Damage Assessment Analytical Quality Assurance Plan Version 3.0, 2011. Although individual analysis may have additional quality assurance requirements, batch quality assurance/quality control included at least the analysis of laboratory blanks, fortified blanks, sample duplicates, and standard reference materials (National Institute of Standards and Technology SRM 2779) as needed.

DNA Extraction. Samples (800–1,400 mL) were filtered through sterile filter units of 47-mm-diameter polyethylsulfone membranes with 0.22-μm pore size (MO BIO Laboratories, Inc.). Eight sample times (days 0, 3, 6, 9, 12, 18, 36, and 64) were analyzed on G3 PhyloChip for 16S rRNA gene-based community analysis. Two replicate oil-treated samples and one unamended control were analyzed for each time point extracted from control and added oil treatments. Three oil-treated samples (days 6, 18, and 64) were selected for metagenome sequencing. These samples represented roughly the three phases of the succession (Fig. S1). For each sample, the same extracted genomic DNA was used for both PhyloChip and metagenomic studies. Genomic DNA was extracted from filters using a modified Miller method (29) described in detail in *Supporting Information*.

Metagenomic Sequencing Library Preparation. DNA was quantified by Qubit fluorometer (Invitrogen) and 200 ng were sheared using a Covaris instrument. The sheared DNA was cleaned, end-repaired, and size-selected using a TruSeq Nano DNA kit (Illumina) targeting fragments around 300 bp. The size and quality of the DNA was checked via Bioanalyzer using a High Sensitivity DNA kit (Agilent Technologies). The size-selected DNA was further processed for library construction according to the manufacturer's instructions. The final libraries (peak at ~440 bp) were assessed via Bioanalyzer using a DNA 7500 kit and sent to Yale Center for Genomic Analysis for 150-bp paired-end sequencing on Illumina HiSeq 2500. The three libraries were pooled and run on a single lane according to the standard protocol performed at the Yale Center for Genomic Analysis.

PhyloChip Analysis. The 16S rRNA gene was amplified using bacterial primers 27F/1492R and archaeal primers 4Fa/1492R (30), with annealing temperatures from 50 to 56 °C. Bacterial PCR product (500 ng) and archaeal PCR product (25 ng) were hybridized to each array following previously described

procedures (4). Detailed protocols are provided in *Supporting Information*. Data from the resulting .CEL files were processed through PhyCA using the same bacterial Stage1 and Stage2 cutoffs as previously described (30).

Metagenome Assembly, Binning, and Annotation. Metagenomic reads quality assessment, reads trimming, contig assembly, and annotation followed the general methods described previously (31). The pipeline is described in *Supporting Information*. Assembled genome fragments were assigned to draft genomes of origin (binned) with a combination of online binning tools (ggkbase.berkeley.edu) and binning software, Maxbin 2.0 (32) and Metabat (33). Subassemblies were obtained by assembling subset of reads (reads were randomly sampled to obtain a 1/10th to 1/80th of total reads) using IDBA_UD. This technique was applied specifically to samples T6 and T18, to obtain near-complete genomes of *Bermanella* sp., *Colwellia*, *Cycloclasticus*, and *Acrobacter*. The genome bins were refined based on tetranucleotide frequency information analyzed using an emergent self-organizing map (34), with refinement of some bins using organism abundance pattern data. Contig abundance was calculated from reads mapping using Bowtie2 (35). Mapping was done with bowtie default parameters including "–sensitive."

This whole genome shotgun project and the associated draft genomes have been deposited at the DNA Data Bank of Japan (DDBJ), European Nucleotide Archive, and GenBank under the accession nos. MAAA00000000–MAAU00000000 and MAAW00000000–MABF00000000. The version described in this paper is version MAAA00000000–MAAU00000000 and MAAW00000000-MABF00000000. The raw reads have been deposited at DDBJ, the European Molecular Biology Laboratory, and GenBank under the accession no. SRP075617. The project description and related metadata are accessible through BioProject PRJNA320927.

In Situ Replication Rates. Bacterial replication rates were calculated from metagenome sequencing with the software iRep (19) using default settings. The measurement is based on the sequencing coverage trend resulting from bidirectional genome replication from a single origin. This method requires high-quality genomes (>75% completeness using single-copy genes), which were selected from our dataset and dereplicated based on 98% nucleotide identity (36). Metagenomic reads were mapped to the selected genomes using Bowtie2 (35) allowing a maximum of three mismatches per read.

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