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Reconstructing metabolic pathways of hydrocarbon-degrading bacteria from the Deepwater Horizon oil spill

Nina Dombrowski¹, John A. Donaho¹, Tony Gutierrez², Kiley W. Seitz¹, Andreas P. Teske³ and Brett J. Baker^{1*}

The Deepwater Horizon blowout in the Gulf of Mexico in 2010, one of the largest marine oil spills¹, changed bacterial communities in the water column and sediment as they responded to complex hydrocarbon mixtures^{2–4}. Shifts in community composition have been correlated to the microbial degradation and use of hydrocarbons^{2,5,6}, but the full genetic potential and taxon-specific metabolisms of bacterial hydrocarbon degraders remain unresolved. Here, we have reconstructed draft genomes of marine bacteria enriched from sea surface and deep plume waters of the spill that assimilate alkane and polycyclic aromatic hydrocarbons during stable-isotope probing experiments, and we identify genes of hydrocarbon degradation pathways. Alkane degradation genes were ubiquitous in the assembled genomes. *Marinobacter* was enriched with *n*-hexadecane, and uncultured *Alpha*- and *Gammaproteobacteria* populations were enriched in the polycyclic aromatic hydrocarbon-degrading communities and contained a broad gene set for degrading phenanthrene and naphthalene. The repertoire of polycyclic aromatic hydrocarbon use varied among different bacterial taxa and the combined capabilities of the microbial community exceeded those of its individual components, indicating that the degradation of complex hydrocarbon mixtures requires the non-redundant capabilities of a complex oil-degrading community.

Marine microorganisms derive energy and carbon from the degradation of petroleum hydrocarbons and drive the bioremediation process during anthropogenic oil spills, such as the Deepwater Horizon (DWH) spill^{2,4,7–9}. Various uncultured bacteria, primarily belonging to *Oceanospirillales* and other *Gammaproteobacteria*, were enriched during the DWH spill and are believed to have played a major role in oil degradation^{2,3,10–12}. However, this assumption relies on the dominance of these organisms in environmental sequencing surveys, whereas the metabolic potential and pathways for degrading hydrocarbons in bacterial populations, which demonstrably assimilate these compounds, remain to be determined. To address this question, we obtained ~113 Gb of shotgun metagenomic data from hydrocarbon-degrading enrichments using stable-isotope probing (SIP) experiments¹³. Enrichments were obtained from weathered hydrocarbons floating on the sea surface and from the subsurface hydrocarbon plume, collected during the DWH oil spill in 2010. The plume sample was incubated with ¹³C-labelled *n*-hexadecane (HEX), and the sea surface sample was separately incubated with ¹³C-labelled naphthalene (NAP) or phenanthrene (PHE) in SIP experiments¹³. *De novo* assembly and (tetranucleotide signature) binning of metagenomic sequences from the ¹³C-labelled fractions allowed the reconstruction of 7 high-quality (completeness of 52–95%) and 17

fragmentary draft genomes (completeness <50%), as well as ~13% of unbinned scaffolds (see Methods, Supplementary Table 1 and Supplementary Fig. 1).

We first annotated genes within all assembled genomes. Taxonomic assignment of annotated genes in the HEX, NAP and PHE assemblies revealed distinct bacterial communities (Supplementary Fig. 2a,b). *Marinobacter* and *Alcanivorax* dominated the HEX-degrading community, and *Alteromonas* and *Cycloclasticus* were abundant in NAP and PHE samples, respectively. This community spectrum coincided with results from the previous SIP-based analysis of the same samples using 16S rRNA clone sequencing¹³. To identify taxa representing the seven high-quality draft genomes, we constructed a phylogenetic tree using up to 15 ribosomal proteins (Fig. 1 and Supplementary Table 1). We recovered a 95% complete *Marinobacter* genome from the HEX enrichment, designated H-Mar (99% inferred protein similarity to *Marinobacter salarius* R9SW1, Fig. 1 and Supplementary Table 1). From the NAP-degrading community we reconstructed two gammaproteobacterial genomes belonging to *Oceanospirillales* (N-Alc, 73% similar to *Alcanivorax* sp. 43B_GOM-46m, 84% complete) and *Alteromonadales* (N-Alt, 86% similar to *Alteromonas macleodii* English Channel 673, 80% complete). An additional 93% complete alphaproteobacterial genome belonged to the order *Rhodospirillales* (N-Tha, 99% similar to *Thalassospira profundimaris* WP0211). Three genomes from the PHE-degrading communities included a bacterium 83% similar to *Cycloclasticus pugetii* PS-134H (P-Cyc, 52% complete), an *Oceanospirillales* member with 73% similarity to *Neptuniibacter caesariensis* MED92 (P-Nep, 86% complete) and a member of the *Alteromonadales* with 64% similarity to *Colwellia psychrerythraea* 34H (P-Col, 70% complete).

The NAP-enriched taxa *Rhodospirillales*, *Oceanospirillales* and *Alteromonadales* (N-Tha, N-Alc and N-Alt) were previously associated with hydrocarbon degradation. For example, isolates of the order *Alteromonadales* showed alkane and polycyclic aromatic hydrocarbon (PAH)-degrading activity in previous SIP experiments¹³. The order *Rhodospirillales* accumulated during the DWH spill in sea surface samples, and isolates of this order, such as *Thalassospira tepidiphila*, degrade PAHs^{3,14}. We also assembled one genome belonging to *Alcanivorax* from the NAP-degrading community (N-Alc), a genus that was barely detected in the spill itself but was present in the previous SIP experiments¹³. Additionally, *Alcanivorax* was abundant in HEX enrichments (Supplementary Fig. 2b). Although *Alcanivorax* isolates degrade alkane hydrocarbons and none have been described to metabolize PAHs^{13,15}, *Alcanivorax* was previously detected in bacterial consortia growing

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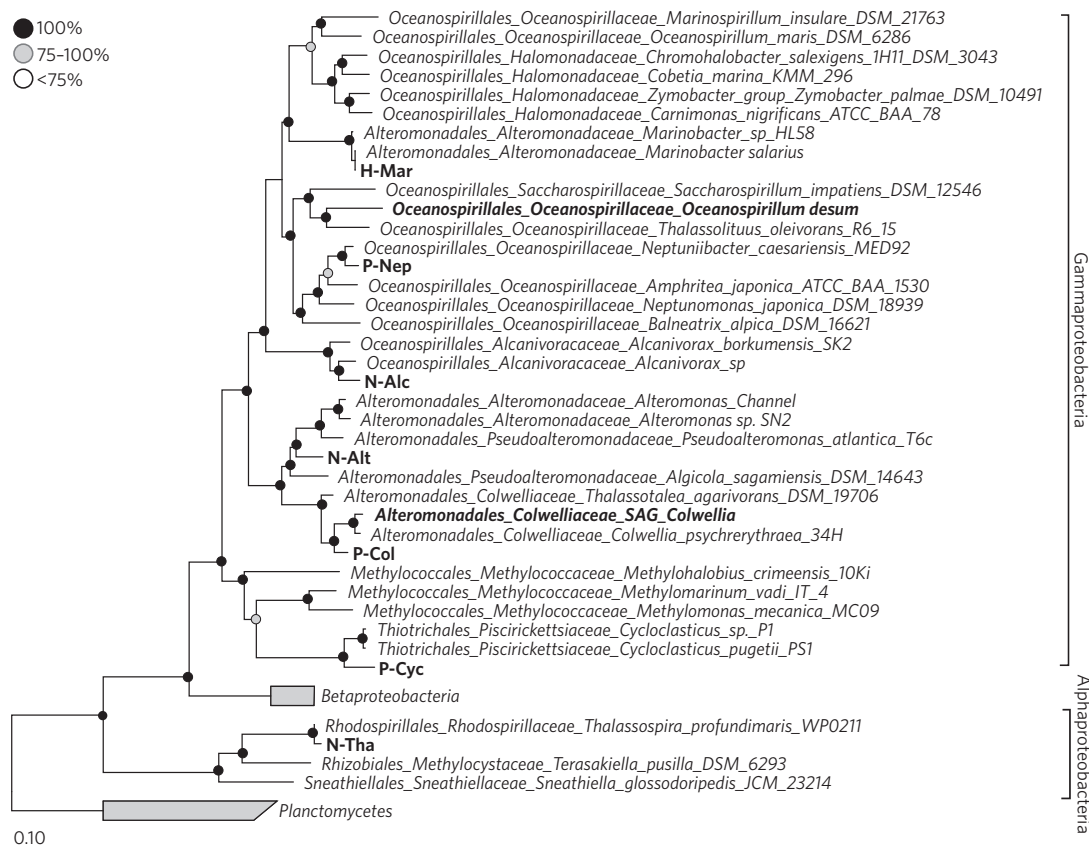


Figure 1 | Phylogenetic characterization of genomic bins reconstructed from three SIP enrichments. Maximum-likelihood-based phylogenetic tree of up to 15 concatenated ribosomal proteins (rpl2, 3, 4, 5, 6, 14, 15, 18, 22, 24 and rp53, 8, 10, 17, 19) from seven high-quality genomes assembled from the *n*-hexadecane (H), naphthalene (N) and phenanthrene (P) SIP enrichments (bold). Two genomes from previously published DWH plume-derived metagenomes are indicated in italics^{10,46}. Bootstrap values were generated using MrBayes. Two *Planctomycetes* strains were used as outgroup. Black, grey and white circles: nodes with bootstrap values of 100, 75-100 and <75% (1,000 replicates).

1 on pyrene as the sole carbon source^{16,17}. Thus, the NAP enrichment
 2 of *Alcanivorax* supports a broader versatility in *Alcanivorax* for
 3 degrading not only alkanes but also PAHs, potentially by participating
 4 in bacterial consortia that fully metabolize PAHs.

5 Focusing on the PHE-degrading community, the genera
 6 *Cycloclasticus*, *Colwellia* and *Neptuniibacter* (corresponding to
 7 P-Cyc, P-Col and P-Nep) represented 66, 13 and 3% of clones in
 8 previous PHE-SIP experiments, respectively¹³. Notably, the
 9 *Cycloclasticus* 16S rRNA phylotype recovered from the previous SIP
 10 study was >99% similar to the most dominant *Cycloclasticus* phylotype
 11 that was enriched at the sea surface during the spill¹³. Concordantly,
 12 *Colwellia* was enriched in laboratory experiments in dispersant-oil
 13 microcosms¹⁸. Thus, the assembled genomes represent abundant, as
 14 well as rare, hydrocarbon-degrading DWH spill community members.

15 16S rRNA gene sequences recovered from the SIP assemblies co-
 16 clusted with sequences derived from the previous SIP experiments
 17 and partially with sequences enriched in surface and plume samples
 18 during the spill (Supplementary Fig. 3)^{2,3,13}. These clustering patterns
 19 therefore indicate that the bacterial lineages present in our metagenomic
 20 assemblies were present during the spill and do not represent artefacts of
 21 the SIP experiment. To confirm this inference, we mapped our SIP
 22 assemblies to published plume-derived metagenomic and metatranscriptomic
 23 data sets¹⁰. The HEX-degrading assembly recruited 2.7
 24 and 0.8% of metagenomic reads from plume and unpolluted samples,
 25 respectively (Supplementary Table 2). Annotated genes from the HEX
 26 assembly matched 6.3 and 1.3% of metatranscriptomic reads from
 27 samples collected proximal and distal, respectively, from the plume
 28 (Supplementary Table 2 and Supplementary Figs 4 and 5). This suggests
 29 that genes from our assembled genomes were enriched and more highly

expressed with increasing hydrocarbon exposure. However, despite the
 percentage of mapped reads, we were unable to locate specific alkane-
 degradation genes, possibly due to insufficient sequencing depth that
 would allow covering non-abundant taxa (Supplementary Fig. 5).
 No metagenomic data set from the sea surface of the spill is available;
 but based on previous 16S rRNA gene studies, *Alphaproteobacteria*
 (that is, order *Rhodospirillales*) as well as *Gammaproteobacteria*
 (orders *Alteromonadales* and *Oceanospirillales*) were enriched in
 oil-contaminated sea surface samples³. Thus, the lineages recon-
 structured in our assemblies reflect (or resemble) those enriched in
 hydrocarbon-contaminated sea surface communities.

To identify genes that enabled bacterial community members to
 be enriched during the DWH spill, we compared the gene content
 between the different hydrocarbon-degrading communities obtained
 in the SIP assemblies. Of the detected 4,756 unique gene functions,
 ~40% were shared and 4, 13 and 22% were unique among the
 HEX, NAP and PHE assemblies, respectively (Supplementary Fig. 6
 and Supplementary Data 1). The PHE enrichment showed selection
 for serine/threonine kinases, while the HEX samples encoded for
 abundant short-chain dehydrogenases and the NAP assembly was
 enriched for tripartite ATP-independent periplasmic (TRAP) trans-
 porters. The serine/threonine kinases phosphorylate serine, threonine
 and tyrosine are proposed mediators of bacteria-bacteria interactions,
 while TRAP transporters function in osmoregulated solute transport^{19,20}.
 Additionally, several functional categories were present in individ-
 ual genomic bins (Fig. 2 and Supplementary Data 2). Five of seven
 genomes carried genes for bacterial chemotaxis and six of seven
 for flagella biosynthesis, hinting at active motility and therefore the
 ability to move towards oil substrates. Additionally, P-Col encoded

Q17

Q2

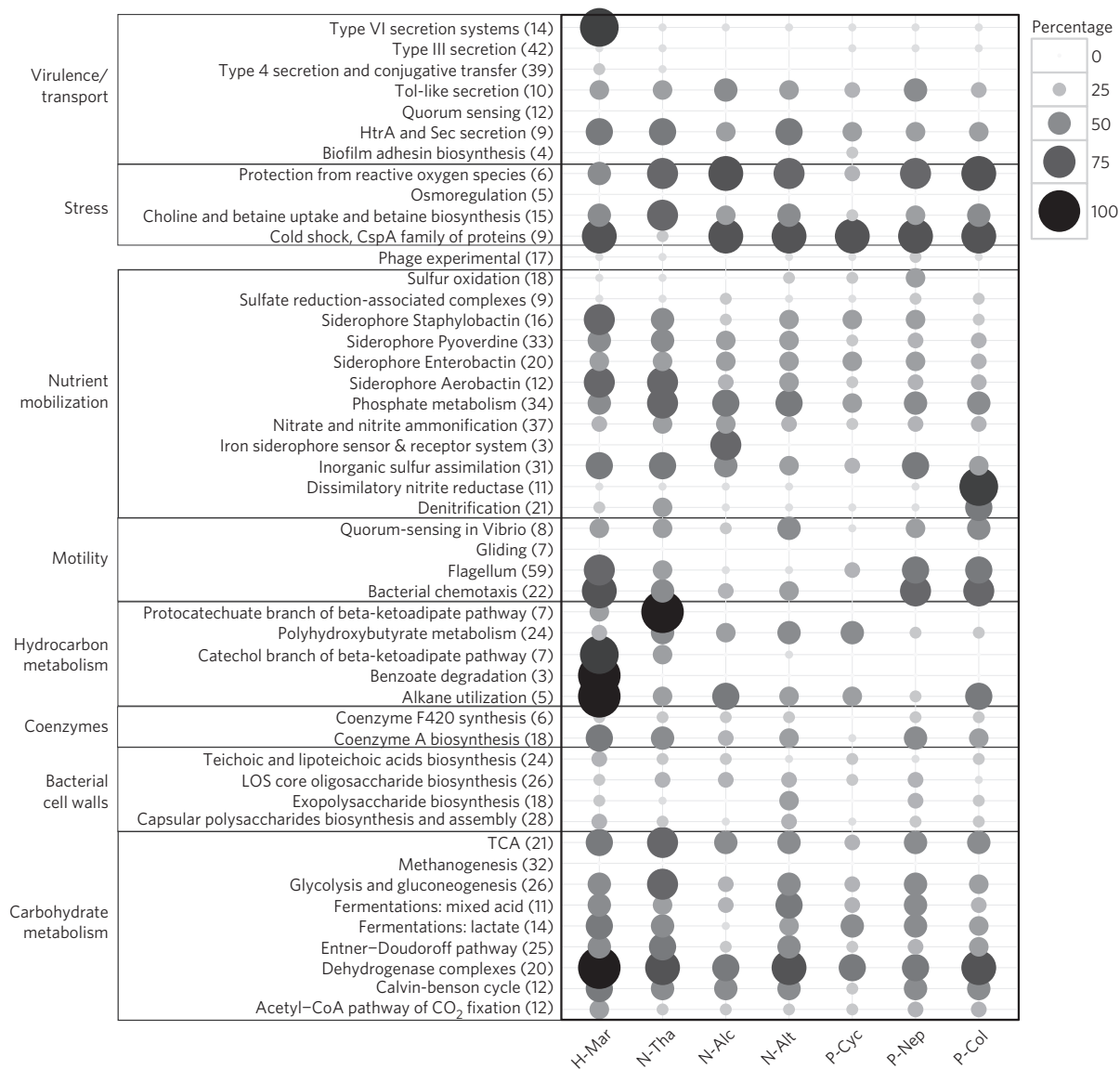


Figure 2 | Abundance of functional categories detected within the reconstructed genomes. Functional categories detected in the seven draft genomes assembled from the *n*-hexadecane (H), naphthalene (N) and phenanthrene (P) SIP enrichments. Functional categories are based on the SEED subsystems³⁶ and were compared against genes annotated within each metagenomic bin using blastp (e-value cutoff of 1e-20). Numbers in parentheses: total number of genes within each functional category. Percentage: per cent of total number of genes per subsystem found within each bin.

1 for several denitrification genes (13 of 21 tested genes), including
 2 *norB/C*, *nosR* and *nosD*, while P-Nep appears able to oxidize
 3 sulfur (that is, including *soxA-D* and sulfite oxidase), implying rel-
 4 evance for degrading sulfur-containing components of oil and the
 5 applied dispersant Corexit. Suggesting physiological adaptations to
 6 phosphorous and iron limitation, most genomic bins contained
 7 genes relevant for phosphate starvation or siderophore biosynthesis,
 8 such as staphylobactin (that is, *phoH* and *sbnA*; Supplementary Data
 9 2). The presence of major genes for motility and for use of scarce
 10 nutrients suggests that the enriched organisms are well adapted
 11 for chemotactic motility towards their hydrocarbon substrates and
 12 for physiological responses to nutrient-limiting conditions that
 13 characterize oil-induced bacterial blooms²¹. Alternatively, these
 14 genes might be of general importance for survival and growth in the
 15 Gulf of Mexico.

16 To resolve the hydrocarbon degradation pathways in the assembled
 17 bacterial genomes, we searched for homologues to known degradation
 18 genes. An entire pathway for alkane degradation was found in H-Mar

(*Marinobacter*), the dominant genus in the HEX-degrading commu-
 19 nity (Fig. 3a and Supplementary Figs 1 and 2). This result is consistent
 20 with isolates of *Marinobacter* being able to degrade HEX, increasing
 21 *Marinobacter* abundance in DWH plume samples as validated by
 22 fluorescence *in situ* hybridization (FISH), and enrichment of
 23 genes for alkane degradation in metatranscriptomes from the
 24 DWH spill and the Gulf coast^{2,10,22,23}. Additionally, we recon-
 25 structed the alkane-degradation pathway in most of the genomes
 26 from the NAP- and PHE-degrading bins (Fig. 3a). However,
 27 based on the SIP approach, only a subset of bacteria appears to
 28 actively employ these pathways—*Marinobacter* (H-Mar)—and
 29 potentially outcompete other alkane-degrading bacteria, such as
 30 *Alcanivorax* (N-Alc). To validate this finding, metatranscriptomic
 31 sequencing could be used to map active gene expression to our
 32 assembled draft genomes. One unique feature of the active alkane-
 33 degrader H-Mar is the presence of genes encoding for the type VI
 34 secretion system^{24,25}, which were mostly absent from all other
 35 genomic bins (Fig. 2). The type VI secretion system is involved in
 36

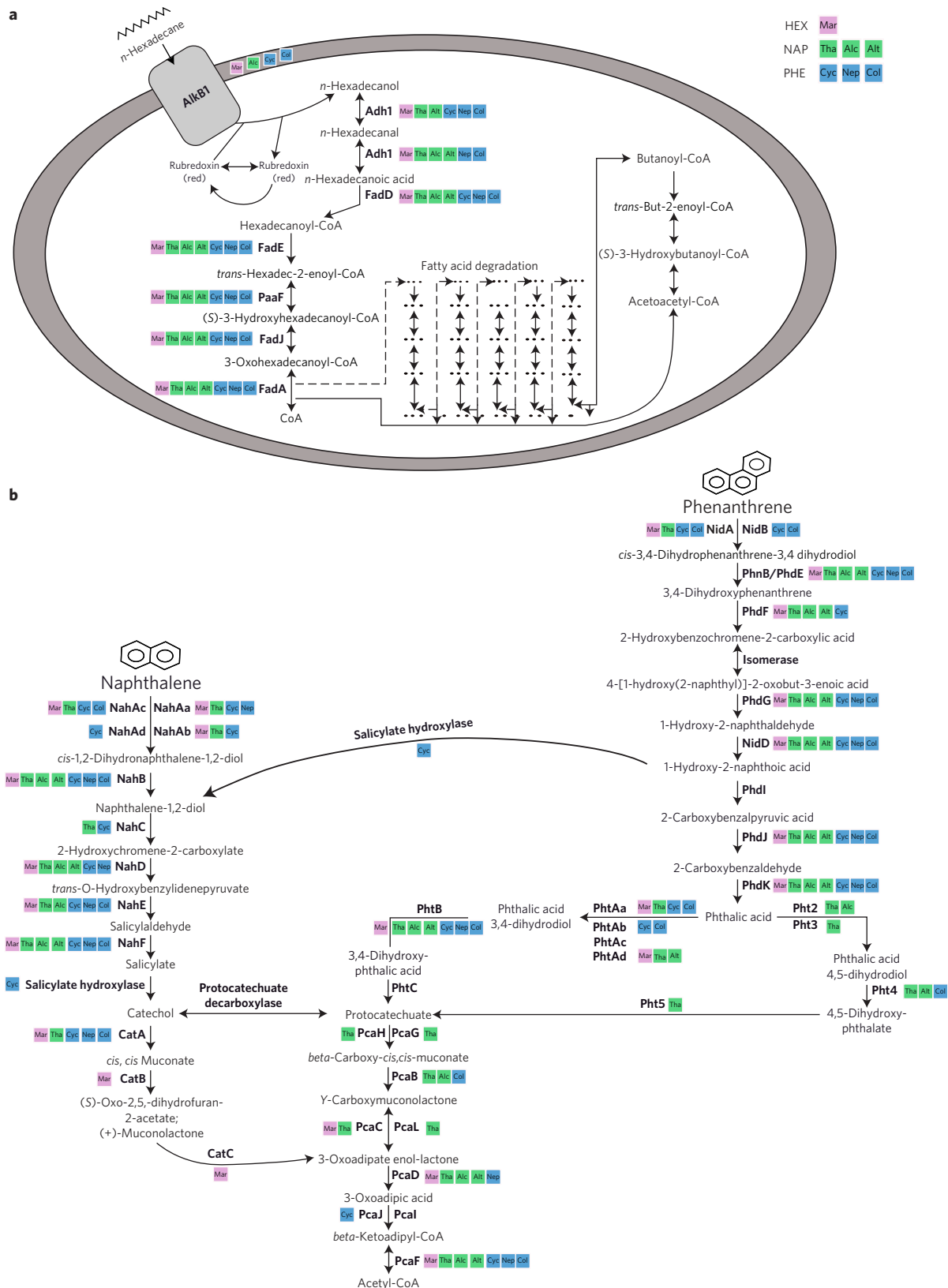


Figure 3 | Distribution of key alkane and polycyclic hydrocarbon degradation pathways in reconstructed genomes. a, Metabolic reconstruction of the *n*-hexadecane (HEX) degradation pathway within seven high-quality metagenomic bins. **b**, Metabolic reconstruction of naphthalene (NAP) and phenanthrene (PHE) degradation pathways within seven high-quality genomes. Purple, green, blue: HEX, NAP and PHE-degrading bins, respectively.

1 inter-bacterial competition and has been discussed to be relevant
2 for alkane assimilation^{24,25}, and thus could provide H-Mar with a
3 competitive advantage over other non-active alkane degraders.

To characterize PAH degradation pathways in SIP-enriched bac- 4
teria, we identified genes encoding for enzymes in both NAP- and 5
PHE-degradation pathways in our assembled genomes (Fig. 3b). 6

1 Of 41 described NAP- and PHE-degradation genes, 19 were present
2 in >50% of genomes, while a subset of genes, such as *nahC*, salicy-
3 late hydroxylase or *phdF*, were only detectable in a few genomes. For
4 example, within N-Tha, N-Alc and N-Alt, we were unable to anno-
5 tate the full known NAP-degradation pathway. N-Tha
6 (*Thalassospira profundimaris*) is equipped to degrade NAP to sali-
7 cylate, but lacks the salicylate hydroxylase, which converts salicylate
8 to catechol. Only the fragmentary genome NAP-25
9 (*Oceanospirillales*) encoded the gene for this step among the
10 assembled genomes from the NAP enrichments. Similarly, the
11 extradiol dioxygenase (*PhdF*), which converts 3,4-dihydroxyphen-
12 anthrene to 2-hydroxybenzochromene-2-carboxylic acid early on
13 in the PHE-degradation pathway, was only detected in P-Cyc
14 (*Cycloclasticus*). Unless all pathway gaps are without exception
15 ascribed to sequencing gaps within the genomic bins, a more pars-
16 monious interpretation of these results suggests that alternative
17 genes and enzymes for these individual steps exist, which are not
18 represented in gene databases. Alternatively, coordinated commu-
19 nity activity might be required to completely degrade polycyclic
20 hydrocarbons during the DWH spill.

21 Our study combines metagenomics and SIP, allowing the
22 assembly of genomes from active hydrocarbon-degrading bacteria
23 present during the DWH spill, and enriched from deep plume and
24 surface samples based on alkane and PAH assimilation. All
25 assembled genomes possessed pathways for alkane and PAH
26 degradation, suggesting that several taxa actively degraded
27 hydrocarbons during the spill (Fig. 3). For example, H-Mar
28 (*Oceanospirillales*) corresponds to *Marinobacter salarius*, which
29 degrades alkanes in the plume, and the bacterium that constitutes
30 N-Tha (*Rhodospirillales*) was responsible for degradation of PAHs
31 at the sea surface. These taxa were enriched during the spill and
32 their genes for hydrocarbon degradation were detected in the
33 plume and sea surface^{2,3,10,22,26}. However, the plume-derived
34 genome (H-Mar) appears to be enriched only at a low relative
35 abundance compared to previous data sets, where an uncharacter-
36 ized *Oceanospirillales* represented >60% of sequence reads¹⁰. This
37 disparity suggests that low-abundant community members were
38 active during the spill, as observed in deep-sea hydrothermal
39 plumes²⁷. Additionally, differences between bacterial taxa detected
40 during the DWH spill compared to SIP enrichments could be a
41 reflection of the experimental set-up. For example, no dispersant
42 was added during the experiment, but Corexit was added during
43 the spill^{13,28} and found to select against *Marinobacter* in micro-
44 cosm experiments¹⁸. Additionally, the SIP procedure required
45 incubation steps at room temperature instead of 4 °C to obtain
46 sufficient incorporation of the ¹³C label into DNA for SIP¹³.
47 Such temperature shifts have been demonstrated to induce
48 changes in plume bacterial community composition³. Whole-
49 community gene expression studies will be required to evaluate
50 the activity of our assembled genomes in their native
51 community context.

52 Within the constraints inherent to the SIP experimental
53 approach, combining SIP with metagenomics enabled us to identify
54 and analyse hydrocarbon-degrading pathways within several uncult-
55 ured bacteria. Bacteria that are usually not associated with PAH
56 degradation (that is, members of *Alcanivorax* in the NAP enrich-
57 ments) persisted at sufficiently high levels for metagenomic recon-
58 struction, indicating that complete pathways for the degradation
59 of PAHs may not be obligatory for individual community
60 members that thrive in PAH-degrading enrichments. Although we
61 cannot exclude that genes for hydrocarbon degradation were
62 missed in our assemblies, the apparent partitioning of key
63 pathway steps to individual community members suggests an alter-
64 nate hypothesis, in that complete degradation of a complex mixture
65 of PAHs requires the coordinated response of a complex bacterial
66 community and the coordination of its metabolic pathways.

Methods

Sample collection and preparation. DNA samples for metagenomic reconstruction
were obtained from a previously published stable-isotope probing (SIP)
experiment¹³. Briefly, an oil-contaminated sea surface sample (original ID PE5) was
collected near a site of the DWH spill (28°44.175' N, 88°22.335' W) in the Gulf of
Mexico on 5 May 2010. Two deep hydrocarbon plume samples (original ID B3
and B6, biological replicates) were collected near the Macondo wellhead
(28°41.686' N, 88°26.081' W) at depths of 1,170 and 1,210 m on 31 May 2010 and
combined for the SIP experiment. Sea surface and plume samples were collected at
~0.86 and 3.5 m from the wellhead. SIP experiments for the plume sample (B3+B6)
were performed with ¹³C-labelled HEX and for the sea surface sample (PE5) with
¹³C-labelled NAP or PHE (yielding a total of three samples). Incubations containing
solely ¹²C-unlabelled substrate were run in parallel to act as unlabelled controls.
Total DNA from labelled and unlabelled incubations from each of the three SIP
experiments was extracted as described previously²⁹. Extracted labelled and
unlabelled DNA (0.7 to 1.0 µg for each sample) was separated using CsCl gradient
ultracentrifugation¹³. To prepare these previously processed SIP samples for
sequencing, DNA concentrations were measured using a Qubit 3.0 Fluorometer and
a final concentration of 10 ng µl⁻¹ for each sample (using a total amount of 100 ng)
from the heavy-labelled fraction was used to prepare libraries for paired-end
Illumina (HiSeq 2500) sequencing.

Metagenomic assembly and binning. Illumina library preparation and sequencing
was performed by the Genome Sequencing and Analysis Facility (GSAF) at the
University of Texas at Austin. For each of the three samples, two Illumina libraries
were prepared and sequenced (technical replicates). Sequencing was performed on
an Illumina HiSeq 2500 with the following specifications: high-throughput run
mode, run type paired end 2 × 125 bp, 6 × 4.0E8 target reads (millions), insert size of
~360–420 bp and ~5% PhiX control spike-in. This sequencing approach provided
~113 Gb of sequencing data (373,279,006, 289,782,708 and 246,243,202 reads from
the HEX, NAP and PHE samples, respectively). Raw Illumina shotgun genomic
reads were separated from Illumina artefacts by removing the adaptors and DNA
spike-ins from the forward and reverse reads. Therefore, a sliding window approach
using a kmer size of 28 and a step size of 1 was used. Reads with ≥3 Ns, an average
score of <Q20 and a read length of <50 bps were removed using cutadapt (yielding
886,823,725 total reads)³⁰. Afterwards, reads were interleaved using interleave_fasta.
py and the interleaved sequences were trimmed using Sickle and a minimum quality
cutoff of 5 (yielding 877,414,119 total reads)³¹. The script for interleave_fasta.py can
be found at https://github.com/jorvis/biocode/blob/master/fasta/interleave_fasta.py.
Metagenomic reads from all SIP samples were individually assembled using
IDBA-UD and the following parameters: -pre_correction, -mink 75, -maxk 105,
-step 10, -seed_kmer 55 (ref. 32). This yielded a total of 2,739,076 scaffolds from all
three SIP enrichments. The minimum and maximum scaffold length ranged from
200 to 130,027 bp.

Metagenomic binning was performed on assembled SIP enrichments using
tetranucleotide frequencies on scaffolds with a length ≥4,000 bp (including a total of
4,874 scaffolds)³³. The resulting emerging self-organizing maps (ESOMs) were
manually sorted and curated (Supplementary Fig. 1)³³. Metagenomic binning was
enhanced by incorporating reference genomes as genetic signatures for the
assembled contigs into ESOMs^{33,34}. In this way we assembled 7 high-quality and 17
fragmentary metagenomic bins (completeness threshold of 50%). The seven high-
quality genomes showed a completeness ranging from 52–95%, included 1,492/4,874
scaffolds with a scaffold length between 4,006 and 257,386 bp. The 17 fragmentary
genomes displayed a completeness below 50% and comprised 2,737/4,874 scaffolds
that ranged from 4,000 to 44,889 bp. Additionally, 645/4,874 scaffolds remained
unbinned (4,002–77,080 bp length). After binning, draft genomes were linked to the
SIP enrichments based on their unique scaffold ID. CheckM was used to evaluate the
accuracy of the binning approach by determining the percentage of completeness
and contamination (Supplementary Table 1)³⁵. Contaminants that were identified
based on their phylogenetic placement (wrong taxonomic assignment compared to
the average taxonomic assignment of the genes assigned to each bin; see section on
taxonomic assignment), GC content (>25% difference compared to the mean of all
scaffolds assigned to each bin) or confidence level (below 0.5) were manually
removed from the bins.

Gene calling, taxonomic assignment and functional characterization. Gene
calling and taxonomic assignment of the three SIP enrichments and seven
high-quality draft genomes were performed using the Joint Genome
Institute–Production Genomics Facility (JGI-PGF) Integrated Microbial Genomes
with Microbiome (IMG/M) system. To achieve this, the complete assemblies
from the three SIP enrichments were uploaded onto the JGI server. The retrieved JGI
output was linked to the seven draft genomes using their unique scaffold IDs.
The JGI output summarizing the taxonomic assignment of all gene annotations was
used to depict the taxonomic composition as shown in Supplementary Fig. 2.
The JGI-based amino-acid sequences were used as a database for blastp searches
(as indicated in the text).

For the functional characterization of our draft genomes, a reference gene
database was assembled by downloading SEED subsystems using the network-based
SEED API (function svr_all_subsystems)³⁶. The SEED-based reference database was

1 manually curated to include only subsystems of interest by downloading the gene
2 functions and gene IDs of individual subsystems (functions svr_subsystem_roles
3 and svr_subsystem_spreadsheet), which were then used to retrieve their respective
4 fasta sequences (function svr_fasta). The SEED subsystems included are shown in
5 Fig. 2, and the numbers in parentheses include the total number of genes screened
6 per subsystem (Fig. 2 and Supplementary Data 2). Additionally, we manually
7 curated a reference database including only hydrocarbon degradation genes by
8 searching the KEGG and NCBI databases for hydrocarbon degradation pathways
9 and corresponding genes (Fig. 3). Both of these reference databases were screened
10 against annotated genes from the seven draft genomes using their JGI-derived
11 amino-acid sequences as input for blastp (e-value threshold of $1e-20$)³⁷.

12 **Phylogenetic analyses.** Phylosift was used to extract marker genes for the
13 phylogenetic placement of the assembled metagenomic bins³⁸. These marker genes
14 consist of up to 15 syntenic ribosomal protein genes that have been demonstrated to
15 undergo limited lateral gene transfer (rpl2, 3, 4, 5, 6, 14, 15, 18, 22, 24 and rpS3, 8,
16 10, 17, 19)³⁹. This gene set was derived from a reference database as detailed in
17 ref. 40. To search for ribosomal protein sequences, all metagenomic bins (fasta files)
18 were used as an input in Phylosift, which was used with default parameters.
19 Moreover, we searched NCBI to include amino-acid sequences from bacterial
20 reference strains for phylogenetic analyses. Amino-acid alignments of the individual
21 ribosomal protein genes were generated using MUSCLE and curated manually⁴¹.
22 Afterwards, the curated alignments of the ribosomal proteins were concatenated for
23 further phylogenetic analyses. In addition to analysing ribosomal proteins, we
24 employed EMIRGE to retrieve 16S rRNA genes from the three SIP enrichments⁴².
25 We therefore ran an EMIRGE analysis comparing the SILVA database against the
26 raw, short reads from the three SIP enrichments using default parameters. Retrieved
27 16S rRNA genes were aligned to the SILVA database using the ARB alignment tool
28 and were curated manually⁴³. For both the ribosomal protein and 16S rRNA gene
29 alignments, phylogenetic trees were generated using a maximum likelihood-based
30 analysis (RAxML; rate distribution models: PROTGAMMA for ribosomal proteins
31 and GTRGAMMA for 16S rRNA gene sequences)⁴⁴. Bootstrap values were
32 calculated using MrBayes with 100,000 generations of Markov chain Monte Carlo
33 (MCMC) analyses with 100 sample and print frequencies⁴⁵. As a point of
34 comparison, we included published 16S rRNA gene sequences from the plume and
35 sea surface as well as from the previous SIP experiment (16S rRNA genes from clone
36 libraries and isolated bacterial strains) for the 16S rRNA phylogenetic tree^{2,3,10,13,46}.

37 **Meta analysis.** To determine whether genes from the assembled metagenomic bins
38 in this study were enriched and active in the plume during the spill, we compared
39 our three SIP enrichments with previously published plume-derived metagenomic
40 and metatranscriptomic data sets (http://mason.eoas.fsu.edu/DWH_plume/)¹⁰. Two
41 reference databases were generated, which were compared against the published
42 metagenomic and metatranscriptomic data sets. Reference database 1 consisted of
43 DNA sequences comprising scaffolds of >2,500 bp that were derived from the three
44 SIP assemblies. Reference database 2 included amino-acid sequences from the seven
45 high-quality draft genomes. Reference database 1 (DNA sequences) was mapped
46 against the published metagenomic and metatranscriptomic data set using BWA.
47 Database 2 (amino-acid sequences) was used to search against the published
48 metatranscriptomic data using Rapsearch2 to retrieve the exact genes that mapped
49 against this data set^{47,48}. For BWA the default parameters were used and an e-value
50 cutoff of 0.001 was used for RapSearch2.

51 **Accession codes.** The genomes are available in NCBI Genbank under BioProjectID
52 PRJNA301966. The whole genome shotgun projects have been deposited under
53 accession nos. LSM000000000 (H-Mar), LSMN000000000 (N-Tha),
54 LSMO000000000 (N-Alc), LSMP000000000 (N-Alt), LSMQ000000000 (P-Cyc),
55 LSMR000000000 (P-Nep) and LSMS000000000 (P-Col). The reference numbers for
56 the original raw sequencing data are SRX1562986 (*n*-hexadecane), SRX1585241
57 (naphthalene) and SRX1586894 (phenanthrene).

58 Received 11 December 2015; accepted 29 March 2016;
59 published XX XX 2016

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Author contributions

N.D., T.G. and B.J.B. conceived this study. N.D. and B.J.B. supervised experiments and
analyses. N.D., J.A.D., K.W.S. and B.J.B. performed analyses. N.D., T.G., A.P.T. and B.J.B.
wrote the paper with contributions from all authors.

Additional information

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