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SHORT COMMUNICATION

Role of methylotrophs in the degradation of hydrocarbons during the Deepwater Horizon oil spill

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The role of methylotrophic bacteria in the fate of the oil and gas released into the Gulf of Mexico during the Deepwater Horizon oil spill has been controversial, particularly in relation to whether organisms such as *Methylophaga* had contributed to the consumption of methane. Whereas methanotrophy remains unqualified in these organisms, recent work by our group using DNA-based stable-isotope probing coupled with cultivation-based methods has uncovered hydrocarbon-degrading *Methylophaga*. Recent findings have also shown that methylotrophs, including *Methylophaga*, were in a heightened state of metabolic activity within oil plume waters during the active phase of the spill. Taken collectively, these findings suggest that members of this group may have participated in the degradation of high-molecular-weight hydrocarbons in plume waters. The discovery of hydrocarbon-degrading *Methylophaga* also highlights the importance of considering these organisms in playing a role to the fate of oil hydrocarbons at oil-impacted sites.
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The Deepwater Horizon disaster of 20 April 2010 is possibly the worst accidental maritime oil spill in the history of the oil and gas industry. Approximately 7×10^5 tonnes of crude oil and gas were released into the Gulf of Mexico over a period of 83 days (Reddy *et al.*, 2012). The spill was unprecedented by virtue of the depth at which it occurred and formation of a deepwater oil plume that became entrained at ~1000–1300 m within the water column and which spanned an impressive $35 \times 2 \times 0.2$ km (Camilli *et al.*, 2010; Diercks *et al.*, 2010). The earliest sampling of the plume (end of May 2010) revealed the dominance of a specific cluster within the *Oceanospirillales*—designated DWH *Oceanospirillales*—that constituted up to 90% of total bacterial 16S rRNA gene clone (Hazen *et al.*, 2010) and pyrosequencing (Yang *et al.*, 2014) libraries. By early June, the plume community had become dominated by members affiliated to *Cycloclasticus* and *Colwellia* (Redmond and Valentine, 2012; Yang *et al.*, 2014). Collectively, these microbial community 'snap-shots', thanks to opportune research cruises that ventured into the Gulf during the spill, have hitherto provided a temporal timeline on the plume microbial dynamics. A contentious and largely unresolved issue, however, relates to the role that methylotrophs had played in the fate of the oil and gas.

During the active phase of the spill (20 April to 15 July), methylotrophic bacteria were not detected near the leaky wellhead or plume (Hazen *et al.*, 2010; Valentine *et al.*, 2010; Yang *et al.*, 2014), whereas these organisms were reported to account for at least 5% of the total bacterial community after the spill (Kessler *et al.* (2011) reported 5–36% in September 2010; Yang *et al.* (2014) reported 0.23–6% and 2% in September 2010 and October 2010, respectively). Kessler *et al.* (2011) postulated that methylotrophs, mainly *Methylophaga*, had contributed to the consumption of methane released during the spill; their involvement was inferred in part from the high numbers of methylotrophs detected in plume waters almost 3 months after capping of the leaky well, and which were thought to be remnants of a July bloom of methane-consuming bacteria (Kessler *et al.*, 2011). This interpretation, however, was severely contested by Joye *et al.* (2011), in part by the fact that the role of *Methylophaga* in methane oxidation remains unsubstantiated as these organisms are not recognized for carrying out methane oxidation or being capable of growth on methane as a carbon and energy source. The enrichment of methylotrophs detected during this post-spill period (September to October 2010)

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appears more likely associated with an eukaryotic phytoplankton bloom that was detected during August 2010 in the vicinity of the spill site (Hu *et al.*, 2011). Phytoplankton blooms can produce large quantities of extracellular high-molecular-weight dissolved organic matter (Biddanda and Benner, 1997) that acts as a rich source of methylated sugars (Panagiotopoulos *et al.*, 2013) exploitable by planktonic bacteria (Baines and Pace, 1991) such as methylotrophs (McCarren *et al.*, 2010). The observed enrichment of these organisms near the Deepwater Horizon spill site during the late summer of 2010 may have thus been associated with a role as terminal degraders of phytoplankton-derived dissolved organic matter (Yang *et al.*, 2014).

Whereas a few studies have reported the enrichment of *Methylophaga* spp. in oil-contaminated field samples and laboratory experiments with oil (Vila *et al.*, 2010 and references therein), the ability of any member of this genus to degrade hydrocarbons has hitherto remained unsubstantiated. The known substrate spectrum for the *Methylophaga* group is confined exclusively to C_1 sources (methanol, methylamine, dimethylsulfide) as sole carbon and energy sources, with the exception of some species that are also capable of utilizing fructose (Janvier and Grimont, 1995). Therefore, the notion of these organisms to have contributed to the degradation of high-molecular-weight hydrocarbons during the Deepwater Horizon spill has, understandably, been brushed aside—at least until now.

Recent work by our group using DNA-based stable-isotope probing with uniformly labeled [^{13}C]*n*-hexadecane, coupled with cultivation-based methods, has uncovered for the first time members of *Methylophaga* with the ability to utilize *n*-hexadecane (Mishamandani *et al.*, 2014; Figure 1). Although *Methylophaga* were detected only in very low abundance within the plume (<0.1% of total 16S rRNA gene sequences in pyrosequencing libraries), these organisms were actually not metabolically 'procrastinating'. A recent report assessing the transcriptional response of water column bacterial communities to the spill revealed that about 1/3 (out of 50) taxa identified in the plume had significantly higher numbers of transcripts (up to 2 orders of magnitude) relative to their levels in non-plume waters (Rivers *et al.*, 2013). All these taxa belonged to the Gammaproteobacteria and included *Methylophaga* (represented by three operational taxonomic units; Table 1) that were reported in a heightened state of metabolic activity, possibly linked to their consumption of methanol produced from methanotrophic oxidation of methane (Rivers *et al.*, 2013). During crude oil enrichment experiments with natural seawater (Mishamandani *et al.*, 2014) and with a non-axenic cosmopolitan marine diatom (Mishamandani *et al.*, unpublished results), we had observed a rapid (within 72 h) and short-lived bloom of *Methylophaga*. Taken collectively, we hypothesize that a bloom of *Methylophaga* with hydrocarbon-degrading qualities may have occurred, but had been missed, during the initial stages (first couple weeks) following the DWH blowout.

The discovery of *Methylophaga* with the capacity to degrade hydrocarbons not only expands the paradigm beyond C1-specific metabolism for this group of methylotrophs, but also adds another dimension to the role these organisms may have had in the fate of the oil during the Gulf

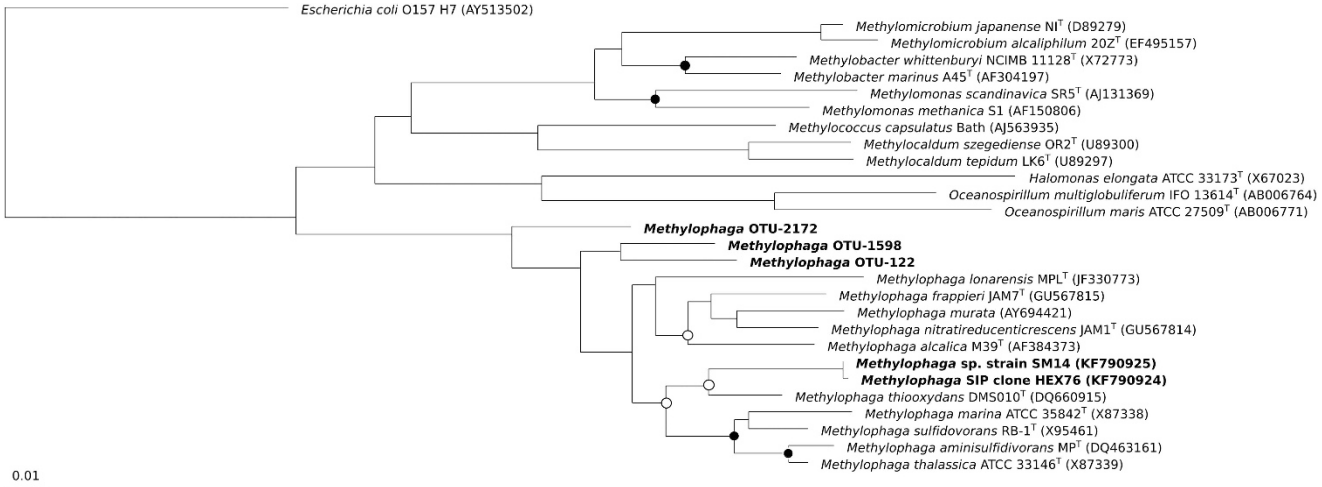

Figure 1 Phylogenetic tree of *Methylophaga* operational taxonomic units (OTUs) 122, 1598 and 2172 identified in oil-contaminated plume waters at Deepwater Horizon (Rivers *et al.*, 2013) and the hydrocarbon-degrading SIP clone HEX76 and isolated strain SM14 identified in a North Carolina surface seawater sample (Mishamandani *et al.*, 2014). These sequences are shown in bold along with related type strains of *Methylophaga* and other methylotrophs in the class Gammaproteobacteria from the SILVA SSU NR 111 NR database (Quast *et al.*, 2013). GenBank accession numbers are in parentheses. The tree was constructed using the neighbour-joining algorithm. Nodes with bootstrap support of at least 65% (○) and 90% (●) are marked (1000 replications). *Escherichia coli* 0157 H7 (AY513502) was used as the outgroup. The scale bar indicates the difference of number of substitutions per site.

Table 1 *Methylophaga* OTUs 122, 1598 and 2172 in plume waters during the active phase of the Deepwater Horizon oil spill (Rivers *et al.*, 2013) and their similarity, based on 16S rRNA gene sequencing, to *Methylophaga* strain SM14

OTU	*Closest BLASTn match*[a]	*% ID to strain SM14*[b]
122	*Methylophaga nitratireducenticrescens* (97%; GU567814)	96.0
1598	*Methylophaga thalassica* (96%; NR036802)	96.3
2172	*Methylophaga frappieri* (96%; GU567815)	94.5

Abbreviation: OTU, operational taxonomic unit.
[a]Results are to the closest type strain; percentage similarity and accession number shown in parentheses.
[b]% ID, percentage identity based on 16S rRNA gene sequencing.

spill—specifically the possibility that these organisms had contributed to a hydrocarbon-degradation cascade.

Conflict of Interest

The authors declare no conflict of interest.

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References

Baines SB, Pace ML. (1991). The production of dissolved organic matter by phytoplankton and its importance to bacteria: Patterns across marine and freshwater systems. *Limnol Oceanogr* **36**: 1078–1090.

Biddanda B, Benner R. (1997). Carbon, nitrogen, and carbohydrate fluxes during the production of particulate and dissolved organic matter by marine phytoplankton. *Limnol Oceanogr* **42**: 506–518.

Camilli R, Reddy CM, Yoerger DR, Van Mooy BAS, Jakuba MV, Kinsey JC *et al.* (2010). Tracking hydrocarbon plume transport and biodegradation at deepwater horizon. *Science* **330**: 201–204.

Diercks A-R, Highsmith RC, Asper VL, Joung DJ, Zhou Z, Guo L *et al.* (2010). Characterization of subsurface polycyclic aromatic hydrocarbons at the Deepwater Horizon wellhead site. *Geophys Res Lett* **37**: L20602.

Hazen TC, Dubinsky EA, DeSantis TZ, Andersen GL, Piceno YM, Singh N *et al.* (2010). Deep-sea oil plume enriches indigenous oil-degrading bacteria. *Science* **330**: 204–208.

Hu C, Weisberg RH, Liu Y, Zheng L, Daly KL, English DC *et al.* (2011). Did the northeastern Gulf of Mexico become greener after the Deepwater Horizon oil spill? *Geophys Res Lett* **38**: L09601.

Janvier M, Grimont PAD. (1995). The genus *Methylophaga*, a new line of descent within phylogenetic branch γ of *Proteobacteria*. *Res Microbiol* **146**: 543–550.

Joye SB, Leifer I, MacDonald IR, Chanton JP, Meile CD, Teske AP *et al.* (2011). Technical comment on 'A persistent oxygen anomaly reveals the fate of spilled methane in the deep Gulf of Mexico'. *Science* **332**: 1033–1034.

Kessler JD, Valentine DL, Redmond MC, Du M, Chan EW, Mendes SD *et al.* (2011). A persistent oxygen anomaly reveals the fate of spilled methane in the deep Gulf of Mexico. *Science* **331**: 312–315.

McCarren J, Becker JW, Repeta DJ, Shi Y, Young CR, Malmstrom RR *et al.* (2010). Microbial community transcriptomes reveal microbes and metabolic pathways associated with dissolved organic matter turnover in the sea. *Proc Natl Acad Sci USA* **107**: 16420–16427.

Mishamandani S, Gutierrez T, Aitken MD. (2014). DNA-based stable isotope probing coupled with cultivation methods implicates *Methylophaga* in hydrocarbon degradation. *Front Microbiol* **5**: 76.

Panagiotopoulos C, Repeta DJ, Mathieu L, Rontani J-F, Sempéré . (2013). Molecular level characterization of methyl sugars in marine high molecular weight dissolved organic matter. *Mar Chem* **154**: 34–45.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P *et al.* (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* **41**: D590–D596.

Reddy CM, Arey JS, Seewald JS, Sylva SP, Lemkau KL, Nelson RK *et al.* (2012). Composition and fate of gas and oil released to the water column during the Deepwater Horizon oil spill. *Proc Natl Acad Sci USA* **109**: 20229–20234.

Redmond MC, Valentine DL. (2012). Natural gas and temperature structured a microbial community response to the *Deepwater Horizon* oil spill. *Proc Natl Acad Sci USA* **109**: 20292–20297.

Rivers AR, Sharma S, Tringe SG, Martin J, Joye SB, Moran MA. (2013). Transcriptional response of bathypelagic marine bacterioplankton to the Deepwater Horizon oil spill. *ISME J* **7**: 2315–2329.

Valentine DL, Kessler JD, Redmond MC, Mendes SD, Heintz MB, Farwell C *et al.* (2010). Propane respiration jump-starts microbial response to a deep oil spill. *Science* **330**: 208–211.

Vila J, Nieto JM, Mertens J, Springael D, Grifoll M. (2010). Microbial community structure of a heavy fuel oil-degrading marine consortium: linking microbial dynamics with polycyclic aromatic hydrocarbon utilization. *FEMS Microbiol Ecol* **73**: 349–362.

Yang T, Nigro LM, Gutierrez T, D'Ambrosio L, Joye SB, Highsmith R *et al.* (2014). Pulsed blooms and persistent oil-degrading bacterial populations in the water column during and after the Deepwater Horizon blowout. *Deep-Sea Res II*; doi:org/10.1016/j.dsr2.2014.01.014.