LETTER



## Simulations predict microbial responses in the environment? This environment disagrees retrospectively

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In their recent study, Hu et al. (1) simulated in the laboratory the deep-sea oil plume of the *Deepwater Horizon* (DWH) disaster, and claim successful enrichment of the dominant oil-degrading bacteria found in the environment. Although the simulation offers valuable insights into microbial succession patterns following the addition of oil, our analysis revealed that the dominant hydrocarbon-degrading bacteria in the simulation was not in the environment, and the one in the environment was not in the simulation.

The disaster in the Gulf of Mexico led to the formation of a deep-sea oil plume in which the first bacterial responders linked to oil degradation were affiliated with the family Oceanospirillaceae (2-4). Cultivation efforts failed to isolate members from these early responders; however, a population genome reconstructed from the oil plume is available (5). In their simulation, Hu et al. (1) reconstructed various population genomes, including an abundant Oceanospirillaceae population. The authors named this population genome "Candidatus Bermanella macondoprimitus," and claim that they were able to enrich "the dominant hydrocarbon-degrading organism that was detected in the initial stage of the DWH [oil] plume" (1), which was missing in previous simulations (6). However, the 16S rRNA gene of Candidatus B. macondoprimitus does not match perfectly to any amplicons from the oil plume (Fig. 1A), and Hu et al. (1) present no evidence to link the simulation to the environment beyond genus-level taxonomic community profiles.

Using genome-wide quantitative read recruitment from metagenomes and metatranscriptomes generated from the oil plume (3), we here demonstrate that

Candidatus B. macondoprimitus was not a dominant member of the oil plume early responders (Fig. 1). In fact, we could not find any evidence for its presence in the oil plume, as its genomic content remained largely undetected (Fig. 1B). Most strikingly, alkB, the only hydrocarbon degradation gene Hu et al. (1) found in Candidatus B. macondoprimitus did not recruit any of the nearly half a billion reads from the oil plume. We then investigated whether DWH Oceanospirillales desum, the highly abundant and active population genome recovered from the oil plume (5), was enriched in the simulation. We found no evidence for its detection (Fig. 1). While the 16S rRNA genes of the two population genomes were 98.9% identical, they only shared an average nucleotide identity of 85.4% over the ~0.3 Mbp of alignment, exposing a substantial gap between the initial microbial response in the environment and simulation. Despite this, the simulation may have better represented later stages of the oil degradation in the environment, yet this cannot be confirmed because of the lack of time-series data from the oil plume.

In summary, based on our findings, we conclude that Hu et al.'s (1) simulation did not enrich the first microbial responders of the oil plume, and the simulation's power to draw conclusions specifically regarding oil degradation in the environment remains to be determined. Simulations are critical research tools to tease apart complex microbial responses in controlled settings, and they should benefit from available environmental omics data to substantiate claims of successful enrichment and predictive power.

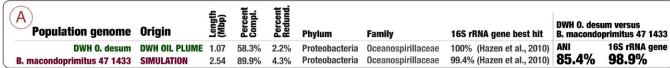
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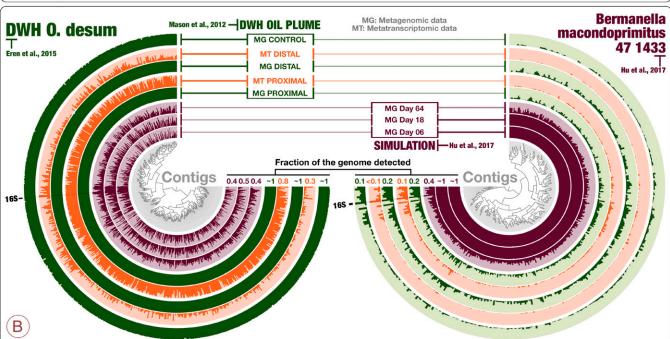
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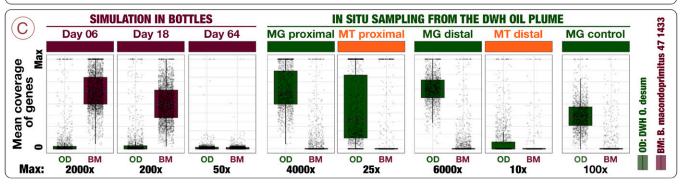


Fig. 1. DWH O. desum vs. Candidatus B. macondoprimitus in the DWH oil plume and the simulation. (A) Description of general features of the two population genomes. CheckM (7) assigned taxonomy, NucMer (8) computed the average nucleotide identity (ANI), and the National Center for Biotechnology Information's blastn measured the 16S rRNA gene similarity [see also Hazen et al. (2)]. (B) Display of the detection of contigs from the two population genomes across metagenomes and metatranscriptomes from the DWH oil plume, as well as the metagenomes from the simulation processed with anvi'o (5). Interactive versions to explore other aspects of data are available at <a href="https://anvi-server.org/merenlab/dbm\_o\_desum">https://anvi-server.org/merenlab/dbm\_o\_desum</a> and <a href="https://anvi-server.org/merenlab/b\_macondoprimitus">https://anvi-server.org/merenlab/b\_macondoprimitus</a>. Contigs are organized based on their tetranucleotide frequencies. In each layer, bars range from 0 (no detection) to 1 (when all nucleotides are covered by at least one read). Proximal, distal, and control samples were collected 1.5, 11, and 40 km from the wellhead by Mason et al. (3). The "165" selections correspond to contigs containing the 16S rRNA gene [see also Hu et al. (1)]. (C) Depiction of the mean coverage of genes in DWH O. desum (n = 1,368) and Candidatus B. macondoprimitus (n = 2,547) across the metagenomes and metatranscriptomes. Genes with less than 50% detection were considered undetected to minimize the impact of nonspecific mapping. We used Prodigal (9) to identify the genes in both population genomes, and R package ggplot2 (10) to visualize their mean coverage.

<sup>1</sup> Hu P, et al. (2017) Simulation of *Deepwater Horizon* oil plume reveals substrate specialization within a complex community of hydrocarbon degraders. *Proc Natl Acad Sci USA* 114:7432–7437.

<sup>2</sup> Hazen TC, et al. (2010) Deep-sea oil plume enriches indigenous oil-degrading bacteria. Science 330:204–208.

<sup>3</sup> Mason OU, et al. (2012) Metagenome, metatranscriptome and single-cell sequencing reveal microbial response to *Deepwater Horizon* oil spill. ISME J 6:1715–1727.

<sup>4</sup> Dubinsky EA, et al. (2013) Succession of hydrocarbon-degrading bacteria in the aftermath of the *Deepwater Horizon* oil spill in the gulf of Mexico. *Environ Sci Technol* 47:10860–10867.

**<sup>5</sup>** Eren AM, et al. (2015) Anvi'o: An advanced analysis and visualization platform for 'omics data. *PeerJ* 3:e1319.

<sup>6</sup> Kleindienst S, et al. (2015) Chemical dispersants can suppress the activity of natural oil-degrading microorganisms. Proc Natl Acad Sci USA 112:14900-14905.

<sup>7</sup> Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW (2015) CheckM: Assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 25:1043–1055.

- 8 Delcher AL, Phillippy A, Carlton J, Salzberg SL (2002) Fast algorithms for large-scale genome alignment and comparison. *Nucleic Acids Res* 30:2478–2483.
  9 Hyatt D, et al. (2010) Prodigal: Prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* 11:119.
- **10** Ginestet C (2011) ggplot2: Elegant graphics for data analysis. J R Stat Soc Ser A 174:245–246.

PNAS Early Edition | 3 of 3 Delmont and Eren