Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts

Supplementary Information

Microbial community analysis of Deepwater Horizon oil-spill impacted sites along the Gulf coast using functional and phylogenetic markers

Jessica K. Looper[1], *Ada Cotto*[1], *Byung-Yong Kim*[3], *Ming-Kuo Lee*[4], *Mark R. Liles*[5], *Sinéad M. Ní Chadhain*[6], *and Ahjeong Son*[1,2,*]

[1]Department of Civil Engineering, Auburn University, Auburn, AL, 36849 USA

[2]Department of Environmental Science and Engineering, Ewha Womans University, Seoul, Republic of Korea

[3]Department of Agricultural Microbiology, National Academy of Agricultural Science, Suwon, Republic of Korea

[4]Department of Geology and Geography, Auburn University, Auburn, AL, 36849 USA

[5]Department of Biological Science, Auburn University, Auburn, AL, 36849 USA

[6]Department of Biology, University of South Alabama, Mobile, AL, 36688 USA

*Correspondence. Address. 238 Harbert Engineering Center, Auburn University, AL 36849, USA; Email. ason@auburn.edu; Phone. +1(334) 844-6260; Fax. +1(334)844-6290

PCR and qPCR reations and thermal cycle conditions

For the *alkB* gene, a touch-down PCR was conducted which contained 2.5 µL of 1× Takara PCR buffer without $MgCl_2$, 1.5 µL of 1.5 mM $MgCl_2$, 2 µL of 0.2 mM dNTPs, 2 µL of 0.8 mM each primer, 0.25 µL of 1.25 U of Takara *Ex Taq* polymerase, and 1 µL of gDNA at 10 – 50 ng/µL. The temperature cycle consisted of: 94°C for 4 min, followed by 10 cycles of 94°C for 30 sec; 65 – 56°C for 30 sec decreasing on a 1°C increment with each cycle; 72°C for 1 min, then 22 cycles of 94°C for 30 sec; 55°C for 30 sec; 72°C for 1 min, and a final extension with 72°C for 10 min. The qPCR assay used a 25 µL reaction volume that consisted of 12.5 µL of 1× Fast SYBR Green Master Mix, 1 µL of 0.5 µM of each primer, and 0.5 µL of gDNA (0.2-1 ng/µL). The temperature cycle consisted of: 95°C for 10 min, followed by 45 cycles of 95°C for 10 sec, 56°C for 30 sec, and the subsequent disassociation analysis.

For the P450 gene, the PCR was conducted using 2.5 µL of 1× Takara PCR buffer, 2.5 µL of 2.5 mM $MgCl_2$, 2 µL of 0.2 mM dNTPs mixture, 1.25 µL of 0.5 mM each primer, 0.25 µL of 1.25 U of *Taq* polymerase, and 2 µL of gDNA (0.5-4 ng/µL). The temperature cycle consisted of: 94°C for 4 min, followed by 32 cycles of 94°C for 45 sec; 58°C for 1 min; 72°C for 1 min, and the extension with 72°C for 5 min. The qPCR assay used a 20 µL reaction volume, which consisted of 10 µL of 1× Fast SYBR Green Master, 1 µL of 0.5 µM of each primer, and 5 µL of gDNA (2-13 ng/µL). The temperature cycle consisted of: 95°C for 10 min, followed by 40 cycles of 95°C for 10 sec, 58°C for 30 sec, and 72°C for 30 sec, followed by the disassociation cycle.

The PCR reaction for the PAH-RHD_α gene consisted of 2.5 µL of 1× Takara PCR buffer without $MgCl_2$, 1.5 µL of 2.5 mM $MgCl_2$, 2 µL of 0.2 mM dNTPs, 0.5 µL of 0.2 mM of each primer, 0.25 µL of 1.25 U of Takara Ex *Taq* polymerase, and 1 µL of gDNA (1-5 ng/µL) at about 1 – 5 ng/µL. The temperature cycle consisted of: 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec, 57°C for 30 sec, and 72°C for 30 sec, and a final extension with 72°C for 5 min. The qPCR assay used a 25 µL reaction volume consisting of 12.5 µL of 1× Fast SYBR Green Master, 1 µL of 0.4 µM of each primer, and 1 µL of gDNA (0.2-2 ng/µL). The temperature cycle of the qPCR assay consisted of: 95°C for 5 min, followed by 40 cycles of 95°C for 30 sec, 58°C for 30 sec, and 72°C for 30 sec, followed by one cycle of 80°C for 10 sec and one cycle of 72°C for 7 min, finished with the disassociation cycle.

The PCR procedure for the *Pseudomonas* genus-specific 16S rRNA target consisted of 5 µL of 1× Takara PCR buffer without $MgCl_2$, 3 µL of 2 mM $MgCl_2$, 2.6 µL of 0.3 mM dNTPs, 4 µL of 0.8 mM each primer, 0.3 µL of 0.75 U of Takara *Ex Taq* polymerase, and 1 µL of gDNA (0.5-2 ng/µL). The temperature cycle consisted of: 95°C for 5 min, followed by 30 cycles of 94°C for 45 sec; 66°C for 1 min; 74°C for 1 min, and a final extension with 74°C for 10 min. The qPCR assay used a reaction volume of 20 µL consisting of 10 µL of 1× Fast SYBR Green Master Mix (Life Technologies; Grand Island, NY),

1.4 µL of 0.7 µM of each primer, and 5 µL of gDNA (0.2-2 ng/µL). The temperature cycle consisted of: 95°C for 15 min, followed by 40 cycles of 95°C for 10 sec, 65°C for 15 sec, and 72°C for 20 sec, following by a disassociation cycle of 95°C for 30 sec, 65°C for 1 min, 95°C for 30 sec.

For the PCR assay of the universal bacterial 16S rRNA gene the reaction consisted of 2.5 µL of 1× Takara PCR buffer without $MgCl_2$, 2 µL of 2 mM $MgCl_2$, 4 µL of 0.4 mM dNTPs, 2.5 µL of 1 mM each primer, 0.3 µL of 1.5 U of Takara *Ex Taq* DNA polymerase, and 1 µL of gDNA at 2 ng/µL. The temperature cycle consisted of: 94°C for 5 min, followed by 30 cycles of 94°C for 1 min; 55°C for 1 min; 72°C for 2 min, and a final extension with 72°C for 15 min. The qPCR assay used a 20 µL reaction volume consisting of 10 µL of 1× TaqMan Universal Master Mix (Life Technologies; Grand Island, NY), 0.8 µL of 0.8 µM of each primer, and 5 µL of gDNA (2-12 ng/µL). The temperature cycle consisted of: 95°C for 2 min, followed by 40 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 2 min."

The rarefaction curve of the pyrosequencing reads

The rarefaction curve was used to determine if the pyrosequencing data represented the microbial diversity found within each sample. As Figure S1 shows, the OTUs formed from the contaminated sample (BJS) represented the overall diversity within the sample leading to high percentages for the Good's coverage values. Similar patterns could be seen for the non-contaminated samples (BJN, SW), indicating that these samples not only had a low number of reads but a lower diversity compared to their high richness values. However, based on the increasing slope of the negative control sample (NCS), there were additional phylotypes within this sample that were not represented in the data. The non-contmainated samples (BD, AL) showed a similar pattern to NCS, indicating that these samples had a higher diversity.

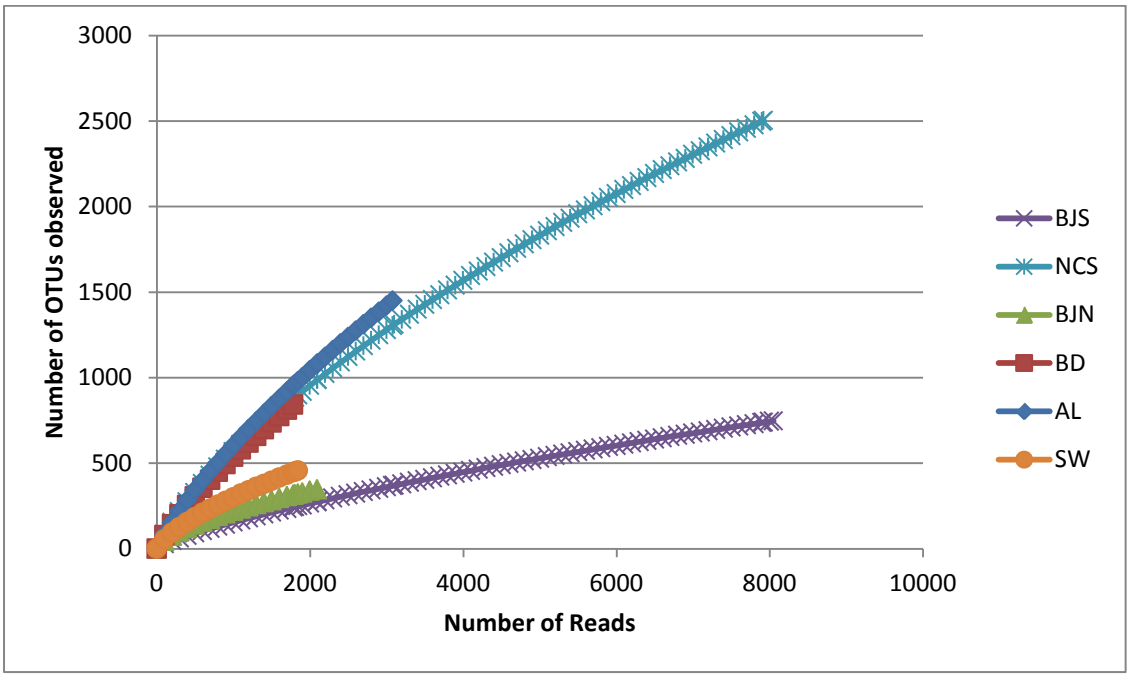

Figure S1. Rarefaction curves for the environmental samples clustering at a 97% similarity cut-off. The curves were generated using 1,000-random samplings without replacement.