# Stable Isotope and Metagenomic Profiling of a Methanogenic Naphthalene-Degrading Enrichment Culture

**Courtney R. A. Toth, Carolina Berdugo-Clavijo, Corynne M. O'Farrell, Gareth M. Jones, Andriy Sheremet, Peter F. Dunfield, and Lisa M. Gieg**

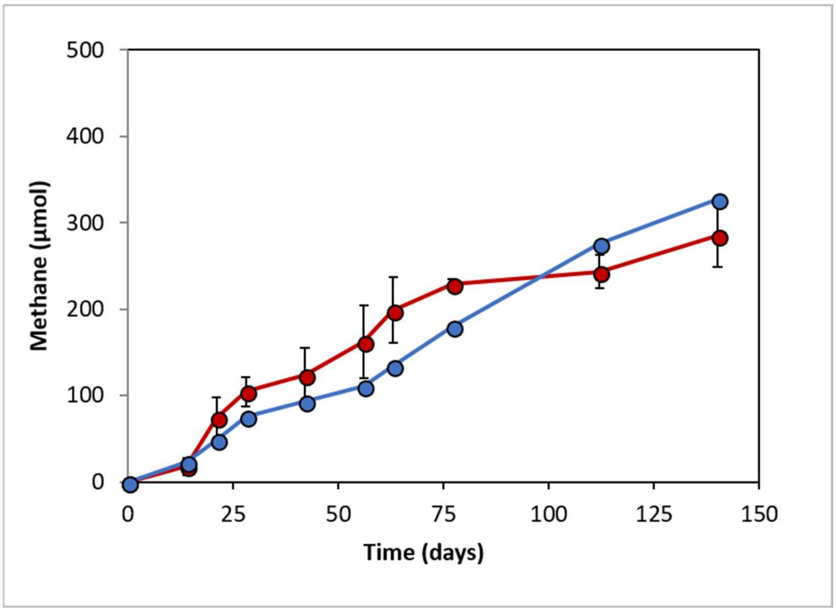

**Figure S1.** Methane production from triplicate parent NDC microcosms amended with naphthalene (blue) relative to an unamended control (red). Adapted from Berdugo Clavijo et al., 2012.

**Table S1.** Primer sets screened for the targeted detection of anaerobic functional genes in a methanogenic naphthalene-degrading enrichment culture.

| **Primer name** | | **Target gene** | **Target lineage** | **Primer sequence (5′ – 3′)** | **Expected amplicon bp** | **Amplification detected** | **References** |
|---|---|---|---|---|---|---|---|
| FAE-B | 7768f<br>8543r | *bssA* | Clostridial *bssA*, *bssA* s.l., *nmsA* | CAAYGATTTAACCRACGCCAT<br>TCGTCRTTGCCCCAYTTNGG | 775 | - | (von Netzer et al., 2013) |
| FAE-N | 7363f<br>7374f<br>8543r | *nmsA* | *nmsA* s. str. | TCGCCGAGAATTTCGAYTTG<br>TTCGAYTTGACGGACAGCGT<br>TCGTCRTTGCCCCAYTTNGG | 1180<br>1169 | - | (von Netzer et al., 2013) |
| Ncr2$_{\mathrm{for}}$<br>Ncr2$_{\mathrm{rev}}$ | | *Ncr* | *Deltaproteobacteria* (NaphS2, N57) | TGGACAAAYAAAMGYACVGAT<br>GATTCCGGCTTTTTTCCAAVT | 320 | - | (Morris et al., 2014) |
| benCarb$_{\mathrm{for}}$<br>benCarb$_{\mathrm{rev}}$ | | *abcA* | | GTGGTCTTCGCACCGTTAAT<br>TCTCCGGTAACTGGGTGTTC | 495 | - | (Fowler, 2014) |

**Table S2.** Summary of draft genomes assembled from pooled $^{13}$C-labelled NDC SIP fractions.

| Draft (meta)genome | IMG Genome ID | Genome size (bp) | Gene count | Protein coding genes with COGs (%) | Scaffolds containing ≥1 PAH-degrading gene orthologs | Scaffolds containing ≥3 PAH-degrading gene orthologs |
|---|---|---|---|---|---|---|
| $^{13}$C-enriched DNA metagenome of NDC | 3300013290 | 68705674 | 104254 | 64.54 | 804 | 73 |
| *Clostridium sp.* Bin 1 | 2724679696 | 5438132 | 5424 | 68.31 | 260 | 28 |
| *Geobacter* sp. Bin 1 | 2724679697 | 4390079 | 4104 | 56.80 | 90 | 9 |
| *Geobacter* sp. Bin 2 | 2724679723 | 3876684 | 3546 | 62.15 | 79 | 9 |
| *Geobacter* sp. Bin 3 | 2724679724 | 2526538 | 2519 | 65.58 | 58 | 7 |
| Unclassified *Rhodospirillales* Bin 1 | 2724679720 | 3473168 | 3651 | 60.81 | 88 | 2 |
| *Desulfovibrio* sp. Bin 1 | 2724679721 | 3088242 | 2959 | 65.12 | 71 | 7 |
| *Anaerolinea* sp. Bin 1 | 2724679725 | 2333788 | 2249 | 56.87 | 52 | 4 |
| *Anaerolinea* sp. Bin 2 | 2724679726 | 2271625 | 2153 | 57.50 | 34 | 4 |
| *Sphaerochaeta* sp. Bin 1 | 2724679727 | 1750954 | 1956 | 51.74 | 41 | 0 |
| *Methanosaeta* sp. Bin 1 | 2724679722 | 2683322 | 2798 | 58.26 | 31 | 3 |

## References

1. Berdugo-Clavijo, C.; Dong, X.; Soh, J.; Sensen, C.W.; Gieg, L.M. Methanogenic biodegradation of two-ringed polycyclic aromatic hydrocarbons. *FEMS Microbiol. Ecol.* **2012**, *81*, 124–133.
2. Fowler, S.J. Syntrophic hydrocarbon metabolism under methanogenic conditions. PhD Thesis. University of Calgary, Calgary, Alberta, 2014.
3. Morris, B.E.; Gissibl, A.; Kummel, S.; Richnow, H.H.; Boll, M. A pcr-based assay for the detection of anaerobic naphthalene degradation. *FEMS Microbiol. Lett.* **2014**, *354*, 55–59.
4. von Netzer, F.; Pilloni, G.; Kleindienst, S.; Kruger, M.; Knittel, K.; Grundger, F.; Lueders, T. Enhanced gene detection assays for fumarate-adding enzymes allow uncovering of anaerobic hydrocarbon degraders in terrestrial and marine systems. *Appl. Environ. Microbiol.* **2013**, *79*, 543–552.