

Microbial toluene removal in hypoxic model constructed wetlands occurs predominantly via the ring monooxygenation pathway

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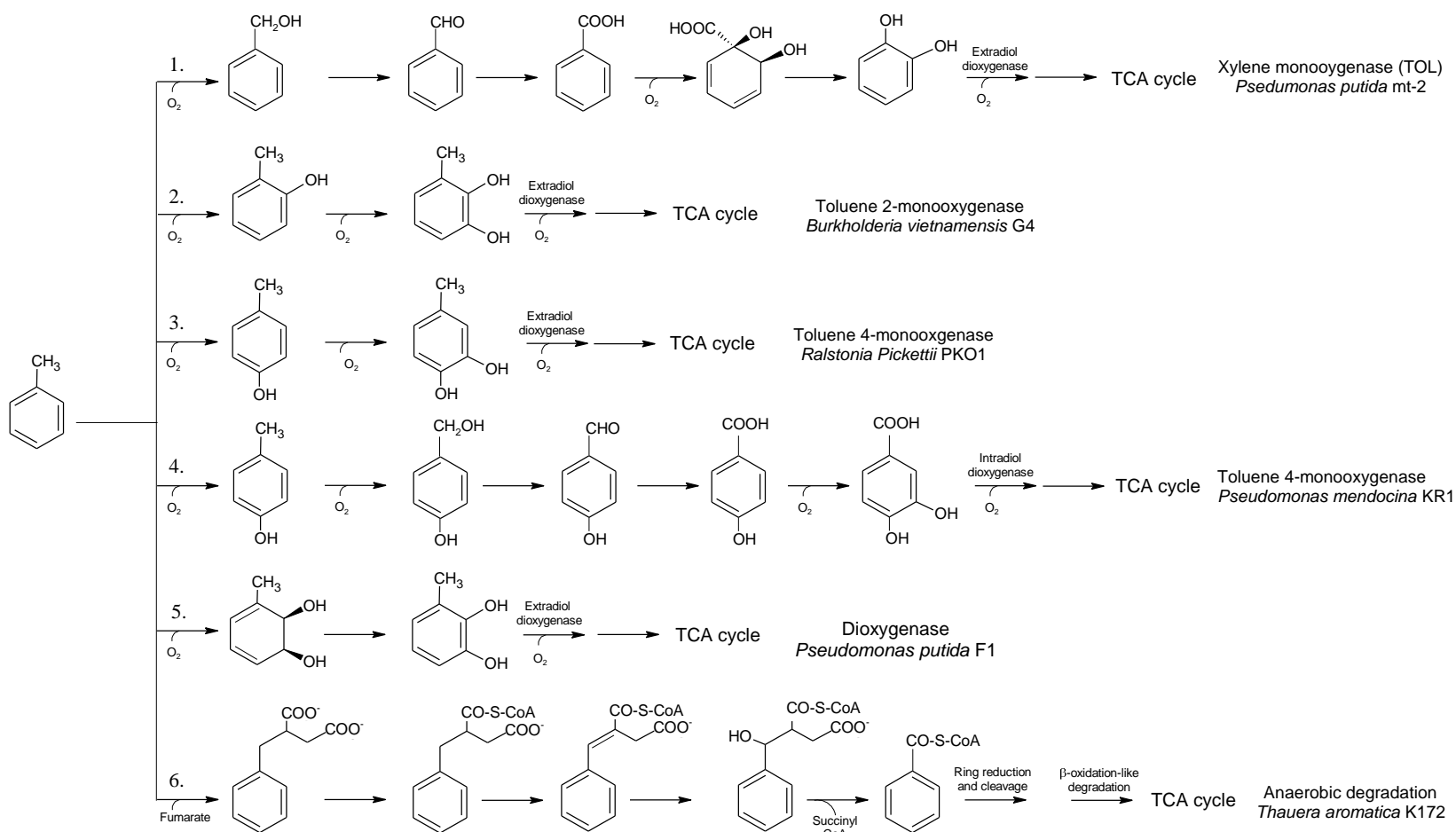


Fig. S1. Schematic representation of aerobic and anaerobic toluene degradation pathways in bacteria. The generic name of the pathway received according to the enzyme catalyzing the first step of the degradation and a model bacterial strain containing the respective pathway is given (Modified from (1)). The catabolic microarray showed that genes belonging to pathways number 1 and 3 were present in PFR-1 and pathways 3 and 6 were present in PFR-2.



Fig. S2. Planted Fixed-bed Reactor (PFR). The reactor consists of a cylindrical glass vessel (diameter: 30 cm; height: 30 cm) containing a metal basket (diameter: 26 cm; height: 28 cm) filled with approximately 20 kg of gravel. The depicted reactor is planted with soft rush (*Juncus effusus*).

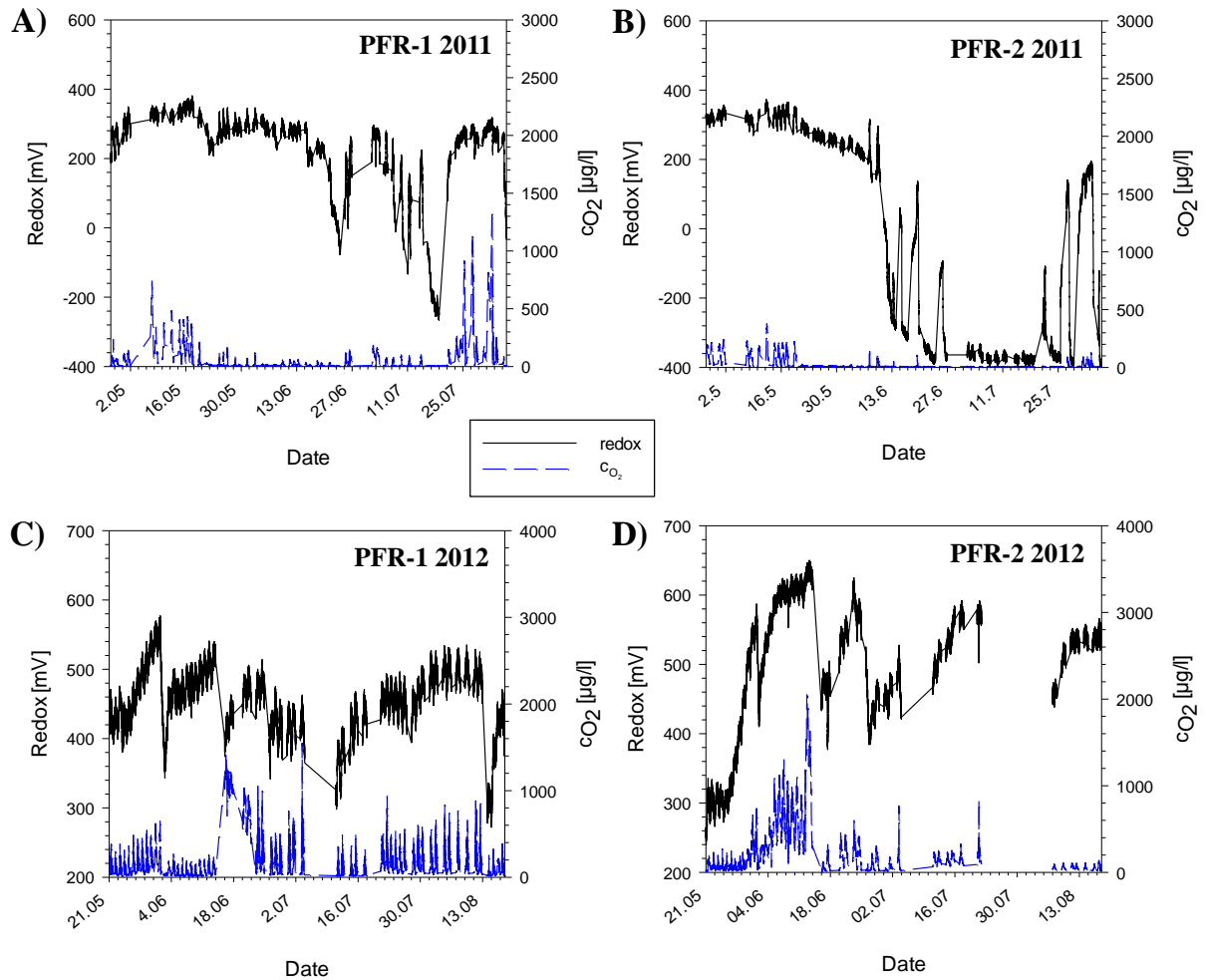


Fig. S3. Redox state and the oxygen concentrations inside the PFR-1 (A and C) and PFR-2 (B and D) monitored during the months of higher photosynthetic plant activity. The redox and dissolved oxygen concentration were constantly measured in the circulation flow and automatically recorded. From 22.07-06.08.2012 no registration of data was performed due to broken electrodes (Panel D).

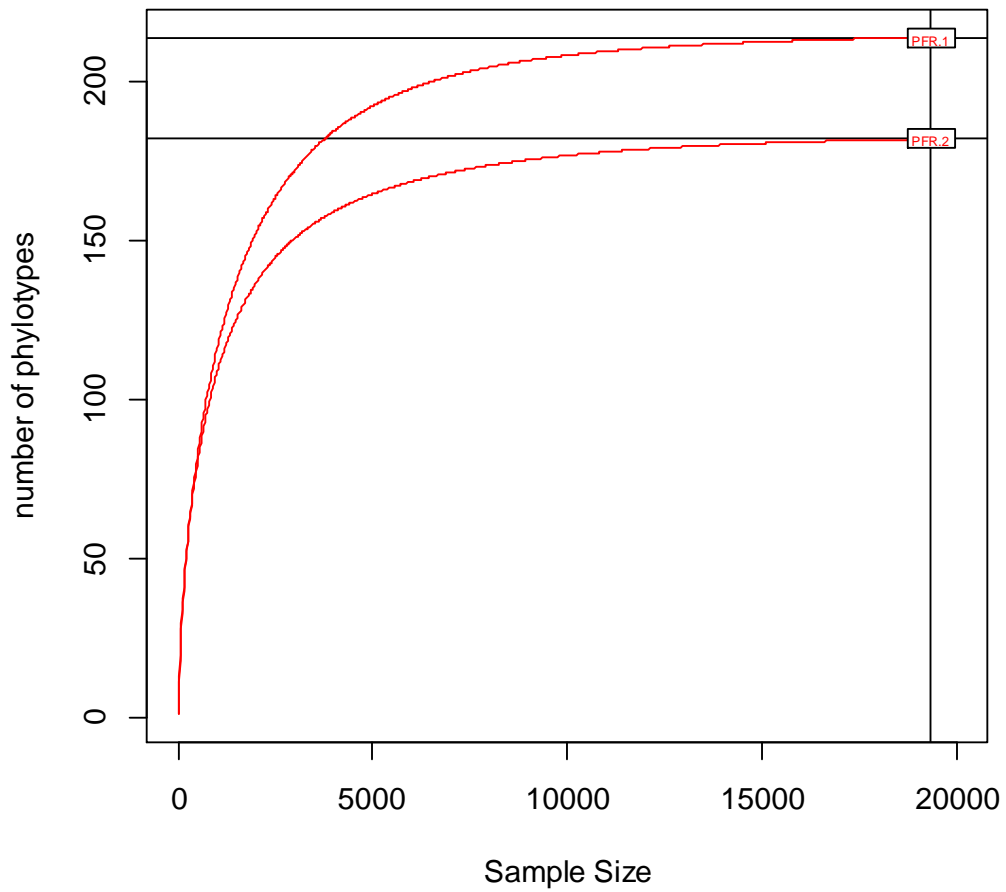


Fig. S4. Rarefaction curves of PFR-1 and PFR-2. The rarefaction curves show the species number against the sampling size for each PFR sampled.

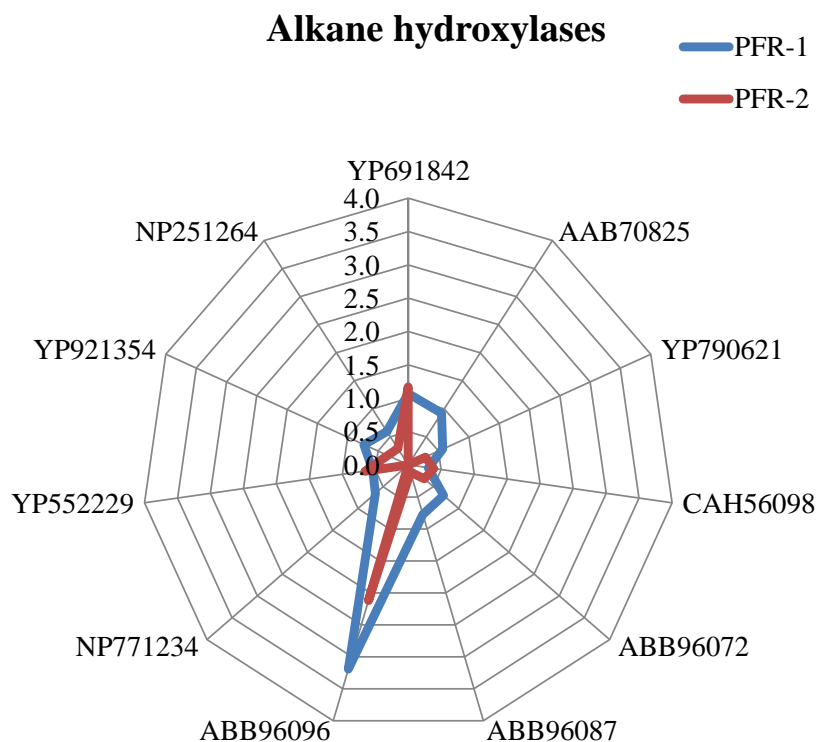


Fig. S5. Abundance of integral-membrane alkane hydroxylases present in PFR-1 and PFR-2 given as normalized intensity. YP_691842: alkane-1 monooxygenase *Alcanivorax borkumensis* SK2; AAB70825: xylene monooxygenase hydroxylase component *Pseudomonas putida*; YP_790621: Alkane-1 monooxygenase *Pseudomonas aeruginosa* UCBPP-PA14; CAH56098: putative alkane hydroxylase Uncultured bacterium; ABB96072, ABB96087, ABB96096: putative alkane monooxygenase Uncultured bacterium; NP_771234: delta 9 acyl-lipid fatty acid desaturase *Bradyrhizobium diazoefficiens* USDA 110; YP_552229: alkane 1-monooxygenase *Polaromonas* sp. JS666; YP_921354: alkane 1-monooxygenase *Nocardioides* sp. JS614; NP_251264: alkane-1 monooxygenase *Pseudomonas aeruginosa* PAO1.

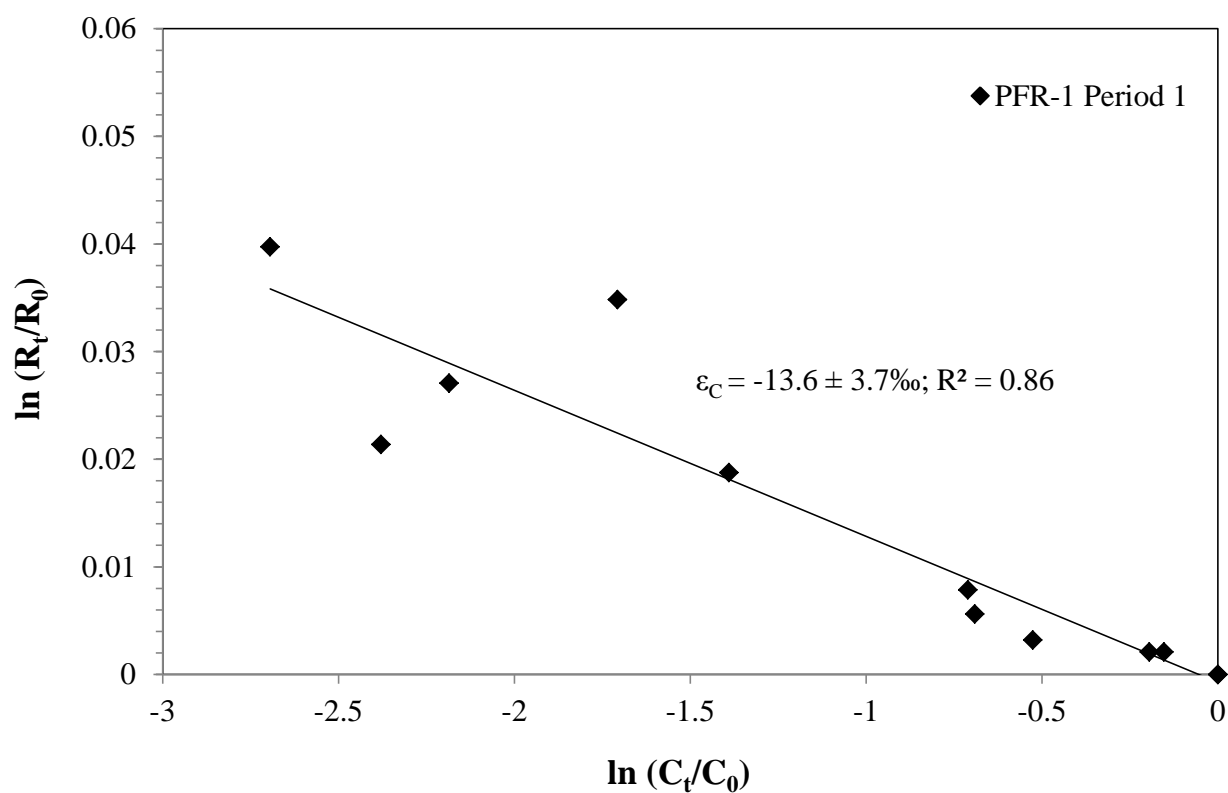


Fig. S6. Double logarithmic plot according to the Rayleigh equation of the hydrogen isotopic composition versus the residual concentration of toluene for PFR-1 during the Period 1, after the first injection pulse of toluene inside the reactor.

Table S1. Description of aerobic isolates representatives obtained from PFRs.

Isolate	Identification (GeneBank N°)	Closest relative ^a (% Identity)	Catabolic genes (GeneBank N°)	Closest relative ^c (% Identity)
AET-5-01, AET-5-2a, AET-5-3b, AET-5-47b	<i>Pseudomonas</i> sp. (KR902601, KR902602, KR902603, KR902604)	<i>Pseudomonas monteilii</i> strain LZU-9 (100)	Xylene monooxygenase (KT021789) Extradiol dioxygenase ^b	xylene monooxygenase <i>Pseudomonas putida</i> (100)
AET-5-VIII, AET-5-3 AET-6-XI, AET-6-18	<i>Pseudomonas</i> sp. (KR902606, KR902605, KR902609, KR902608)	<i>Pseudomonas putida</i> strain YN3 (100)	Toluene dioxygenase (KT021793) Extradiol dioxygenase (KT021794)	toluene dioxygenase <i>Pseudomonas</i> sp. KA-08 (100) 3-methylcatechol 2,3-dioxygenase <i>Pseudomonas putida</i> group (100)
AET-6-14	<i>Ralstonia</i> sp. (KR902607)	<i>Ralstonia</i> sp. DT 4-07 (100)	Toluene monooxygenase (KT021790) Phenol monooxygenase (KT021791) Extradiol dioxygenase (KT021792)	toluene-4-monooxygenase <i>Ralstonia pickettii</i> PKO1(99) methane/phenol/toluene hydroxylase <i>Ralstonia pickettii</i> 12D (100) catechol-2,3-dioxygenase <i>Ralstonia pickettii</i> 12D (97)

^aClosest relative according to 16S rRNA gene (blastn)

^bDNA of the isolate AET-5-01 showed a positive reaction with Exdo-A primers (Brenerova et al., 2009). However, it was not possible to retrieve a clean sequence to describe this gene.

^cClosest relative of nucleotide catabolic gene sequence against protein database (blastx)

Table S2. Primer sequences designed and used in this study to detect catabolic genes present in aerobic isolates through qPCR analysis.

Primer name	Sequence (5'-3')	Target gene (abbreviation ^a)	Annealing T (°C)	qPCR Efficiency (%)	Reference
Nad-F Nad-R	TCCTACGGGAGGCAGCAGT GGACTACCAGGGTATCTAATCCTGTT	16S rRNA Eubacteria	57	91	(2)
xyIM-AET01-F xyIM-AET01-R	GTGATCCGAACCGAGACATTG GCCTCAATCTTTATCGCATCTTTG	xylene monooxygenase isolate AET-5-01 (<i>xyIM</i>)	55	94	This study
TOD-AET18-F TOD-AET18-R	GCTACCACGGGTGGGCTTACG GCGGGCTCCATTCCTTCTGTTC	toluene dioxygenase AET-5-VIII (<i>tod</i>)	58	101	This study
EXDO-AET18-F EXDO-AET18-R	GCAAGCTAATGGCATGGATGAC GGCGTGGCACCGTAGAAC	catechol-2,3-dioxygenase isolate AET-5-VIII (<i>exdo-AET-18</i>)	56	99	This study
TMO-AET14-F TMO-AET-14-R	GGATCGTCGGGCAGTTTGAAC GTGCTGGTAGTCGAACCTCGTTG	toluene monooxygenase isolate AET-6-14 (<i>tmo</i>)	56	99	This study
PHE-AET14-F PHE-AET14-R	ACCTGTGTTCGTGCCGTTC ATCCTGCTCCAGCATGAACCTG	phenol hydroxylase isolate AET-6-14 (<i>phe</i>)	58	92	This study
EXDO-AET14-F EXDO-AET14-R	GAGGTGCTGGGCTTCTTC ACGTCGTGCCATGAATCC	catechol-2,3-dioxygenase isolate AET-6-14 (<i>exdo-AET-14</i>)	60	93	This study

^a This abbreviation is used to report the qPCR results

Table S3. Nucleotide sequence and description of catabolic gene probes hybridizing with PFRs DNA.

ID Gene Bank number	Nucleotide sequence	Description
Mono- and Di-oxygenases		
YP_001110001	TGGGAGATGTACGTGCGAGCAGAACGGCGCGCGTGTTC AAGGATCTTGC	methane/phenol/toluene hydroxylase <i>Burkholderia vietnamiensis</i> G4
AAL50373	CTAGAACAGGATCCGGACAACGTGCCCATCGTGCAGCGTGGATCGACAA	TomA3 <i>Burkholderia cepacia</i>
NP_251202	GCCACCGTGCAGCACCCGAGCAGGTCGAGGCGGAGCGCGCGCGTGGC	anthranilate dioxygenase large subunit <i>Pseudomonas aeruginosa</i> PAO1
BAB21463	CGAAGGGCGAGAGCGCCGAGAACCGCCACGCGCATCCGTCAGTACGAA	chlorobenzoate 1,2-dioxygenase <i>Burkholderia</i> sp. NK8
YP_587013	GCGCTGGGCTAACC CGAAGATCGTCCGAACCTCGCCCGCCGCGAGGT	benzoate 1,2-dioxygenase <i>Cupriavidus metallidurans</i> CH34
NP_887278	CGAGCGCCGAGGTGGGCGTCAAGGGCGCGCGCAACAGGCTGGAATAC	hydroxylating alpha subunit of a dioxygenase system <i>Bordetella bronchiseptica</i> RB50
Extradiol dioxygenases		
BAD72667	AGCATGACCGACACCTCGATCGACATCGGCCGACCCGCCACGGCCTGAC	catechol 2,3-dioxygenase Uncultured bacterium
BAD83302	TGGGCTTCAACCTGGCCGAGCAGGTTGTGCGCGTCCGACAAGCTGCAG	catechol 2,3-dioxygenase Uncultured bacterium
BAD72738	GAAGAAGAAGGTGTCGCTCGATCTCGGCCGACCCGCCACGGCATCACCC	catechol 2,3-dioxygenase Uncultured bacterium
BAH89638	AGGCCCGGGCAGTGAGCATCCGGTGTGACATGGACGAGGCGCTGTT	catechol 2,3-dioxygenase Uncultured bacterium
AAW81687	GGGCGTGAAGAGGTCAACCCAGAGCCTGGCCCGCGATCTGAAGGGTA	catechol 2,3-dioxygenase <i>Pseudomonas fluorescens</i> PC36
AAW81688	GATTTCTATCACGACCGTGTGCTTACCGAGAGTTTCTGACCGTGATGA	catechol 2,3-dioxygenase <i>Pseudomonas fluorescens</i> PC37
AAS75778	GTGCGATGCTGCAGTTCAATCTGCCGACGGCCATGAGATGCGCCTGTAC	catechol 2,3-dioxygenase <i>Comamonas testosteroni</i> CC F3
BAH90217	AGGCGCATCTCGTCCCGTGGGCGCCTGAATGCAGCATGCGGCCGCT	2,3-dihydroxybiphenyl-1,2-dioxygenase Uncultured bacterium
BAH90326	TGGCCGCTGGGCGAGGTGAACCTGCAGCATGCGGCCGCTGGAGGGCAGCT	catechol 2,3-dioxygenase Uncultured bacterium
JE0127	GACCCGAAGGCAACATGCAGGCGCGCACCTGGATGGCCCGCACCCACAG	catechol 2,3-dioxygenase (EC 1.13.11.2) <i>Pseudomonas</i> sp. Y 2
BAH90119	TGCTTGCCGAACGTGAGCACGAGCGCGCGCGCGCTCTTCCACACCTT	2,3-dihydroxybiphenyl-1,2-dioxygenase Uncultured bacterium
BAA25604	CCTGTCTCATGCGTCAAGCCGCTCGCAGCGGGCACGCCGCTGGAACGGAT	2,3-dihydroxybiphenyl 1,2-dioxygenase <i>Rhodococcus erythropolis</i> TA421
ACG59214	ACACCGTCAGGGTTTGAGCTTGAGTTTGGTTGGGGCGCGCTGAGGTCA	3-isopropylcatechol-2,3-dioxygenase-like protein Uncultures bacterium
Anaerobic toluene degradation		
BAD42366	TGGCCAAGTCGCACATCGAACAGGCCAGCCGAGATGGGCGCGTTCGCCCT	putative benzylsuccinate synthase alpha subunit <i>Magnetospirillum</i> sp. TS-6
AAx84174	ACCAGCCAGTGC GCGTGGGGTCCGGCCAGTTCTCGAGAACATCGCGCG	putative benzoyl-CoA reductase <i>Thaueria aromatica</i>
Alkane Hydroxylases		
YP_691842	ACTGCTTTGGGGTTGGCAGGGGGTGGTGTCTTCTTCTGGGGCAGAGTTTCT	alkane-1 monooxygenase <i>Alcanivorax borkumensis</i> SK2
AAB70825	AGCGGGATGGCCACTGTGGTGTGAAAGTGAAC TGGGGCGGGCGGGCTAG	xylene monooxygenase hydroxylase component <i>Pseudomonas putida</i>
YP_790621	GTGTGGGCCGCTGGCGTGTTCGTCGCTTCCAGGAGTGGGGCTGGCTCGG	Alkane-1 monooxygenase <i>Pseudomonas aeruginosa</i> UCBPP-PA14
CAH56098	CGCCTCGCTTCGGTGAGAGTTTCTGGACTTCC TGGCCGCGAGTGTGTGG	putative alkane hydroxylase Uncultured bacterium
ABB96072	TCAACGCTGGCTGATGTCGGTGTCTCTTCCGGG TGTGCTCGTGGTGTG	putative alkane monooxygenase Uncultured bacterium
ABB96087	CGCCTGGGCGATGTCGGTGGTGTCTGGGGCGCATTTGTTGCTGCTTTCG	putative alkane monooxygenase Uncultured bacterium
ABB96096	GCTCAACGCGTGGCTGATGTCGGTGTCTTGTGGG TGGGCTGATGGTGT	putative alkane monooxygenase Uncultured bacterium
NP_771234	TTCTGGGCGCTGCTCTGGGCAAGGAGGCAAGGATGAACTGGAACGCCCG	delta 9 acyl-lipid fatty acid desaturase <i>Bradyrhizobium diazoefficiens</i> USDA 110
YP_552229	TTCTTGGGAGGATGGTGTGGATGCAAGCCTGCGTCA CCGTGAACCACA	alkane 1-monooxygenase <i>Polaromonas</i> sp. JS666
YP_921354	TGTGGGGCGCGCTGGTCCCTGGCTGGCGCGCGGCTGCTGCCGTACCTG	alkane 1-monooxygenase <i>Nocardioides</i> sp. JS614
NP_251264	GTCTGGGCCGCTGGCGTGTTCGTCGCTTCCAGGAGTGGGGCTGGCTCGG	alkane-1 monooxygenase <i>Pseudomonas aeruginosa</i> PAO1

Table S4. Phylogenetic assignment of phylotypes. Classification of all phylotypes based on 16S rRNA analysis using RDP.

Phylotype	Abundance (%)		Phylogenetic affiliation based on 16S rRNA gene sequence					
	PFR-1	PFR-2	Phylum	Class	Order	Family	Genus	
1	33.5	17.0	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Curvibacter	
2	9.6	9.1	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Simplicispira	
3	6.2	12.0	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Rudaea	
4	5.6	10.4	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Rudaea	
5	6.4	5.2	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae		
6	2.6	1.4	TM7				TM7_genera_incertae_sedis	
7	2.4	1.9	Proteobacteria	Betaproteobacteria	Burkholderiales			
9	2.4	0.1	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Rhodoblastus	
10	0.5	5.9	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Magnetospirillum	
11	1.6	0.5	Actinobacteria	Actinobacteria	Actinomycetales	Sporichthyaceae		
12	1.1	1.8	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae		
13	1.5	1.1	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	
14	1.2	0.1	Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	Clostridium sensu stricto	
15	1.3	0.3	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Aquitalea	
16	0.8	0.0	Proteobacteria	Gammaproteobacteria				
17	0.1	2.4	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Zoogloea	
19	0.0	2.9	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Thermomonas	
20	0.6	0.8	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Sphingobacteriaceae	Mucilaginibacter	
21	1.3	0.0	Chlamydiae	Chlamydiae	Chlamydiales	Parachlamydiaceae	Parachlamydia	
22	0.6	0.1	Acidobacteria	Holophagae	Holophagales	Holophagaceae	Geothrix	
23	0.1	2.0	Proteobacteria	Betaproteobacteria				
24	0.8	0.0	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Cupriavidus	
25	0.6	0.1	Verrucomicrobia	Subdivision3	Subdivision3_genera_incertae_sedis	Subdivision3_genera_incertae_sedis	Subdivision3_genera_incertae_sedis	
26	0.6	0.0	Actinobacteria	Actinobacteria	Actinomycetales			
27	0.5	0.6	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae		
28	0.4	0.9	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	
29	0.5	0.1	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Pandoraea	
31	0.2	1.2	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae		
32	0.5	0.0	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	
33	0.5	0.0						
36	0.5	0.0	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	
37	0.4	0.1						
38	0.5	0.0						
39	0.4	0.1	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Azospira	
40	0.4	0.2	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	Steroidobacter	
41	0.4	0.1	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	

Phylotype	Abundance (%)		Phylogenetic affiliation based on 16S rRNA gene sequence				
	PFR-1	PFR-2	Phylum	Class	Order	Family	Genus
42	0.2	0.6	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Ralstonia
43	0.1	1.0	Acidobacteria	Holophagae	Holophagales	Holophagaceae	Geothrix
44	0.3	0.1	Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	Mycobacterium
45	0.3	0.1	Proteobacteria	Alphaproteobacteria	Rhizobiales		
46	0.0	2.0	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia
47	0.2	0.6	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	
48	0.2	0.2	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	
49	0.3	0.0	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Massilia
50	0.2	0.1	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Leifsonia
52	0.2	0.4	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	Chitinophaga
53	0.2	0.2	Actinobacteria	Actinobacteria	Actinomycetales	Cellulomonadaceae	Cellulomonas
54	0.0	1.0	Acidobacteria	Holophagae	Holophagales	Holophagaceae	Geothrix
55	0.2	0.1	Proteobacteria	Gammaproteobacteria			
56	0.2	0.2	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium
57	0.2	0.1					
58	0.2	0.1	Verrucomicrobia	Subdivision3	Subdivision3_genera_incertae_sedis	Subdivision3_genera_incertae_sedis	Subdivision3_genera_incertae_sedis
60	0.2	0.0	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Paucimonas
61	0.2	0.2	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonas
62	0.2	0.5	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Cupriavidus
63	0.2	0.1	Proteobacteria				
64	0.0	0.7	Proteobacteria	Gammaproteobacteria			
65	0.1	0.0	Proteobacteria	Alphaproteobacteria	Alphaproteobacteria_incertae_sedis	Rhizomicrobium	Rhizomicrobium
66	0.2	0.1	Actinobacteria	Actinobacteria	Actinomycetales	Kineosporiaceae	
68	0.1	0.1	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis
69	0.2	0.1	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Paludibacter
70	0.2	0.1	Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	Clostridium sensu stricto
71	0.2	0.0	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Clostridium III
72	0.0	0.5	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Undibacterium
73	0.1	0.6	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	
74	0.1	0.1	Acidobacteria	Holophagae	Holophagales	Holophagaceae	Geothrix
75	0.1	0.0					
76	0.1	0.2	Proteobacteria	Alphaproteobacteria	Alphaproteobacteria_incertae_sedis	Rhizomicrobium	Rhizomicrobium
78	0.2	0.0	Verrucomicrobia	Subdivision3	Subdivision3_genera_incertae_sedis	Subdivision3_genera_incertae_sedis	Subdivision3_genera_incertae_sedis
79	0.1	0.4	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiales_incertae_sedis	Thiomonas
81	0.1	0.1					
82	0.1	0.2	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Massilia
83	0.0	0.5	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas
84	0.1	0.0	Actinobacteria	Actinobacteria	Actinomycetales	Cellulomonadaceae	Cellulomonas
85	0.1	0.0	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	

Phylotype	Abundance (%)		Phylogenetic affiliation based on 16S rRNA gene sequence				
	PFR-1	PFR-2	Phylum	Class	Order	Family	Genus
86	0.1	0.0	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae	
87	0.0	0.3	Proteobacteria	Alphaproteobacteria	Rhizobiales		
88	0.1	0.0	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	
89	0.1	0.1	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Dechloromonas
90	0.1	0.0	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Aquitalea
91	0.0	0.2	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	
93	0.1	0.2	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	
94	0.1	0.0	Proteobacteria				
95	0.1	0.0	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	
96	0.1	0.1	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium
97	0.0	0.4	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geobacter
98	0.1	0.1					
99	0.1	0.1	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	
100	0.2	0.0	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	
101	0.1	0.0	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Undibacterium
103	0.1	0.0	Verrucomicrobia	Opitutae			
104	0.1	0.0	Firmicutes	Bacilli	Bacillales	Paenibacillaceae 2	Ammoniphilus
105	0.1	0.1	Acidobacteria	Acidobacteria_Gp1	Gp1	Gp1	Gp1
106	0.1	0.0	Chlamydiae	Chlamydiae	Chlamydiales		
108	0.1	0.0					
110	0.1	0.0	Actinobacteria	Actinobacteria	Actinomycetales	Propionibacteriaceae	
111	0.1	0.1	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas
112	0.1	0.0	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Sphingobacteriaceae	Mucilaginibacter
113	0.1	0.3	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas
114	0.1	0.2	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Shuttleworthia
115	0.1	0.2	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	
117	0.1	0.0					
118	0.1	0.0	Chloroflexi	Anaerolineae			
119	0.1	0.1	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Gemmata
120	0.1	0.0	TM7	TM7_genera_incertae_sedis	TM7_genera_incertae_sedis	TM7_genera_incertae_sedis	TM7_genera_incertae_sedis
121	0.0	0.4	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	
122	0.1	0.0	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Castellaniella
123	0.1	0.0	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Magnetospirillum
124	0.0	0.1	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	
125	0.1	0.0	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	
126	0.1	0.0	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Magnetospirillum
127	0.1	0.0	Proteobacteria	Alphaproteobacteria			
128	0.1	0.0	Proteobacteria	Gammaproteobacteria			
129	0.1	0.0					

Phylotype	Abundance (%)		Phylogenetic affiliation based on 16S rRNA gene sequence				
	PFR-1	PFR-2	Phylum	Class	Order	Family	Genus
130	0.1	0.2	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Sphingobacteriaceae	Mucilaginitacter
132	0.1	0.0	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Planctomyces
133	0.0	0.2	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	Acidisoma
134	0.1	0.0	Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	Clostridium sensu stricto
135	0.1	0.1	Proteobacteria	Deltaproteobacteria			
136	0.0	0.3	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia
137	0.0	0.2	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Bordetella
140	0.0	0.3	Bacteroidetes				
142	0.0	0.3	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	
144	0.1	0.1	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae	Methylocystis
145	0.1	0.0	Proteobacteria	Gammaproteobacteria			
146	0.1	0.0	Proteobacteria	Alphaproteobacteria			
147	0.1	0.0					
149	0.1	0.0	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	
150	0.1	0.0	Proteobacteria	Betaproteobacteria			
152	0.0	0.0	Proteobacteria	Deltaproteobacteria	Myxococcales		
153	0.0	0.2	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	
154	0.0	0.2	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Prosthecoacter
155	0.1	0.0	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Singulisphaera
157	0.1	0.0					
158	0.0	0.2	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Pelomonas
159	0.1	0.0	Proteobacteria				
160	0.0	0.2	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	
161	0.1	0.1	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	
162	0.1	0.0					
164	0.1	0.0					
165	0.0	0.1	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	
166	0.0	0.1	Bacteroidetes				
167	0.1	0.0	Firmicutes	Negativicutes	Selenomonadales		
168	0.1	0.1	Chlamydiae	Chlamydiae	Chlamydiales		
169	0.1	0.0	Actinobacteria	Actinobacteria	Acidimicrobiales		
170	0.0	0.0					
171	0.0	0.2	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	Chitinophaga
173	0.1	0.1	Actinobacteria	Actinobacteria	Acidimicrobiales		
174	0.1	0.0	Proteobacteria	Betaproteobacteria	Burkholderiales		
175	0.1	0.0	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	
177	0.0	0.0	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	
178	0.1	0.0	Proteobacteria	Alphaproteobacteria	Alphaproteobacteria_incertae_sedis	Rhizomicrobium	Rhizomicrobium
179	0.0	0.1	Proteobacteria	Alphaproteobacteria	Rickettsiales	Rickettsiaceae	

Phylotype	Abundance (%)		Phylogenetic affiliation based on 16S rRNA gene sequence				
	PFR-1	PFR-2	Phylum	Class	Order	Family	Genus
180	0.0	0.1	Proteobacteria	Gammaproteobacteria			
181	0.0	0.1	Proteobacteria	Betaproteobacteria			
182	0.0	0.1	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	
184	0.1	0.0	Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	Clostridium sensu stricto
185	0.0	0.1	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	
186	0.0	0.0					
187	0.0	0.1	Chlamydiae	Chlamydiae	Chlamydiales		
189	0.0	0.0	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Paludibacter
190	0.0	0.0	Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	Clostridium sensu stricto
191	0.0	0.0	Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	Legionella
193	0.1	0.0	Firmicutes	Bacilli	Bacillales	Bacillaceae 1	Bacillus
194	0.0	0.0	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Achromobacter
195	0.0	0.0	Proteobacteria				
196	0.0	0.1	Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	
198	0.1	0.0	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium
199	0.0	0.0	Proteobacteria	Alphaproteobacteria	Alphaproteobacteria_incertae_sedis	Rhizomicrobium	Rhizomicrobium
201	0.0	0.1	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	
203	0.1	0.1	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Rhizobium
204	0.0	0.0	Proteobacteria	Alphaproteobacteria			
205	0.0	0.0	Proteobacteria	Gammaproteobacteria			
206	0.0	0.0	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	
207	0.0	0.1	Chlorobi	Ignavibacteria	Ignavibacteriales	Ignavibacteriaceae	Ignavibacterium
208	0.0	0.0	Proteobacteria	Alphaproteobacteria	Rhizobiales		
210	0.0	0.0	Acidobacteria	Acidobacteria_Gp3	Gp3	Gp3	Gp3
211	0.0	0.0	Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	Clostridium sensu stricto
213	0.0	0.1	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium
214	0.0	0.1	Chlorobi	Ignavibacteria	Ignavibacteriales	Ignavibacteriaceae	Ignavibacterium
215	0.0	0.0					
216	0.0	0.0	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	
217	0.0	0.0					
218	0.0	0.0	Firmicutes	Bacilli	Bacillales	Paenibacillaceae 1	
219	0.0	0.0	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Paludibacter
220	0.0	0.0	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Zoogloea
221	0.0	0.0	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae	Methylocystis
222	0.0	0.0	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	
223	0.0	0.1	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	
224	0.1	0.0	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	Anaerolinea
225	0.0	0.0	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	
226	0.0	0.0	Actinobacteria	Actinobacteria	Actinomycetales	Propionibacteriaceae	

Phylotype	Abundance (%)		Phylogenetic affiliation based on 16S rRNA gene sequence				
	PFR-1	PFR-2	Phylum	Class	Order	Family	Genus
228	0.1	0.0	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Singulisphaera
229	0.0	0.0	Proteobacteria	Alphaproteobacteria	Rhodospirillales		
230	0.0	0.0	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	
232	0.0	0.0	Proteobacteria	Betaproteobacteria			
234	0.0	0.0	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiales_incertae_sedis	Aquabacterium
235	0.0	0.1	Armatimonadetes	Armatimonadetes_gp5	Armatimonadetes_gp5	Armatimonadetes_gp5	Armatimonadetes_gp5
238	0.0	0.1	Proteobacteria	Alphaproteobacteria	Rhizobiales		
239	0.0	0.0	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Afipia
240	0.0	0.0	WS3	WS3_genera_incertae_sedis	WS3_genera_incertae_sedis	WS3_genera_incertae_sedis	WS3_genera_incertae_sedis
241	0.0	0.0	Firmicutes	Bacilli	Bacillales	Pasteuriaceae	Pasteuria
242	0.0	0.0	Proteobacteria	Deltaproteobacteria			
243	0.0	0.0	Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	Clostridium sensu stricto
244	0.0	0.0	Deinococcus-Thermus	Deinococci	Thermales	Thermaceae	Meiothermus
245	0.0	0.0	Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	Fervidicella
247	0.1	0.0	Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	Mycobacterium
248	0.0	0.0	Proteobacteria	Alphaproteobacteria	Alphaproteobacteria_incertae_sedis	Rhizomicrobium	Rhizomicrobium
249	0.0	0.1	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geobacter
250	0.0	0.1	Armatimonadetes	Armatimonadetes_gp5	Armatimonadetes_gp5	Armatimonadetes_gp5	Armatimonadetes_gp5
251	0.0	0.0	TM7	TM7_genera_incertae_sedis	TM7_genera_incertae_sedis	TM7_genera_incertae_sedis	TM7_genera_incertae_sedis
252	0.0	0.0					
253	0.0	0.0	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	Sediminibacterium
254	0.0	0.0	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Simplicispira
255	0.1	0.0	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Pseudoxanthomonas
257	0.0	0.0	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	
258	0.0	0.0	Proteobacteria	Deltaproteobacteria	Myxococcales		
259	0.0	0.0	Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	Legionella
260	0.0	0.0	Chloroflexi				
262	0.0	0.0	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	
263	0.0	0.1	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	
264	0.0	0.0	Acidobacteria	Acidobacteria_Gp3	Gp3	Gp3	Gp3
265	0.0	0.0	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Uliginosibacterium
267	0.0	0.0	Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	Legionella
268	0.0	0.1	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
270	0.0	0.0	Verrucomicrobia	Subdivision3	Subdivision3_genera_incertae_sedis	Subdivision3_genera_incertae_sedis	Subdivision3_genera_incertae_sedis
271	0.0	0.1	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	
274	0.0	0.0	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geobacter
275	0.0	0.1	Proteobacteria	Betaproteobacteria			
276	0.1	0.0	Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	Clostridium sensu stricto
280	0.0	0.1	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Sphingobacteriaceae	Mucilagibacter

Phylotype	Abundance (%)		Phylogenetic affiliation based on 16S rRNA gene sequence				
	PFR-1	PFR-2	Phylum	Class	Order	Family	Genus
281	0.0	0.0	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	
282	0.0	0.0	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Singulisphaera
286	0.0	0.0	Chlamydiae	Chlamydiae	Chlamydiales	Parachlamydiaceae	
287	0.0	0.0	Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	Mycobacterium
290	0.0	0.0	Proteobacteria	Gammaproteobacteria			
291	0.0	0.1	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	
299	0.0	0.0	Proteobacteria	Gammaproteobacteria			
302	0.0	0.0	Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	Clostridium sensu stricto
304	0.0	0.0	Verrucomicrobia	Subdivision3	Subdivision3_genera_incertae_sedis	Subdivision3_genera_incertae_sedis	Subdivision3_genera_incertae_sedis
308	0.0	0.1	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geobacter
311	0.0	0.1	Chlorobi	Ignavibacteria	Ignavibacteriales	Ignavibacteriaceae	Ignavibacterium
312	0.0	0.1	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Singulisphaera
319	0.0	0.0	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	
324	0.0	0.0	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	
328	0.0	0.1	Proteobacteria	Deltaproteobacteria	Syntrophobacteriales	Syntrophobacteraceae	Desulfovirga
333	0.0	0.1	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia
334	0.0	0.1	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Achromobacter
337	0.0	0.0	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Malikia
343	0.0	0.1	Proteobacteria	Betaproteobacteria	Nitrosomonadales	Nitrosomonadaceae	Nitrosospira
345	0.0	0.1	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	Bdellovibrio
354	0.0	0.0	Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	Legionella
355	0.0	0.0	Cyanobacteria/Chloroplast	Chloroplast	Chloroplast	Chloroplast	Chlorarachniophyceae
362	0.0	0.0	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	
381	0.0	0.1	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	
400	0.0	0.0	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geobacter
401	0.0	0.1	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Pseudolabrys
454	0.0	0.1	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia

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