

SUPPLEMENTARY ONLINE MATERIAL

Diversity of benzylsuccinate synthase-like (*bssA*) genes in hydrocarbon-polluted marine sediments suggest substrate-dependent clustering

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The supplementary material includes :

Figures S1 to S5

Tables S1 to S4

References

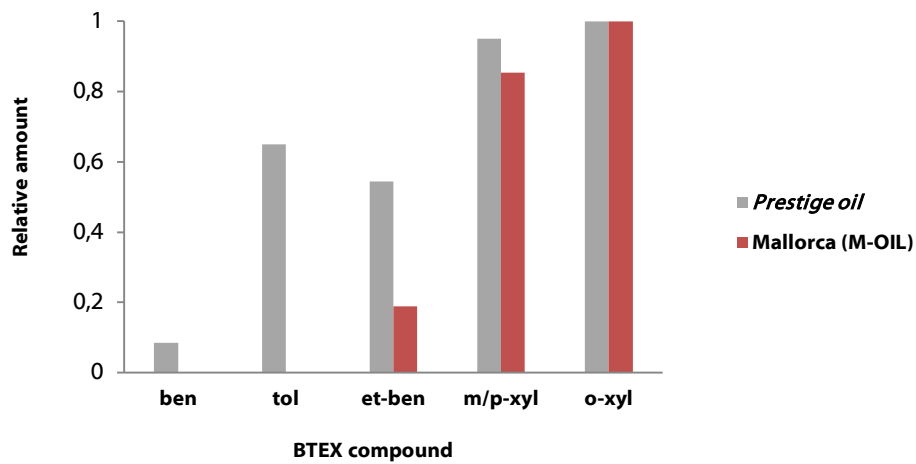


Figure S1a. Relative distribution of BTEX in M-OIL sample and in the original *Prestige oil* used in Mallorca microcosms. Ben (benzene), tol (toluene), et-ben (ethyl-benzene), *m/p-xyl* (*m*-xylene and *p*-xylene), *o-xyl* (*o*-xylene).

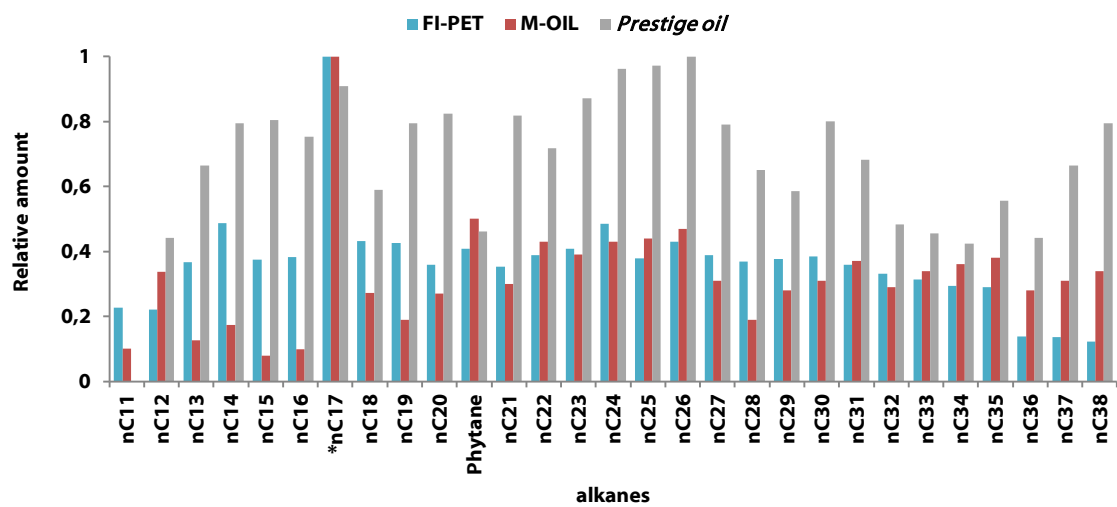


Figure S1b. Relative distribution of alkanes in most polluted samples. Data are compared with the *Prestige oil* fingerprinting described in Alzaga *et al.*, 2004 (1). *sum of nC17 and pristane.

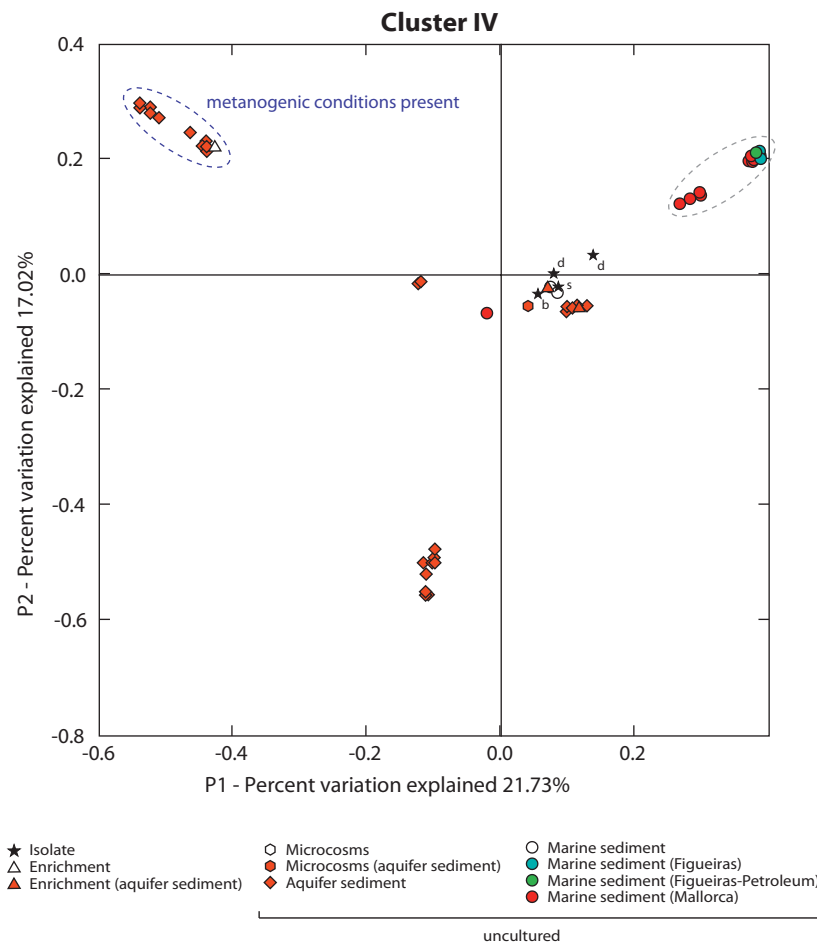


Figure S2. PCoA (Principal coordinate analysis) of distances between *assA* sequences present in the sediment samples collected from marine sediments (Figueiras beach, Rodas beach and Mallorca) and sequences available in the databases (see table S1). The positions of the sequences corresponding to isolates are labeled with a letter that indicates the taxonomic affiliation: *Betaproteobacteria* (b), *Desulfobacterales* (*Desulfatibacillum alkenivorans* AK-01) (d) and *Syntrophobacterales* (*Desulfoglaeba alkanexedens*) (s). The percentage of the variation between the samples by principal coordinate is indicated on the axes. A sequence cutoff of 2% of amino acid similarity (OTU_{0.02}) was used to select the sequences for the analysis.

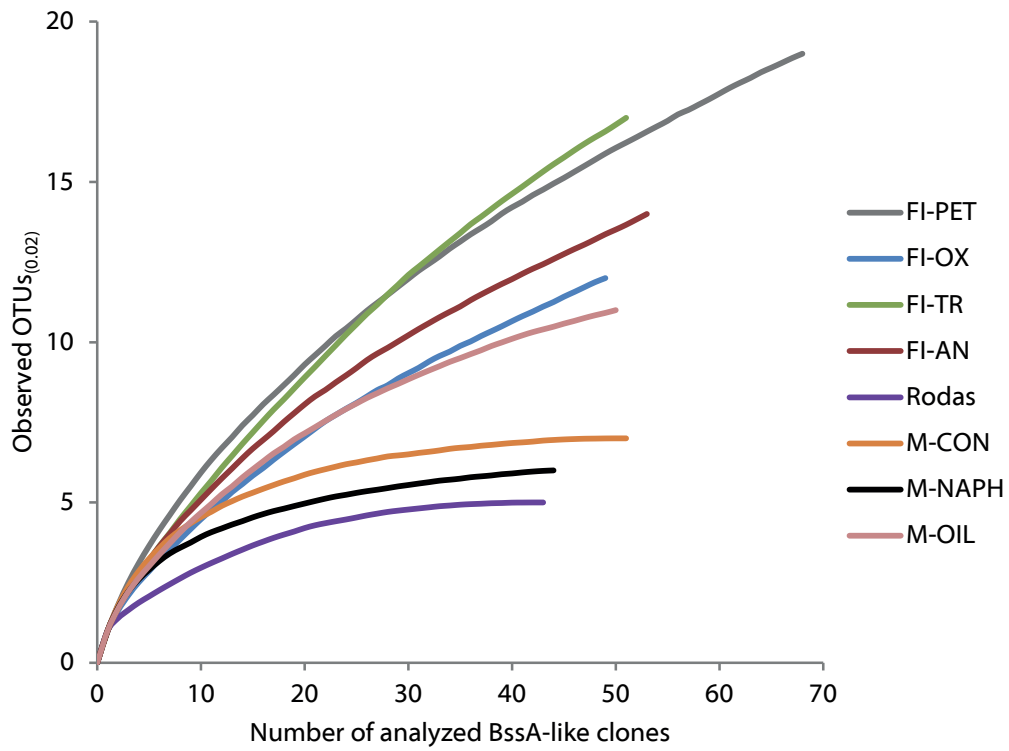
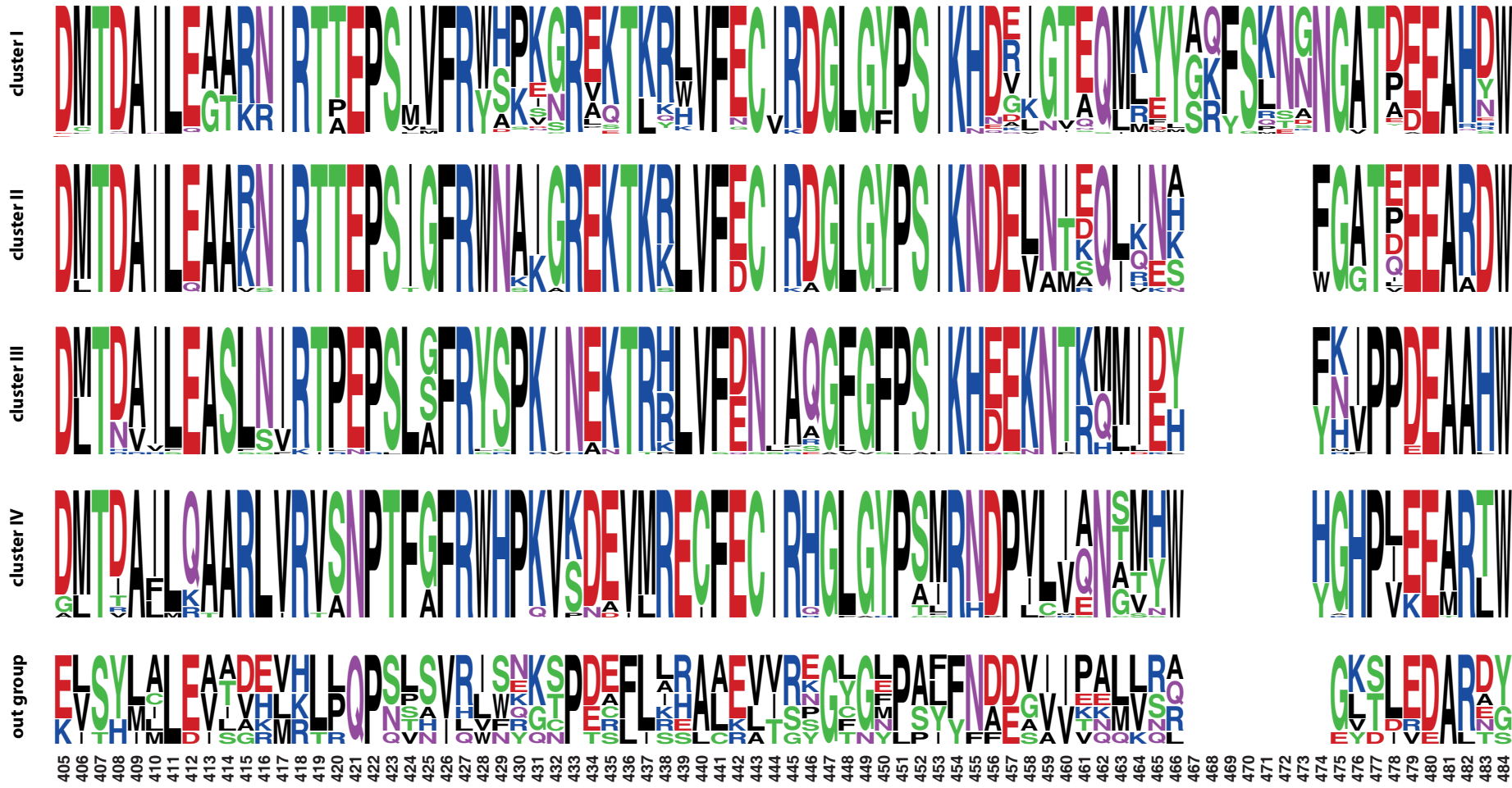
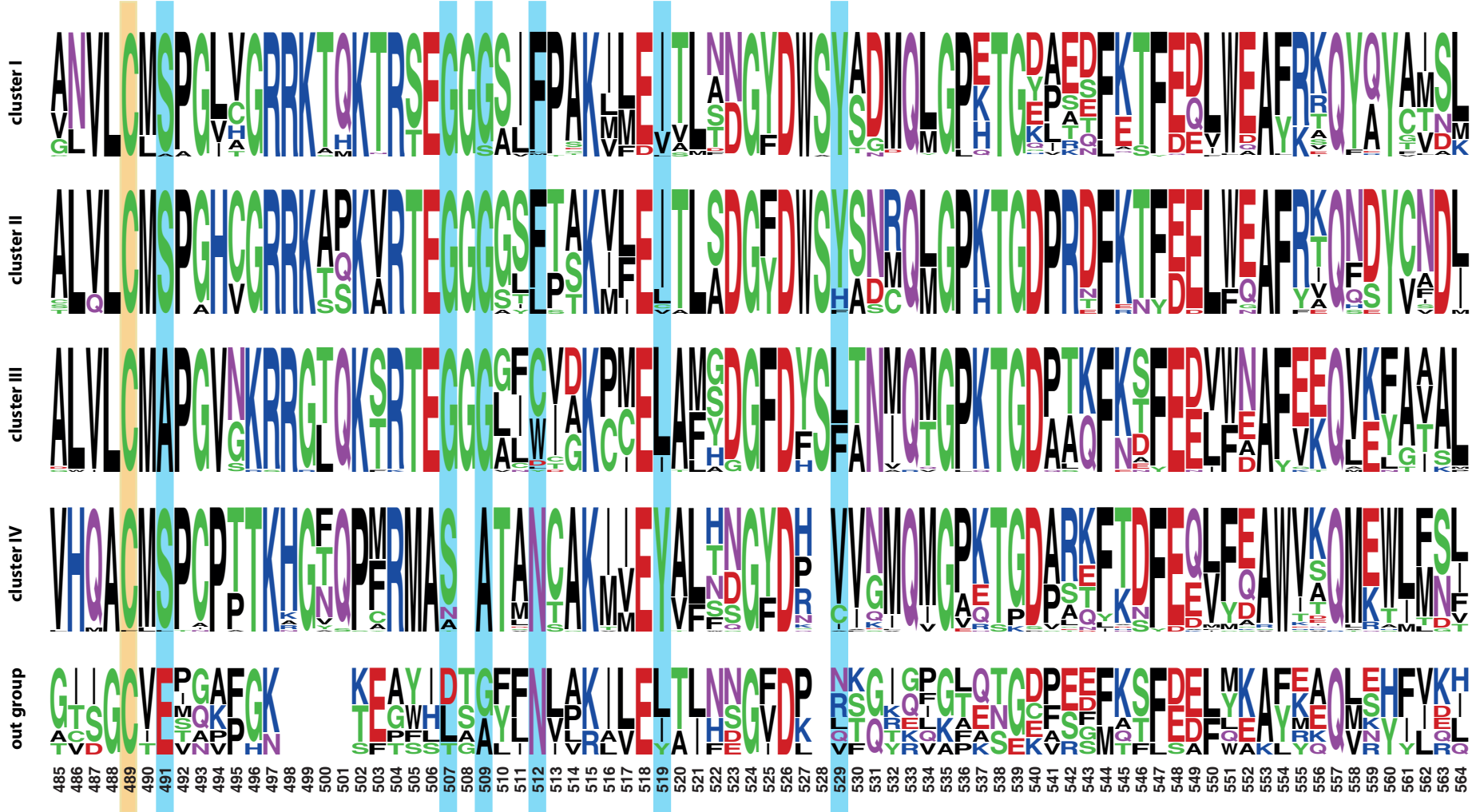
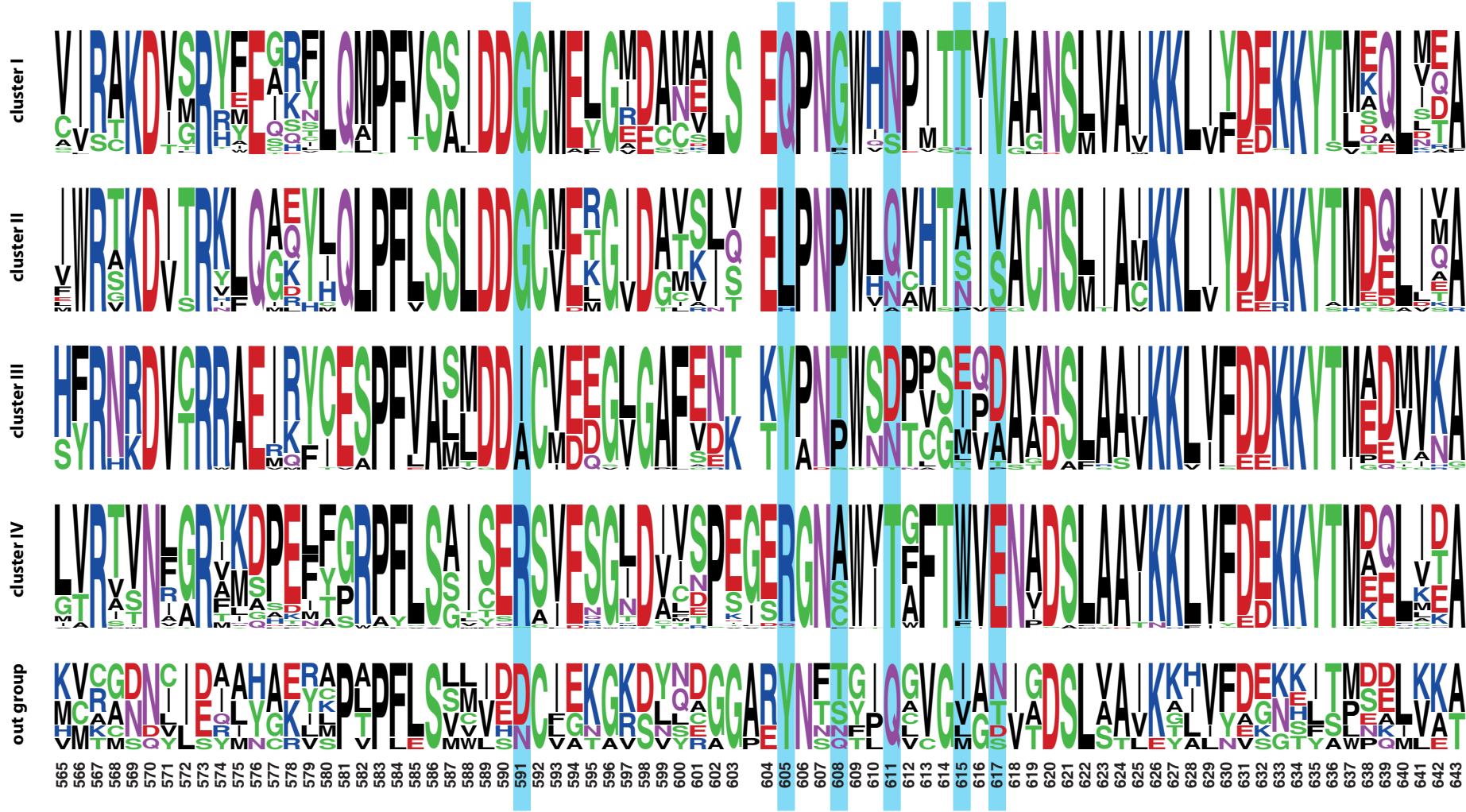


Figure S3. Rarefaction curves of the BssA-like sequences from sediments samples (FI, Figueiras; M, Mallorca) with a sequence cutoff of 2% of amino acid similarity (OTU_{0.02}).







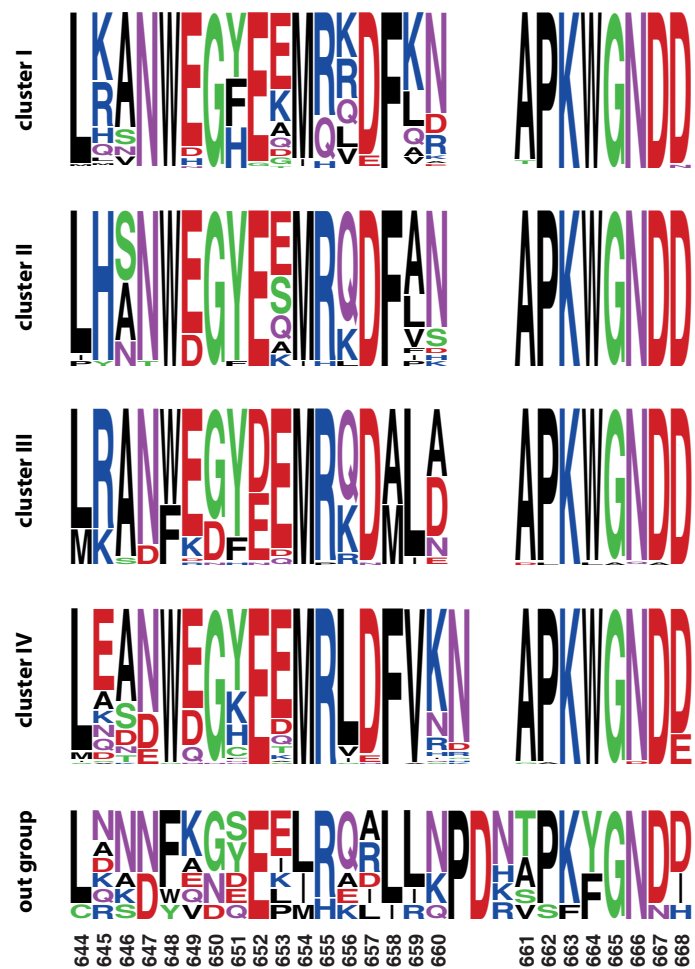


Figure S4. Alignment of the consensus sequences of the different clusters determined in the study showing the frequency profiles of amino acids at each position (logo). Position numbers refer to *Thauera aromatica* K172 sequence. The active site cysteine residue is highlighted in yellow, while the specificity relevant residues shown in Figure 4 are highlighted in blue.

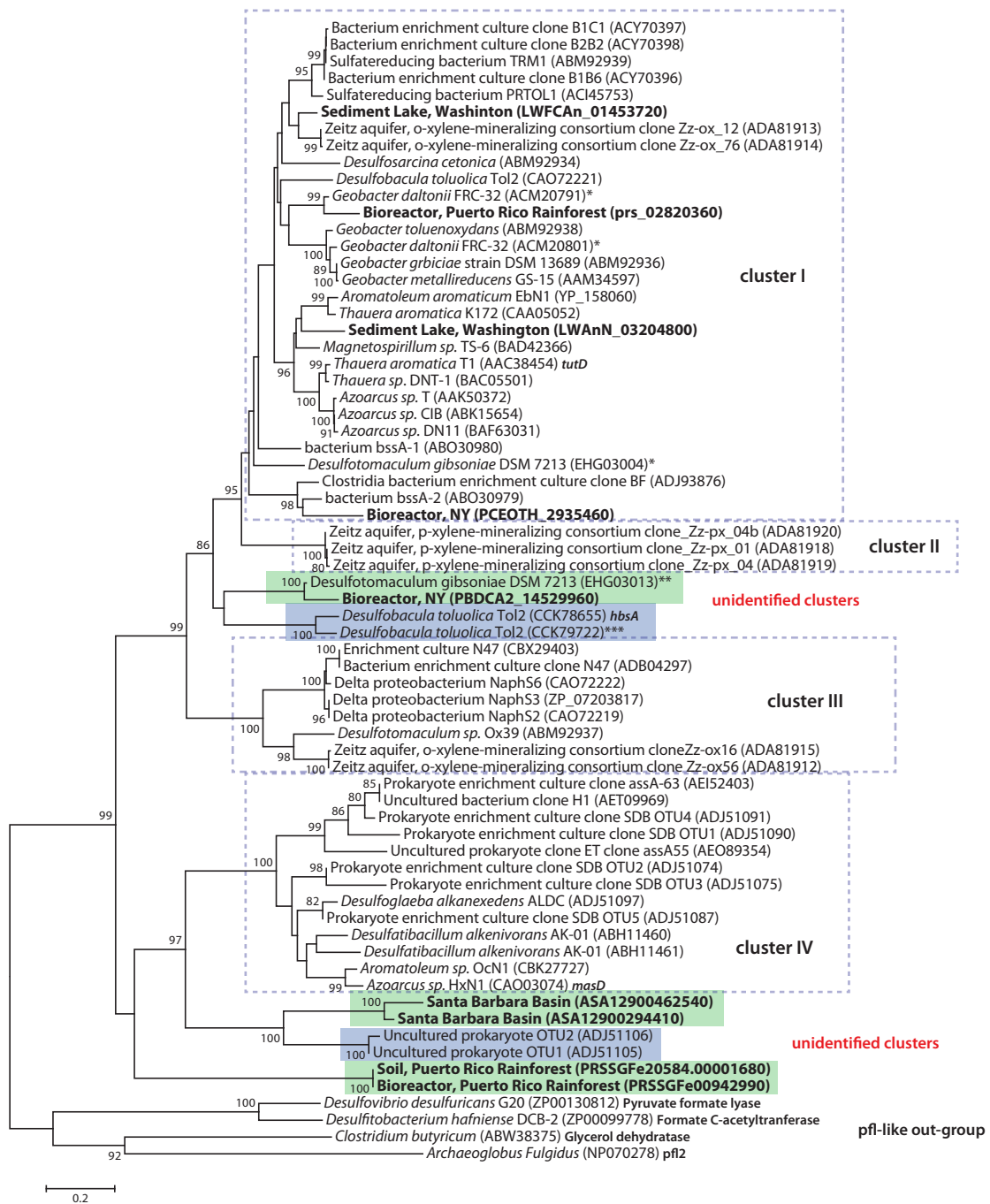


Figure S5. Unidentified clusters found in our analysis. The phylogenetic tree shows all BssA-like sequences from isolated strains and enrichment cultures deposited in databases and sequences annotated as putative pyruvate-formate lyase (pfl) enzymes from genomes and metadata (metagenomes). The analysis suggests the existence of non-classified clusters (highlighted in blue and green). Asterisks denote the sequences annotated as pfl from aromatic oxidizing strains that belong to cluster I (*) or unidentified cluster (**). A third sequence (BssA3) from *Desulfobacula toluolica* Tol2 annotated as BssA (2) groups in the HbsA cluster (***) . The sequences retrieved from metagenomes are written in bold. Two sequences annotated as putative glycol radical enzymes from Callaghan *et al.*, 2010 (3) are included in the analysis (ADJ51105 and ADJ51106). Numbers in parentheses represents protein GenBank accession numbers or gene object ID (metagenomic data).

References

1. **Alzaga R, Montuori P, Ortíz L, Bayona JM, Albaigés J.** 2004. Fast solid-phase extraction-gas chromatography-mass spectrometry procedure for oil fingerprinting: Application to the *Prestige* oil spill. *J Chromatogr A* **1025**:133-138.
2. **Wöhlbrand L, Jacob JH, Kube M, Mussmann M, Jarling R, Beck A, Amann R, Wilkes H, Reinhardt R, Rabus R.** 2012. Complete genome, catabolic sub-proteomes and key-metabolites of *Desulfobacula toluolica*Tol2, a marine, aromatic compound-degrading, sulfate-reducing bacterium. *Environmental Microbiology*:n/a-n/a.
3. **Callaghan AV, Davidova IA, Savage-Ashlock K, Parisi VA, Gieg LM, Suflita JM, Kukor JJ, Wawrik B.** 2010. Diversity of Benzyl- and Alkylsuccinate Synthase Genes in Hydrocarbon-Impacted Environments and Enrichment Cultures. *Environmental Science & Technology*.

Table S1. BssA-like sequences retrieved from the databases and used in the analysis.

<i>bssA</i> like*	Protein acc. number	No. seq	Sample source	Reported contaminants in the isolation source	notes	Ref.
<i>Thauera aromatica</i> strain T1	AAC38454	1	Untreated gasoline spill soil, California (USA)	Gasoline		(1)
<i>Thauera aromatica</i> K172	CAA05052	1	Municipal sewage plant in Konstanz (Germany)		Anaerobic sludge	(2)
<i>Azoarcus</i> sp. T	AAK50372	1	Anaerobic, toluene-degrading Laboratory aquifer column.	Toluene	Column filled with material from the interface of a river-groundwater infiltration site.	(3)
<i>Geobacter metallireducens</i> GS15	AAM34597	1	Freshwater surficial bottom sediments, Potomac River.			(4)
<i>Aromatoleum aromaticum</i> EbN1	YP_158060	1	Anoxic freshwater mud sampled in Bremen, Germany			(5)
<i>Thauera</i> sp. DNT-1	BAC05501	1	Anaerobic sludge from a wastewater treatment plant			(6)
<i>Magnetospirillum</i> sp. TS6	AD42366	1	Soils from damp ground and rice paddies		Denitrifying <i>Alphaproteobacteria</i>	(7)
<i>Azoarcus</i> sp. CIB	ABK15654	1				Blazquez et al., 2006 ¹
<i>Azoarcus</i> sp. DN11	BAF63031	1	Contaminated subsurface aquifer, Kumamoto (Japan)	Gasoline-contaminated groundwater		(8)
<i>Desulfosarcina cetonica</i> DSM 7267	ABM92934	1	Oil recovery water, Azerbaijan			
<i>Geobacter grbiciae</i> strain DSM 13689	ABM92936	1	Freshwater sediment, estuary of the Potomac River, USA.			(9)
<i>Desulfotomaculum</i> sp. OX39	ABM92937	1	Sediment of a drilling core taken at a former gasworks plant near Stuttgart (Germany)	Monocyclic aromatics, PAHs, and heterocyclic compounds.		

<i>Geobacter toluenoxydans</i> TMJ1	ABM92938	1	Sediment from a near Stuttgart (Germany)	Tar-oil-contaminated site		
Sulfate reducer TRM1	ABM92939	1	Soil percolation column filled with material from a contaminated aquifer (Stuttgart)	BTEX		
<i>Desulfobacula toluolica</i> strain Tol2	CAO72221	1	Marine sediment, Eel Pond			
Sulfate-reducing bacterium PRTOL1	EU780921	1	Naval Air Station, Patuxent River	Aviation fuel-contaminated soil		(10)
<i>Georgfuchsia toluolica</i> strain G5G6T	CBH30982	1	Polluted aquifer, Banisveld (The Netherlands)	BTEX-containing landfill leachate		(11)
<i>Geobacter daltonii</i> FRC-32	ACM20791 ACM20801	2	Contaminated shallow subsurface	mixed radionuclides, heavy metal and hydrocarbon contamination	Annotated as Formate C-acetyltransferase	Lucas et al., 2009 ¹
<i>Desulfotomaculum gibsoniae</i> DSM 7213	EHG03004	1	freshwater mud		Annotated as pyruvate formate-lyase (PFL)	Lucas et al., 2011 ¹
Bacterium bssA-2	ABO30979	1	Sand deposits at Pensacola aquifer (California)	Nitrogen heterocycles, PAHs and phenols	toluene-degrading methanogenic consortium	(12)
Bacterium bssA-1	ABO30980	1				
Bacterium enrichment culture	ADA81912- ADA81917	6	heavily contaminated former industrial field site Zeitz (Germany)	BTEX	<i>o</i> -xylene-mineralizing consortium	(13)
	ADA81918- ADA81925	8				
Bacterium enrichment culture	ACY70396- ACY70398	3	BTEX-contaminated aquifer near Zeitz (Germany)	BTEX	Groundwater Enriched consortium Zz5-7	(14)
Clostridia enrichment (clone BF)	ADJ93876	1	Soil of a former coal gasification site in Gliwice, Poland	Bencene	benzene-degrading and iron-reducing clone	(15)
uncultured bacterium	ABM91877- ABM91886	10	Former gasworks site, Testfeld Süd near Stuttgart (Germany)	Tar-oil-contaminated	Contaminated aquifer sediments	(9)
	ABM91887- ABM91896	10	Contaminated Flingern aquifer sediments, Düsseldorf (Germany)			
	ABM91897- ABM91912	16	Gasworks site in Pasing, Munich (Germany)			

uncultured bacterium	ADG27767- ADG27785	19	Gasworks site, aquifer sediment, Testfeld Süd, (Germany)	Coal processing products, BTEX, PAHs	Toluene-degrading 'heavy' DNA (SIP)	(16)
uncultured prokaryote	ADJ51098- ADJ51100, ADJ51102, ADJ51103	5*	Oil refinery in Casper (Wyoming)	Fuel and liquid propane gas, gasoline, heavy fuel oil, kerosene, asphalt	Contaminated aquifer sediment	(17)
	ADJ51101, ADJ51104	2	Gas condensate-contaminated Aquifer, South Platte alluvial aquifer, Fort Lupton (Colorado)	96% w/w C5-C15 compounds (18% w/w BTEX)		
uncultured bacterium	ACR82309- ACR82335	27	Groundwater, rural wooded area in South Glens Falls (NY)	Gas condensate, BTEX	Coal tar waste- contaminated groundwater	(18)
uncultured <i>Thauera sp.</i>	AFB74483, AFB74485- AFB74487	4	Granular sludge nitrate amended (Washington)	BTEX	Toluene degrading microcosms	(19)
	AFB74484	1	Agricultural soil nitrate amended (Michigan)			
uncultured <i>desulfobacterium</i>	AFB74488	1	Contaminated soil sulfate amended (Michigan)			
uncultured bacterium	AFB74489	1				
uncultured bacterium	AEO79008- AEO79010	3	Oily sludge	Oil	Incubated for 500-days under methanogenic conditions without any carbon sources	(20)
uncultured prokaryote	AEY68269	1	Production water from Shengli oilfield, Shangdong (China)	moderately heavy oil	Methanogenic enrichments cultures	(21)
uncultured bacterium	AFX62232- AFX62236	5	Amon mud volcano station 929			(22)
	AFX62237	1	Gulf of Mexico, hydrocarbon seep dive 161			
	AFX62238- AFX62277, AFX73928- AFX73934	47	Contaminated Flingern aquifer sediments, Düsseldorf (Germany).	Tar-oil contaminated	Contaminated aquifer sediments. Sampling from 2009.	

<i>nmsA</i> like*	Protein acc. number	No. seq	Sample source	Reported contaminants in the isolation source	notes	Ref.
delta proteobacterium NaphS2	CAO72219	1	North Sea sediment (Germany)		Anaerobically incubated medium with sulfate and naphthalene.	(23)
delta proteobacterium NaphS3	CAO72220	1	Etang de Berre marine sediment, Mediterranean lagoon (France)			
delta proteobacterium NaphS6	CAO72222	1				
bacterium enrichment culture clone N47	ADB04297	1	contaminated aquifer near Stuttgart (Germany)		Sulfate reducing conditions with naphthalene.	(24)
uncultured bacterium	AFX62278	1	Amon mud volcano station 929		Contaminated aquifer sediments. Sampling from 2009.	(22)
	AFX62279- AFX62293	15	Gulf of Mexico, hydrocarbon seep dive 161			
	AFX62294- AFX62301	8	Guaymas hydrocarbon seep, dive 4573			
	AFX62302- AFX62320	19	Contaminated Flingern aquifer sediments, Düsseldorf (Germany).	Tar-oil contaminated		
<i>hbsA</i>	Protein acc. number	No. seq	Sample source	Reported contaminants in the isolation source	notes	Reference
<i>Desulfobacula toluolica</i> strain Tol2	CCK78655	1	Marine sediment, Eel Pond		Hydroxybenzylsuccinate synthase subunit A	(25)
	CCK79722	1			Annotated as <i>bssA</i> in the NCBI database	
<i>assA</i>	Protein acc. number	No. seq	Sample source	Reported contaminants in the isolation source	notes	Reference
<i>Desulfatibacillum alkenivorans</i> AK-01 (sulfate-reducing bacterium AK-01)	ABH11460	2	Estuarine sediment, Arthur Kill waterway (NY/NJ)	Oil contaminated sediments		(26)
	ABH11461					
<i>Desulfoglaeba alkanexedens</i> ALDC	ADJ51097	1	Naval storage facility, VA (USA)	Oily wastewater		(17)
<i>Aromatoleum</i> sp. OcN1	CBK27727	1	Sediment samples from ditches in Bremen (Germany)			(27)
<i>Azoarcus</i> sp. HxN1	CAO03074	1				(28)

prokaryote enrichment	ADJ51074, ADJ51075, ADJ51087, ADJ51090, ADJ51091	5	Marine sediments, Paletta Creek site in San Diego Bay (CA)	Oil	Methanogenic, octacosane (C ₂₈ H ₅₈)-degrading enrichment culture	(17)
uncultured prokaryote	ADJ51096	1	North of Zodletone Mountain in the Anadarko Basin, OK (USA)		Sulfate-reducing conditions with propane	
uncultured prokaryote	AEO89352- AEO89354	3	Oily sludge incubated for 500-days		Methanogenic conditions	(20)
prokaryote enrichment	AEI52403- AEI52425	23			Methanogenic alkane degrading enrichment culture	
uncultured bacterium	AET09969- AET09982	14	Contaminated soil, Shengli oil field, eastern China	Oil	Methanogenic hexadecane-degrading consortium enriched with crude oil	Cheng et al., 2011 ¹
uncultured prokaryote	ADJ51068, ADJ51077, ADJ51079- ADJ51081, ADJ51084- ADJ51086	8	Contaminated aquifer sediment, Passaic River (NJ)			
	ADJ51076, ADJ51078, ADJ51083, ADJ51088, ADJ51089	5	Contaminated river sediment, Gowanus Canal (NY)	Sites have been negatively impacted by petroleum spills		(17)
	ADJ51082, ADJ51094	2	Contaminated river sediment, Newtown Creek (NY)			
	ADJ51069- ADJ51072, ADJ51095	5	Contaminated river sediment, Arthur Kill waterway (NY/NJ)			

	ADJ51073, ADJ51092, ADJ51093	3	Gas condensate-contaminated aquifer, South Platte alluvial aquifer, Fort Lupton (Colorado)	96% w/w C5-C15 compounds and 18% w/w BTEX	Sulfate- reducing and methanogenic conditions	
	ADJ51096	1	Zodletone spring water, Anadarko Basin (OK)	Methane, ethane, and propane	Anoxic water	
uncultured bacterium	AFX62321- AFX62322	2	Guaymas hydrocarbon seep, dive 4573			(22)
Unidentified clusters (annotated as pfl like)	Protein acc. number	No. seq	Sample source	Reported contaminants in the isolation source	notes	Reference
uncultured prokaryote	ADJ51105- ADJ51106	2	Gas condensate-contaminated aquifer, South Platte alluvial aquifer, Fort Lupton (Colorado)	96% w/w C5-C15 compounds and 18% w/w BTEX	Sediments under sulfate- reducing and methanogenic conditions	(17)
<i>Desulfotomaculum gibsoniae</i> DSM 7213	EHG03013	1	freshwater mud		Annotated as pyruvate formate-lyase (PFL)	Lucas et al., 2011 ¹
Unidentified clusters (Metagenomic projects)	Gene object ID		Sample source	Reported contaminants in the isolation source	Notes	Reference
Poplar biomass microbial communities from Brookhaven National Lab, NY	PBDCA2_14529960		Bioreactor	Solid waste	Sample from total biomass decay community	
Methane oxidizing archaeal communities from Santa Brabara Basin	ASA129_00294410 ASA129_00462540		Marine sediment, Santa Barbara Basin (ANME Sed A12 9-12 cm)			IMG/M database ¹
Microbial communities from Puerto Rico rain forest that decompose switchgrass	PRSSGFe_00942990 PRSSGFe2_0584.000016 80		Soil			
Discharged <i>bssA</i> like² (Metagenomic projects)	Gene object ID		Sample source	Reported contaminants in the isolation source	Notes	Reference
Microbial communities from Lake Washington	LWFCAn_01453720 LWAnN_03204800		Sediment, Lake Washington, Seattle (USA)		Methane and Nitrogen Cycles (Flow sorted aerobic + nitrate)	IMG/M database ¹

Green-waste compost microbial community from solid state bioreactor, Puerto Rico	prs_02820360	Compost	Solid waste
PCE-dechlorinating microbial communities from Ithaca, NY	PCEOTH_2935460	Bioreactor	Tetrachloroethylene and derivatives

* *bssA* like and *nmsA* like refers to BssA and NmsA *sensu lato* sequences, respectively.

¹ unpublished

² these sequences do not share $\geq 80\%$ of the same region. They were included only for the analysis of Figure S6.

Table S2a. BTEX composition in the sediment samples.

	BTEX compound (ppb)				
	Ben	EtBen	Tol	m/p-xyl	o-xyl
FI-PET	nd	nd	nd	blq	blq
M-OIL	nd	16.1 ± 2.1	nd	73.6 ± 13.5	86.2 ± 14.3
Prestige Oil (x10³)	0.16 ± 0.02	1.26 ± 0.16	1.04 ± 0.11	1.82 ± 0.16	1.91 ± 0.12

Ben (benzene); **EtBen** (Ethylbenzene), **Tol** (toluene), **m/p-xyl** (m-xylene and p-xylene), **o-xyl** (o-xylene)

nd = not detected

blq = below limit of quantification (<1 ppb)

Table S2b. PAHs composition in the sediment samples.

	PAH compound (ppm)					
	N	1-MN	2-MN	N2	N3	N4
RI	1.28 ± 0.96	nd	0.12 ± 0.17	nd	nd	nd
FI-OX	0.10 ± 0.04	0.12 ± 0.3	1.5 ± 1.09	nd	nd	nd
FI-TR	0.07 ± 0.02	nd	0.42 ± 0.21	nd	nd	nd
FI-AN	0.05 ± 0.01	nd	0.03 ± 0.01	nd	nd	nd
FI-PET	4.3 ± 1.91	2.52 ± 1.07	27.55 ± 6.1	22.5 ± 3.94	46.2 ± 13.2	25.1 ± 4.8
M-CON	0.0064 ± 0.00	nd	nd	nd	nd	nd
M-NAP	0.053 ± 0.00	nd	blq	nd	nd	nd
M-OIL	0.71 ± 0.22	0.07 ± 0.04	0.91 ± 0.37	0.21 ± 0.06	0.09 ± 0.02	nd
Prestige Oil^a	345		1076 ¹	1232	1017	no data

Naphthalene (**N**), 1-methylnaphthalene (**1-MN**), 2-methylnaphthalene (**2-MN**), dimethylnaphthalene (**N2**), trimethylnaphthalene (**N3**), tetramethylnaphthalene (**N4**).

¹ Sum of 1-MN and 2-MN.

^a Data for the Prestige oil fingerprinting analysis described in Alzaga et al., 2004.

*Methylated compounds with C1, C2 and C3 groups

nd = not detected

blq = below limit of quantification (<1 ppb)

Table S3. Most Probable Number of aromatic degrading bacteria in the Mallorca sediment microcosms and Atlantic Islands sediments.

		Carbon source						
	Treatment	Oil (CI (95%))	Ben (CI (95%))	Tol (CI (95%))	An (CI (95%))	Naph (CI (95%))	Ac (CI (95%))	
Mallorca	SRB (x10 ³)	None	23 (10-170)	9.2 (1-17)	9.2 (1-17)	2.3 (0.6-8)	0.21 (0.0-0.7)	92 (10-170)
		Naph	42 (10-170)	4.2 (1-17)	4.2 (1-17)	2.3 (0.6-8)	0.92 (0.2-3)	42 (10-170)
		Oil	92 (10-170)	15 (10-170)	15 (10-170)	14 (10-170)	0.92 (0.2-3)	150 (10-170)
	IRB (x10)	None	-	-	-	-	-	230 (66-800)
		Naph	2.3 (0.6-8)	-	-	4.2 (1-17)	-	70 (16-300)
		Oil	-	2.3 (0.6-8)	0.92 (0.2-3.7)	-	-	210 (61-730)
	MnRB (x10)	None	2.3 (0.6-8)	-	0.92 (0.2-3.7)	-	-	210 (61-730)
		Naph	2.3 (0.6-8)	0.36 (0.05-2.5)	0.92 (0.2-3.7)	0.92 (0.2-3.7)	-	23 (6.6-80)
		Oil	-	0.36 (0.05-2.5)	2.3 (0.6-8)	-	-	150 (4.1-52)
Atlantic Islands ^a	SRB (x10 ³)	FI ^b	nd	1.0 (1.9-49)	4.1 (1.0-27)	0.33 (0.1-0.9)	4.9 (3-7.1)	67 (44-100)
		FI-PET	nd	12.8 (3.2-50)	7.6 (2-26.8)	12.8 (3.2-50)	12.8 (3.2-50)	48.9 (3.5-57.5)
		RI	nd	1.3 (0.5-3.6)	0.03 (0.01-0.09)	0.02 (0.008-0.07)	0.8 (0.3-1.8)	3.5 (1.3-9)
	IRB (x10)	FI ^b	nd	0.3 (0.04-2.4)	1.1 (0.3-3.5)	-	1.6	8.0
		FI-PET	nd	4.8 (1.3-17.4)	2.4 (0.5-10.9)	3.0 (0.7-12.3)	3.0 (0.7-12.3)	30.6 (7.6-122)
		RI	nd	-	-	-	-	-

^a Data taken from Acosta-González *et al.*, 2013 (29).

^b Average values from FI-OX, FI-TR and FI-AN.

nd, not determined; -, no growth detected.

Table S4. Specificity of the *bssA* probe in each sample.

Site	Sample	Number of clones ¹	
		(+)	(-)
Mallorca	M-CON	51	19
	M-NAPH	44	26
	M-OIL	50	20
Figueiras	FI-PET	68	2
	FI-OX	49	21
	FI-TR	51	19
	FI-AN	53	17
	RI	43	27

¹(+) *bssA*-like sequences; (-) unrelated, non-*bssA* sequences

References

1. **Coschigano PW, Young LY.** 1997. Identification and sequence analysis of two regulatory genes involved in anaerobic toluene metabolism by strain T1. *Applied and Environmental Microbiology* **63**:652-660.
2. **Leuthner B, Leutwein C, Schulz H, Horth P, Haehnel W, Schiltz E, Schagger H, Heider J.** 1998. Biochemical and genetic characterization of benzylsuccinate synthase from *Thauera aromatica*: a new glycyl radical enzyme catalysing the first step in anaerobic toluene metabolism. *Molecular Microbiology* **28**:615-628.
3. **Achong GR, Rodriguez AM, Spormann AM.** 2001. Benzylsuccinate Synthase of *Azoarcus* sp. Strain T: Cloning, Sequencing, Transcriptional Organization, and Its Role in Anaerobic Toluene and m-Xylene Mineralization. *J Bacteriol* **183**:6763-6770.
4. **Kane SR, Beller HR, Legler TC, Anderson RT.** 2002. Biochemical and genetic evidence of benzylsuccinate synthase in toluene-degrading, ferric iron-reducing *Geobacter metallireducens*. *Biodegradation* **13**:149-154.
5. **Kube M, Heider J, Amann J, Hufnagel P, Kühner S, Beck A, Reinhardt R, Rabus R.** 2004. Genes involved in the anaerobic degradation of toluene in a denitrifying bacterium, strain EbN1. *Archives of Microbiology* **181**:182-194.
6. **Shinoda Y, Sakai Y, Uenishi H, Uchihashi Y, Hiraishi A, Yukawa H, Yurimoto H, Kato N.** 2004. Aerobic and Anaerobic Toluene Degradation by a Newly Isolated Denitrifying Bacterium, *Thauera* sp. Strain DNT-1. *Applied and Environmental Microbiology* **70**:1385-1392.
7. **Shinoda Y, Akagi J, Uchihashi Y, Hiraishi A, Yukawa H, Yurimoto H, Sakai Y, Kato N.** 2005. Anaerobic degradation of aromatic compounds by magnetospirillum strains: isolation and degradation genes. *Biosci Biotechnol Biochem* **69**:1483-1491.
8. **Kasai Y, Kodama Y, Takahata Y, Hoaki T, Watanabe K.** 2007. Degradative Capacities and Bioaugmentation Potential of an Anaerobic Benzene-degrading Bacterium Strain DN11. *Environmental Science & Technology* **41**:6222-6227.
9. **Winderl C, Schaefer S, Lueders T.** 2007. Detection of anaerobic toluene and hydrocarbon degraders in contaminated aquifers using benzylsuccinate synthase (*bssA*) genes as a functional marker. *Environmental Microbiology* **9**:1035-1046.
10. **Beller HR, Kane SR, Legler TC, McKelvie JR, Sherwood Lollar B, Pearson F, Balsler L, Mackay DM.** 2008. Comparative Assessments of Benzene, Toluene, and Xylene Natural Attenuation by Quantitative Polymerase Chain Reaction Analysis of a Catabolic Gene, Signature Metabolites, and Compound-Specific Isotope Analysis. *Environmental Science & Technology* **42**:6065-6072.
11. **Weelink SAB, Van Doesburg W, Saia FT, Rijpstra WIC, Röling WFM, Smidt H, Stams AJM.** 2009. A strictly anaerobic betaproteobacterium *Georgfuchsia toluolica* gen. nov., sp. nov. degrades aromatic compounds with Fe(III), Mn(IV) or nitrate as an electron acceptor. *FEMS Microbiology Ecology* **70**:575-585.
12. **Washer CE, Edwards EA.** 2007. Identification and Expression of Benzylsuccinate Synthase Genes in a Toluene-Degrading Methanogenic Consortium. *Applied and Environmental Microbiology* **73**:1367-1369.
13. **Herrmann S, Vogt C, Fischer A, Kuppardt A, Richnow H-H.** 2009. Characterization of anaerobic xylene biodegradation by two-dimensional isotope fractionation analysis. *Environmental Microbiology Reports* **1**:535-544.
14. **Jehmlich N, Kleinsteuber S, Vogt C, Benndorf D, Harms H, Schmidt F, Von Bergen M, Seifert J.** 2010. Phylogenetic and proteomic analysis of an anaerobic toluene-degrading community. *Journal of Applied Microbiology* **109**:1937-1945.
15. **Abu Laban N, Selesi D, Rattei T, Tischler P, Meckenstock RU.** 2010. Identification of enzymes involved in anaerobic benzene degradation by a strictly anaerobic iron-reducing enrichment culture. *Environ Microbiol* **12**:2783-2796.
16. **Winderl C, Penning H, Netzer Fv, Meckenstock RU, Lueders T.** 2010. DNA-SIP identifies sulfate-reducing *Clostridia* as important toluene degraders in tar-oil-contaminated aquifer sediment. *ISME J* **4**:1314-1325.
17. **Callaghan AV, Davidova IA, Savage-Ashlock K, Parisi VA, Gieg LM, Suflita JM, Kukor JJ, Wawrik B.** 2010. Diversity of Benzyl- and Alkylsuccinate Synthase Genes in Hydrocarbon-Impacted Environments and Enrichment Cultures. *Environmental Science & Technology*.

18. **Yagi JM, Suflita JM, Gieg LM, DeRito CM, Jeon C-O, Madsen EL.** 2010. Subsurface Cycling of Nitrogen and Anaerobic Aromatic Hydrocarbon Biodegradation Revealed by Nucleic Acid and Metabolic Biomarkers. *Applied and Environmental Microbiology* **76**:3124-3134.
19. **Sun W, Cupples AM.** 2012. Diversity of Five Anaerobic Toluene-Degrading Microbial Communities Investigated Using Stable Isotope Probing. *Applied and Environmental Microbiology* **78**:972-980.
20. **Wang L-Y, Li W, Mbadinga SM, Liu J-F, Gu J-D, Mu B-Z.** 2012. Methanogenic Microbial Community Composition of Oily Sludge and Its Enrichment Amended with Alkanes Incubated for Over 500 Days. *Geomicrobiology Journal* **29**:716-726.
21. **Mbadinga S, Li K-P, Zhou L, Wang L-Y, Yang S-Z, Liu J-F, Gu J-D, Mu B-Z.** 2012. Analysis of alkane-dependent methanogenic community derived from production water of a high-temperature petroleum reservoir. *Applied Microbiology and Biotechnology*:1-12.
22. **von Netzer F, Pilloni G, Kleindienst S, Krüger M, Knittel K, Gründger F, Lueders T.** 2012. Enhanced gene detection assays for fumarate-adding enzymes allow uncovering anaerobic hydrocarbon degraders in terrestrial and marine systems. *Applied and Environmental Microbiology*.
23. **Musat F, Galushko A, Jacob J, Widdel F, Kube M, Reinhardt R, Wilkes H, Schink B, Rabus R.** 2009. Anaerobic degradation of naphthalene and 2-methylnaphthalene by strains of marine sulfate-reducing bacteria. *Environ Microbiol* **11**:209-219.
24. **Meckenstock RU, Annweiler E, Michaelis W, Richnow HH, Schink B.** 2000. Anaerobic Naphthalene Degradation by a Sulfate-Reducing Enrichment Culture. *Applied and Environmental Microbiology* **66**:2743-2747.
25. **Wöhlbrand L, Jacob JH, Kube M, Musmann M, Jarling R, Beck A, Amann R, Wilkes H, Reinhardt R, Rabus R.** 2012. Complete genome, catabolic sub-proteomes and key-metabolites of *Desulfobacula toluolica*Tol2, a marine, aromatic compound-degrading, sulfate-reducing bacterium. *Environmental Microbiology*:n/a-n/a.
26. **Callaghan AV, Wawrik B, Ni Chadhain SM, Young LY, Zylstra GJ.** 2008. Anaerobic alkane-degrading strain AK-01 contains two alkylsuccinate synthase genes. *Biochemical and Biophysical Research Communications* **366**:142-148.
27. **Zedelius J, Rabus R, Grundmann O, Werner I, Brodkorb D, Schreiber F, Ehrenreich P, Behrends A, Wilkes H, Kube M, Reinhardt R, Widdel F.** 2011. Alkane degradation under anoxic conditions by a nitrate-reducing bacterium with possible involvement of the electron acceptor in substrate activation. *Environmental Microbiology Reports* **3**:125-135.
28. **Grundmann O, Behrends A, Rabus R, Amann J, Halder T, Heider J, Widdel F.** 2008. Genes encoding the candidate enzyme for anaerobic activation of n-alkanes in the denitrifying bacterium, strain HxN1. *Environmental Microbiology* **10**:376-385.
29. **Acosta-González A, Rosselló-Móra R, Marqués S.** 2013. Characterization of the anaerobic microbial community in oil-polluted subtidal sediments: aromatic biodegradation potential after the Prestige oil spill. *Environ Microbiol* **15**:77-92.