

Figure S1 Anaerobic growth and substrate utilization profiles of *Magnetospirillum* sp. pMbN1 with binary substrate mixtures of (A) succinate and 4-hydroxybenzoate, (B) succinate and phenylacetate, (C) succinate and acetate, and (D) 4-methylbenzoate and phenylacetate. The respective adaptation substrate is always listed first.



Figure S2 Anaerobic growth and substrate utilization profiles of *Magnetospirillum* sp. pMbN1 with binary substrate mixtures of (A) fumarate and benzoate, (B) L-malate and benzoate, (C) oxaloacetate and benzoate, and (D) 4-hydroxybenzoate and succinate. The respective adaptation substrate is always listed first.



Figure S3 Anaerobic growth and substrate utilization profiles of *Magnetospirillum* sp. pMbN1 with binary substrate mixtures of (A) acetate and benzoate, (B) acetate and 4-methylbenzoate, (C) pyruvate and benzoate, and (D) pyruvate and 4-methylbenzoate. The respective adaptation substrate is always listed first.

Table S1 Fold change in the abundance of soluble proteins during anaerobic growth of *Magnetospirillum* sp. pMbN1 with selected substrate mixtures (reference: succinate-adapted cells).

		Fold change in abundance compared to that of cells grown with succinate <sup>b</sup>														
		Single su	ıbstrate <sup>c</sup>	Substr	ate mixtu	re <sup>c</sup>										
				S + B			S + M		M + B			M + B	+ S			
Protein <sup>a</sup>	Predicted function <sup>a</sup>	В	М	Ph 1	Ph2	Ph3	Phla	Ph1b	Ph 1	Ph2	Ph3	Ph1	Ph2a	Ph2b	Ph3a	Ph3b
Proteins re	elated to anaerobic benzoate degradation															
BclA BcrA BcrB BcrC BcrD Dch-1	Benzoate-CoA ligase Benzoyl-CoA reductase, α-subunit Benzoyl-CoA reductase, β-subunit Benzoyl-CoA reductase, γ-subunit Benzoyl-CoA reductase, δ-subunit Cyclohex-1,5-diene-1-carbonyl-CoA hydratase 6-Hydroxycyclohex-1-ene-1-carbonyl-CoA		3.7 20.0 9.4 3.4 8.0 9.8	3.4 18.8 12.1 3.9 11.5 12.2	3.2 18.4 11.0 3.7 10.3 10.6	2.8 12.3 8.1 2.9 7.6 8.1	2.2 9.5 4.5 1.7 3.7 4.8	2.8 12.7 7.0 2.6 5.6 7.1	5.1 29.0 17.1 5.6 15.7 16.9	4.9 27.8 15.9 5.2 14.1 15.2	3.7 23.3 12.0 4.1 11.0 12.3	4.7 24.3 15.4 5.0 14.0 15.1	4.6 26.1 15.2 4.8 14.0 15.0	4.2 21.7 13.1 4.4 11.5 13.4	3.7 19.5 11.0 3.7 9.9 11.5	3.3 16.9 9.6 3.3 8.2 10.2
Had-1 Oah-1 KorA KorB	6-Dydroxycyclonex-1-ene-1-carbonyl-CoA dehydrogenase 6-Oxocyclohex-1-ene-1-carbonyl-CoA hydrolase 2-Oxoacid:ferredoxin oxidoreductase; α-subunit 2. Oxoacid:ferredoxin oxidoreductase; β subunit	<b>3.3</b> <b>8.9</b> 2.1 2.0	<b>2.9</b> <b>5.7</b> 2.3	<b>3.1</b> <b>6.5</b> 1.6	<b>2.9</b> <b>5.8</b> 1.6	<b>2.5</b> <b>4.8</b> 1.5	1.8 <b>2.9</b> 1.6	2.0 4.0 1.8	<b>4.2</b> <b>8.9</b> 1.9	<b>3.9</b> <b>8.4</b> 2.0	<b>3.3</b> <b>7.2</b> 2.2 2.0	<b>3.6</b> <b>7.8</b> 1.7	<b>3.5</b> <b>7.5</b> 1.7	<b>3.3</b> <b>7.0</b> 1.8 1.7	<b>2.8</b> <b>6.6</b> 1.8 1.7	<b>2.8</b> <b>5.9</b> 2.0
Proteins re	elated to uptake and anaerobic degradation of 4-me.	2.0 thvlbenzoa	te	1.5	1.0	1.5	1.4	1.0	1.0	1.0	2.0	1.0	1.0	1.7	1.7	1.7
Orf870	ABC-type transport system, periplasmic solute-binding protein	-1.6	6.2	1.2	-1.2	1.7	4.0	4.7	1.5	6.4	13.6	2.1	1.4	3.8	6.0	5.7
MclA MbrA MbrB MbrC MbrD Dch-2	Putative 4-methylbenzoate-CoA ligase 4-Methylbenzoyl-CoA reductase, α-subunit 4-Methylbenzoyl-CoA reductase, β-subunit 4-Methylbenzoyl-CoA reductase, γ-subunit 4-Methylbenzoyl-CoA reductase, δ-subunit 4-Methylcyclohex-1,5-diene-1-carbonyl-CoA	-1.1 -1.3 <b>4.2</b> 1.2 1.3 1.5	7.2 113.8 54.0 47.1 12.0 4.1	-1.2 -1.8 2.3 1.5 1.2 1.2	1.1 -1.0 <b>2.8</b> 1.3 1.2 1.4	1.2 -1.8 <b>2.9</b> 1.4 1.0 1.4	4.6 47.5 25.3 20.4 9.4 3.1	5.1 69.3 30.7 27.4 17.7 3.2	1.5 <b>10.1</b> <b>7.3</b> <b>6.1</b> 1.8 1.6	3.2 29.3 14.9 12.1 3.7 1.8	4.1 77.3 30.4 27.9 9.2 2.5	1.6 <b>11.6</b> <b>10.4</b> <b>6.5</b> 1.9 1.7	1.3 9.3 6.7 5.5 1.8 1.6	2.9 27.9 16.0 13.3 4.3 1.9	4.0 42.6 22.6 19.3 6.4 2.2	4.9 60.1 30.9 27.4 7.5 2.6
Had-2	4-Methyl-6-hydroxycyclohex-1-ene-1-carbonyl-CoA dehydrogenase	-1.1	1.8	-1.0	1.0	1.1	1.4	1.5	1.0	1.2	1.3	1.1	1.0	1.2	1.3	1.3
Oah-2	4-Methyl-6-oxocyclohex-1-ene-carbonyl-CoA hydrolase	1.6	86.3	1.2	-1.3	-1.1	48.1	61.5	10.6	22.5	39.6	10.4	9.0	22.6	31.5	42.5
Proteins re	Proteins related to succinate uptake and catabolism															
DctP	Periplasmic solute-binding protein of C <sub>4</sub> -dicarboxylate TRAP transporter		-6.0	-2.1	-1.5	1.4	-1.7	-1.9	-5.7	-4.8	-4.4	-3.4	-3.4	-2.4	-2.0	-1.7
PdhB LpdA	Pyruvate dehydrogenase, E1 component Pyruvate dehydrogenase, E3 component		-10.5 -6.2	-3.7 -2.9	-2.1 -2.0	-1.1 -1.2	-1.5 -1.4	-1.9 -1.9	-9.9 -4.9	-9.1 -5.6	-7.5 -5.3	-9.9 -6.2	-10.3 -7.0	-2.7 -2.5	-2.4 -2.4	-2.6 -2.5

<sup>a</sup> For accession numbers and functional annotation of respective proteins see Additional file 1: Table S4. <sup>b</sup> Fold changes in protein abundance as determined by 2D DIGE. Values marked in bold were above the applied threshold for significantly changed abundances (>|2.5|). <sup>c</sup> Abbreviations: B, benzoate; M, 4-methylbenzoate; S, succinate; Ph, phase. For further details see Figure 3.

#### Table S2 Abundance changes (ratios) of selected transcripts in cells of *Magnetospirillum* sp. pMbN1 anaerobically grown with benzoate, 4-methylbenzoate or succinate (reference) as single substrates, or ternary substrate mixture (see Figures 1D and 2).

Ratio in transcript abundance<sup>a</sup>

	Single substra	tes	Mixture of 4-methylbenzoate, benzoate and succinate (sampling points with cultivation time after inoculation)													
			Benzoate u	tilization ph	ase (phase 1)	)		Diauxic lag (phase 2)	g phase	4-Methylbenzoate and bulk succinate utilization phase (phase 3)						
Gene name	4-Methyl- Benzoate benzoate		14.8 h	19.9 h	19.9 h 20.9 h 21.9 h 22.9 l		22.9 h	23.4 h 24.9 h		26.4 h	28.0 h	28.9 h				
Genes related to benz	coate utilization															
bclA	8 ± 3	$3 \pm 2$	6 ± 3	4 ± 1	5 ± 0	6 ± 2	5 ± 1	3 ± 1	1 ± 1	1 ± 0	$2 \pm 0$	4 ± 1				
bcrC	69 ± 25	34 ±14	53 ± 21	48 ± 22	61 ± 16	81 ± 26	60 ± 7	13 ± 3	$2 \pm 0$	5 ±1	8 ± 1	16 ±5				
benK	6 ± 3	2 ± 1	2 ± 1	2 ± 1	$3 \pm 0$	3 ± 1	2 ± 0	1 ± 1	$-1 \pm 0$	-1 $\pm 0$	$-1 \pm 0$	$2 \pm 0$				
Genes related to 4-me	ethylbenzoate uti	lization														
mclA	-2 ± 1	282 ± 78	3 ± 1	3 ± 1	5 ±1	9 ± 1	16 ± 5	139 ± 45	282 ± 51	209 ± 26	230 ± 22	394 ± 139				
mbrC	-1 ± 1	396 ± 109	$2 \pm 0$	2 ± 1	3 ± 1	6 ± 2	7 ± 1	126 ± 45	596 ± 86	548 ± 93	571 ± 87	1046 ± 235				
orf870	-2 ± 1	53 ± 16	2 ± 1	2 ± 0	$2 \pm 0$	$2 \pm 0$	6 ± 1	34 ± 15	108 ± 39	49 ± 11	57 ± 24	62 ± 29				
Genes related to succ	inate utilization															
pdhB	-13 ± 6	-17 ± 8	-19 ± 8	-14 ± 3	-11 ± 3	-14 ± 1	-16 ±4	-19 ± 6	-1 ± 1	-5 ±1	-12 ± 3	-7 ± 2				
maeB	-9 ±5	-11 ± 6	-5 ± 2	-5 ±1	-4 ± 1	-4 ± 1	-3 ± 1	$-3 \pm 1$	$-2 \pm 0$	$-2 \pm 1$	-3 ± 1	-1 ± 1				
dctP	-18 ± 8	-19 ± 7	-6 ± 2	-8 ± 3	$-4 \pm 0$	-4 ± 2	-4 ± 2	-4 ± 1	$-2 \pm 0$	$-2 \pm 0$	$-2 \pm 0$	-1 ± 2				
dctM	-14 ± 4	-15 ± 6	-5 ± 2	-6 ±0	-5 ±1	-4 ± 1	-3 ± 1	$-3 \pm 1$	$-1 \pm 0$	$-2 \pm 0$	-2 ± 1	-1 ± 1				
dctA	-18 ± 7	-26 ±12	-6 ± 3	-6 ± 2	-3 ± 1	-4 ± 1	-3 $\pm 1$	-5 ± 2	-4 ± 2	-3 $\pm 1$	-3 ± 1	-2 ± 1				

<sup>a</sup> Ratio of transcript abundance between test and reference (succinate) state. Data used for calculation of transcript abundances is provided in Table S7.

Ratio: > 500 > 250 > 50 > 10  $\geq$  5 > -5  $\leq$  -5  $\leq$  -10 < -20

# Table S3 Selected proteins identified from membrane protein-enriched fractions of *Magnetospirillum* sp. pMbN1 grown anaerobically with benzoate, 4-methylbenzoate and succinate as single substrates, or ternary mixture (for 1DE gel image see Additional file 1: Figure S4).

		Single substra	ites		Ternary subs		
Protein name <sup>a</sup>	Predicted function <sup>a</sup>	Benzoate	4-Methyl- benzoate	Succinate	Phase 1	Phase 2b	Phase 3b
Proteins related to benzoate	uptake						
BenK	Benzoate/H <sup>+</sup> symporter	476	277		304	325	301
Proteins related to 4-methyll	benzoate uptake						
Orf960	ABC-type transport system, permease component						100
Orf950	ABC-type transport system, permease component		74			62	185
Orf930	ABC-type transport system, ATPase component		115				58
Orf920	ABC-type transport system, ATPase component		153			46	133
Proteins related to $C_4$ -dicarb	poxylate uptake and degradation						
DctQ	TRAP-type C <sub>4</sub> -dicarboxylate transport system, permease	82		252		129	157
DctM	TRAP-type C <sub>4</sub> -dicarboxylate transport system, permease			98	63		
DctA	C <sub>4</sub> -dicarboxylate/Na <sup>+</sup> symporter			254	65	113	137
MaeB	NADP <sup>+</sup> -dependent malic enzyme			93	89		
PdhA	Pyruvate dehydrogenase, E1 component, α-subunit			45			
Central metabolism							
GltA	Citrate synthase	114	93		226	99	
AcnB	Aconitase	80	127		126		
Icd	Isocitrate dehydrogenase	244	284		545	174	147
SucC	Succinyl-CoA ligase, β-subunit	269	297	217	500	252	281
SucD	Succinyl-CoA ligase, $\alpha$ -subunit		76		151	75	
SdhB	Succinate dehydrogenase, β- subunit				35		
SdhC	Succinate dehydrogenase, y- subunit	206	166	122		136	172
SdhD	Succinate dehydrogenase, δ- subunit	102	102	93	35	73	118
FumA	Fumarate hydratase class I	126			249		
Mdh	Malate dehydrogenase	305	241	173	398	290	178
		Mascots	score: not de	tected	> 20	> 200	> 500

<sup>a</sup> For accession numbers and functional annotation of respective proteins see Additional file 1: Table S4.

		-			<u> </u>						
		Accession	Destain	Length						Accession	
Gene identifier	Status <sup>a</sup>	No. <sup>b</sup>	name	(anno acids)	INTERPRO	Gene name	Organism	Identity	e-Value	no. <sup>d</sup>	Predicted function
Genes related to ana	erobic 4-me	thylbenzoate u	utilization								
pmbn1w00890	F	KF941519	MclA	549	IPR000873	bclA	Thauera aromatica	26%	6×10 <sup>-24</sup>	Q8GQN9	4-Methylbenzoate-CoA ligase
pmbn1w00500	F	KF941520	MbrC	390	IPR010327	bcrC	Thauera aromatica	33%	1×10 <sup>-59</sup>	O87874	4-Methylbenzoyl-CoA reductase, γ-subunit
pmbn1w00490	F	KF941521	MbrB	450	IPR010327	bcrB	Thauera aromatica	33%	3×10 <sup>-46</sup>	O87875	4-Methylbenzoyl-CoA reductase, β-subunit
pmbn1w00510	F	KF941522	MbrA	269	IPR002731, IPR008275	bcrA	Thauera aromatica	46%	3×10 <sup>-49</sup>	O87876	4-Methylbenzoyl-CoA reductase, α-subunit
pmbn1w00520	F	KF941523	MbrD	291	IPR002731, IPR008275	bcrD	Thauera aromatica	33%	$1 \times 10^{-32}$	O87877	4-Methylbenzoyl-CoA reductase, δ-subunit
pmbn1w00830	F	KF941524	Dch-2	257	IPR001753, IPR017602	dch	Thauera aromatica	64%	9×10 <sup>-80</sup>	O87873	4-Methylcyclohex-1,5-diene-1-carbonyl-CoA hydratase
pmbn1w00850	F	KF941525	Had-2	324	IPR002085, IPR002328, IPR011032, IPR013149, IPR013154, IPR016040, IPR017614	had	Thauera aromatica	64%	$1 \times 10^{-103}$	O87871	6-Hydroxy-4-methylcyclohex-1-ene-1-carbonyl-CoA dehydrogenase
pmbn1w00840	F	KF941526	Oah-2	379	IPR001753, IPR017613,	oah	Thauera aromatica	75%	$1 \times 10^{-172}$	087872	4-Methyl-6-oxocyclohex-1-ene-1-carbonyl-CoA hydrolase
pmbn1w00880	F	KF941527	Orf880	222	IPR001647, IPR009057, IPR011075, IPR015893, IPR023772	mb3439c	Mycobacterium bovis	37%	3×10 <sup>-7</sup>	P67443	TetR-type transcriptional regulator
pmbn1w00870	F	KF941528	Orf870	381	IPR028081, IPR028082	amb1552	Magnetospirillum magneticum	60%	7×10 <sup>-156</sup>	Q2W719	ABC-type transport system, periplasmic solute-binding protein
pmbn1w00920	F	KF941529	Orf920	266	IPR003439, IPR003593, IPR017871, IPR027417	livF	Salmonella typhimurium	51%	$8 \times 10^{-70}$	P0A191	ABC-type transport system, ATP-binding protein
pmbn1w00930	F	KF941530	Orf930	271	IPR003439, IPR003593, IPR017871, IPR027417	braF	Pseudomonas aeruginosa	42%	$4 \times 10^{-65}$	P21629	ABC-type transport system, ATP-binding protein
pmbn1w00950	F	KF941531	Orf950	290	IPR001851	livH	Salmonella typhimurium	31%	$6 \times 10^{-47}$	P0A2J1	ABC-type transport system, permease component
pmbn1w00960	F	KF941532	Orf960	348	IPR001851	braE	Pseudomonas aeruginosa	33%	$4 \times 10^{-47}$	P21628	ABC-type transport system, permease component
Genes related to ana	erobic benz	oate utilizatio	n								
pmbn1C394_0012	F	KF941495	BclA	528	IPR000873, IPR025110, IPR011957	bclA	Thauera aromatica	60%	0.0	Q8GQN9	Benzoate-CoA ligase (EC 6.2.1.25)
pmbn1w00160	F	KF941533	BcrC	391	IPR010327, IPR011955	bcrC	Thauera aromatica	62%	$1 \times 10^{-154}$	O87874	Benzoyl-CoA reductase, y-subunit
pmbn1w00150	F	KF941534	BcrB	432	IPR010327, IPR011955	bcrB	Thauera aromatica	75%	0.0	O87875	Benzoyl-CoA reductase, β-subunit
pmbn1w00140	F	KF941535	BcrA	430	IPR002731, IPR008275, IPR011954	bcrA	Thauera aromatica	72%	$1 \times 10^{-178}$	O87876	Benzoyl-CoA reductase, a-subunit
pmbn1w00130	F	KF941536	BcrD	279	IPR002731, IPR008275, IPR011956	bcrD	Thauera aromatica	74%	$1 \times 10^{-107}$	O87877	Benzoyl-CoA reductase, δ-subunit
pmbn1w00120	F	KF941537	Fdx	79	IPR017896, IPR017900	fdx	Thauera aromatica	60%	6×10 <sup>-15</sup>	O88151	Ferredoxin
pmbn1w00200	F	KF941538	KorA	578	IPR002869, IPR002880, IPR005476, IPR009014, IPR015941, IPR019752, IPR022367	korA	Thauera aromatica	60%	0.0	O87870	2-Oxoglutarate:ferredoxin oxidoreductase, $\alpha$ -subunit
pmbn1w00210	F	KF941539	KorB	279	IPR011766	korB	Thauera aromatica	67%	1×10 <sup>-111</sup>	Q8RJQ9	2-Oxoglutarate:ferredoxin oxidoreductase, β-subunit
pmbn1w00170	F	KF941540	Dch-1	259	IPR001753, IPR017602	dch	Thauera aromatica	66%	4×10 <sup>-82</sup>	O87873	Cyclohex-1,5-diene-1-carbonyl-CoA hydratase
pmbn1w00190	F	KF941541	Had-1	358	IPR002085, IPR002328, IPR011032, IPR013149, IPR013154, IPR016040, IPR017614	had	Thauera aromatica	63%	1×10 <sup>-114</sup>	O87871	6-Hydroxycylohex-1-ene-1-carbonyl-CoA dehydrogenase
pmbn1w00180	F	KF941542	Oah-1	377	IPR001753, IPR017613	oah	Thauera aromatica	79%	1×10 <sup>-179</sup>	087872	6-Oxocyclohex-1-ene-1-carbonyl-CoA hydratase
pmbn1w04893	D	KF941494	BenK	453	IPR005829, IPR011701, IPR016196, IPR020846	benK	Acinetobacter sp.	49%	$1 \times 10^{-152}$	O30513	Benzoate/ H <sup>+</sup> symporter
Genes related to succ	inate utiliz	ation									
pmbn1C3_00100	F	KF941496	DctP	337	IPR004682, IPR018389	dctP	Rhodobacter capsulatus	69%	6×10 <sup>-153</sup>	P37735	TRAP-type periplasmic $C_4$ -dicarboxylate-binding protein
pmbn1C3_00110	F	KF941497	DctQ	233	IPR007387	dctQ	Rhodobacter capsulatus	52%	7×10 <sup>-64</sup>	D5AQF8	TRAP-type C4-dicarboxylate transport system, permease component
pmbn1C3_00120	F	KF941498	DctM	427	IPR004681, IPR010656	dctM	Rhodobacter capsulatus	77%	0.0	D5AQF7	TRAP-type $C_4$ -dicarboxylate transport system, permease component

#### Table S4 Anntotation of genes related to anaerobic degradation of 4-methylbenzoate, benzoate and succinate in Magnetospirillum sp. pMbN1.

#### Table S4 continued

				Tanadh			relevant BLAS	STP hit <sup>c</sup>			
		Accession	Protein	Length (amino						Accession	
Gene identifier	Status <sup>a</sup>	No. <sup>b</sup>	name	acids)	INTERPRO	Gene name	Organism	Identity	e-Value	no. <sup>d</sup>	Predicted function
pmbn1C3_00090	F	KF941499	DctS	651	IPR000014, IPR000700, IPR001610, IPR003594, IPR003661, IPR005467, IPR009082, IPR013767, IPR004358	dctS	Rhodobacter capsulatus	42%	2×10 <sup>-149</sup>	P37739	Sensory histidine kinase of $C_4$ -dicarboxylate-responsive sensory/regulatory system
pmbn1C3_00080	F	KF941500	DctR	200	IPR000792, IPR001789, IPR011006, IPR011991	dctR	Rhodobacter capsulatus	51%	6×10 <sup>-58</sup>	P37740	DNA-binding response regulator of $C_4$ -dicarboxylate-responsive sensory/regulatory system
pmbn1w02526	D	KF941501	DctA	415	IPR001991, IPR018107	dctA	Sinorhizobium meliloti	63%	$4 \times 10^{-172}$	P20672	C4-dicarboxylate/ Na <sup>+</sup> symporter
pmbn1w03530	D	KF941502	DctB	581	IPR003594, IPR003661, IPR005467, IPR009082, IPR017055, IPR004358	dctB	Rhizobium leguminosarum	34%	1×10 <sup>-93</sup>	P10047	Sensory histidine kinase of $\mathrm{C}_4\text{-}dicarboxylate\text{-}responsive sensory/regulatory system$
pmbn1w3533	D	KF941503	DctD	446	IPR001789, IPR002078, IPR002197, IPR003593, IPR009057, IPR011006, IPR025943, IPR025944, IPR027417	dctD	Sinorhizobium meliloti	48%	3×10 <sup>-137</sup>	P13632	DNA-binding response regulator of $\mathrm{C}_4\text{-}\mathrm{dicarboxylate\text{-}responsive sensory/regulatory system}$
pmbn1d03865	D	KF941505	PdhA	332	IPR017597, IPR001017	pdhA	Sinorhizobium meliloti	68%	$3 \times 10^{-154}$	Q9R9N5	Pyruvate dehydrogenase, E1 component, α-subunit
pmbn1d03867	D	KF941506	PdhB	440	IPR000089, IPR003016, IPR005475, IPR005476, IPR009014, IPR011053, IPR015941, IPR027110	pdhB	Sinorhizobium meliloti	63%	0.0	Q9R9N4	Pyruvate dehydrogenase, E1 component, $\beta$ -subunit
pmbn1d03876	D	KF941507	LpdA	462	IPR004099, IPR006258, IPR012999, IPR013027, IPR016156, IPR023753	lpdA	Rhizobium etli	61%	0.0	O05940	Pyruvate dehydrogenase, E3 component
pmbn1w00775	D	KF941504	MaeB	762	IPR002505, IPR012188, IPR012301, IPR012302, IPR016040	maeB	Escherichia coli	62%	0.0	P76558	NADP*-dependent malic enzyme
Genes related to cent	ral metabo	lism									
pmbn1w00530	D	KF941508	GltA	438	IPR002020, IPR010953, IPR016141, IPR016142, IPR016143, IPR019810, IPR024176	gltA	Sinorhizobium meliloti	73%	0.0	O33915	Citrate synthase (EC 2.3.3.1)
pmbn1w03702	D	KF941509	AcnB	861	IPR001030, IPR004406, IPR015928, IPR015929, IPR015930, IPR015931, IPR015932 IPR018136 IPR015933, IPR015937	acnB	Pseudomonas aeruginosa	73%	0.0	Q9I2V5	Aconitase 2 (EC 4.2.1.3)
pmbn1w00603	D	KF941510	Icd	741	IPR004436, IPR024084	icd	Azotobacter vinelandii	75%	0.0	P16100	Isocitrate dehydrogenase (EC 1.1.1.42)
pmbn1w06208	D	KF941511	SucC	398	IPR005809, IPR005811, IPR011761, IPR013650, IPR013815, IPR013816, IPR016102	sucC	Magnetospirillum magneticum	97%	0.0	Q2W063	Succinyl-CoA ligase, β-subunit (EC 6.2.1.5)
pmbn1w06210	D	KF941512	SucD	291	IPR003781, IPR005810, IPR005811, IPR016040, IPR016102, IPR017440	sucD	Rickettsia bellii	78%	5×10 <sup>-166</sup>	Q1RH56	Succinyl-CoA ligase, α-subunit (EC 6.2.1.5)
pmbn1w04352	D	KF941513	SdhA	593	IPR003952, IPR003953, IPR011281, IPR014006, IPR015939, IPR027477	sdhA	Paracoccus denitrificans	72%	0.0	Q59661	Succinate dehydrogenase, flavoprotein subunit (EC 1.3.99.1)
pmbn1w04353	D	KF941514	SdhB	259	IPR001041, IPR004489, IPR006058, IPR009051, IPR012285, IPR012675, IPR017900, IPR017896, IPR025192	sdhB	Rickettsia bellii	72%	4×10 <sup>-139</sup>	Q1RGP3	Succinate dehydrogenase, iron-sulfur subunit
pmbn1w04350	D	KF941515	SdhC	109	IPR000701, IPR014314, IPR014361, IPR018495	sdhC	Paracoccus denitrificans	45%	$4 \times 10^{-24}$	Q59659	Succinate dehydrogenase, cytochrome $b_{556}$ subunit
pmbn1w04351	D	KF941516	SdhD	126	IPR000701, IPR014312	sdhD	Rickettsia bellii	39%	$8 \times 10^{-19}$	Q1RHB6	Succinate dehydrogenase, hydrophobic membrane anchor subunit
pmbn1w01837	D	KF941517	FumA	538	IPR004646, IPR004647, IPR011167, IPR020557	fumA	Salmonella typhimurium	67%	0.0	P40720	Fumarate hydratase class I (Fumarase) (EC 4.2.1.2)
pmbn1w06206	D	KF941518	Mdh	319	IPR001236, IPR001557, IPR011275, IPR015955, IPR016040, IPR022383	mdh	Magnetospirillum magneticum	99%	0.0	Q2W064	Malate dehydrogenase (EC 1.1.1.37)

<sup>a</sup> Status of DNA sequence: F, finished; D, draft.

<sup>b</sup> GenBank accession number.

<sup>c</sup> Proteins related to the anaerobic degradation of 4-methylbenzoate and benzoate were compared to protein sequences of biochemically characterized proteins of Thauera aromatica K172.

<sup>d</sup> UniProtKB/Swiss-Prot accession number.

		Growth with single substrates									
From experiment with binary	mixture of:	Adaptati	on substrate	Co-su	ıbstrate						
Adaptation substrate	<b>Co-substrate</b>	<b>OD</b> <sub>max</sub>	$\mu_{max}(h^{-1})$	<b>OD</b> <sub>max</sub>	$\mu_{max} \left( \mathbf{h}^{-1} \right)$						
Cells adapted to succinate											
Succinate (5 mM)	Benzoate (1 mM)	0.38	0.21	0.18	0.21						
Succinate (5 mM)	4-Methylbenzoate (1 mM)	0.38	0.21	0.15	0.13						
Succinate (5 mM)	4-Hydroxybenzoate (1 mM)	0.36	0.20	ND	ND						
Succinate (5 mM)	Phenylacetate (1 mM)	0.36	0.20	0.19	0.16						
Succinate (5 mM)	Acetate (8 mM)	0.36	0.20	0.30	0.22						
Cells adapted to 4-methylbenzod	ate										
4-Methylbenzoate (1.5 mM)	Benzoate (1.5 mM)	0.27	0.16	0.28	0.15						
4-Methylbenzoate (1.5 mM)	Phenylacetate (1.5 mM)	0.27	0.16	0.32	0.16						
Cells adapted to other aliphatic	acids										
Acetate (8 mM)	Benzoate (1 mM)	0.27	0.18	0.17	0.20						
Acetate (8 mM)	4-Methylbenzoate (1 mM)	0.27	0.18	0.18	0.19						
Pyruvate (6 mM)	Benzoate (1 mM)	0.34	0.18	0.18	0.21						
Pyruvate (6 mM)	4-Methylbenzoate (1 mM)	0.34	0.18	0.19	0.16						
Fumarate (5 mM)	Benzoate (1 mM)	0.37	0.27	0.19	0.17						
L-Malate (5 mM)	Benzoate (1 mM)	0.37	0.33	0.16	0.25						
Oxaloacetate (6 mM)	Benzoate (1 mM)	0.27	0.20	0.17	0.23						
Cells adapted to other aromatic	acids										
4-Hydroxybenzoate (1 mM)	Succinate (5 mM)	0.13	0.20	0.34	0.10						

## Table S5 Anaerobic growth of *Magnetospirillum* sp. pMbN1 with aliphatic and aromatic acids supplied as single substrates.

Preferentially utilized substrates are highlighted in boldface (see Table 1). Applied substrate concentrations are indicated in parentheses. Values for maximal optical density ( $OD_{max}$ ) and maximum specific growth rates ( $\mu_{max}$ ) are based on at least two replicate cultures yielding a standard deviation of <5%.  $\mu_{max}$  was calculated from the slope of the active growth phase (*m*):  $\mu_{max} = m \times 1/\Delta OD$ . ND, not determined.

Primer	Sequence $(5' \rightarrow 3')$	Target gene	Product length (bp)	Real-time detection temperature (°C)
benK 391F benK 550R	GTGGCGCTGATGACCGAATACG GAACCGCCGCCACGAAGAACAC	benK	160	84
berC 809F berC 963R	CAGCTTCCAGATGCCCGACAGGTT TCATCCACGGCGACGCTTACTATCA	bcrC	155	83
bclA 125F bclA 276R	CCGACGGCAGCCACACCTAC CTTGACGGCGCCCCAGAAC	bclA	152	83
orf870 457F orf870 615R	TCGGGGCGTGAGCAGAC GGCCCCCGTCCCGAATAC	orf870	159	82
mbrC 723F mbrC 872R	ATAAAATGCGGGTGGTGTTGGAAGG GGTCGCCCGCAGTATTGATGTCC	mbrC	150	83
mclA 217F mclA 399R	CGCTTCGCCCGTGGTCTCTAC GCCCGCATGTCGCAATAAGGTC	mclA	183	83
dctA 728F dctA 879R	TGCTGGTCGGCTTCAACATCTTCA CACCAGGCCGACCACCGACTT	dctA	152	84
dctP 319F dctP 495R	TCTTCGACCTGCCCTACATCTTCC GTCGGCGGGGGGTCTTGAGC	dctP	176	84
dctM 181F dctM 344R	TTCTTCATCCTGGCGGGCAACTT ACGGTGGCGGGGGCTGGAG	dctM	164	87
pdhB 716F pdhB 877R	GCGCCCAGCACAGCCAGGATTA TCTGGCCGTAGAGCAGTTCGTTTTC	pdhB	161	84
maeB 1121F maeB 1286R	CCTTCGGGCCGGAATACCTGA CCGAGCGGAACACGAACTGGT	maeB	165	84

### Table S6 Primers used for reverse transcription real-time PCR.

	Table S7 Average cycle threshold	(C <sub>T</sub> ) v	alues and efficiencies (	(E)	obtained from reverse trans	scription real-time PCR reactions.
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	Average	e $C_T$ values	and effici	encies <sup>a</sup> :																						
	Single s	substrate					Ternary	y mixture	of 4-met	hylbenzoa	ite, benzo	pate and s	uccinate	(cultivatio	on time a	fter inocula	tion)									
							Benzoate utilization phase (phase 1)											lag phase	(phase 2	2)	4-Methylbenzoate and bulk succinate utilization phase (phase 3)					
Gene name	Ben	zoate	4-M ben	ethyl- zoate	Succinate		14.8 h		19.9 h		20.9 h		21	.9 h	22.9 h		23.4 h		24.9 h		26.4 h		28.0 h		28.9 h	
	$C_{T}$	Е	CT	Е	CT	Е	CT	Е	$C_T$	Е	$C_{T}$	Е	CT	Е	CT	Е	CT	Е	CT	Е	CT	Е	CT	Е	$C_{T}$	Е
bclA	24.4	1.79	25.5	1.81	27.4	1.78	24.4	1.72	25.1	1.77	24.8	1.84	24.5	1.77	24.6	1.82	25.4	1.86	27.5	1.81	27.1	1.79	26.4	1.83	25.1	1.82
	±1.0	±0.04	±0.9	±0.04	±0.6	±0.06	±0.7	±0.05	±0.3	±0.06	±0.1	±0.07	±0.5	±0.05	±0.5	±0.06	±0.4	±0.05	±0.3	±0.03	±0.2	±0.04	±0.3	±0.06	±0.4	±0.07
bcrC	20.8 ±0.8	1.85 ±0.03	21.7 ±0.6	1.83 ±0.04	27.7 ±0.6	1.77 ±0.06	21.0 ±0.6	1.74 ±0.08	21.1 ±0.6	1.78 ±0.05	20.8 ±0.4	1.86 ±0.02	20.3 ±0.5	1.77 ±0.04	20.8 ±0.2	1.73 ±0.06	23.4 ±0.4	1.88 ±0.06	$\begin{array}{c} 26.8 \\ \pm 0.0 \end{array}$	1.83 ±0.03	25.0 ±0.3	1.79 ±0.07	24.2 ±0.3	1.77 ±0.05	23.0 ±0.5	1.88 ±0.04
benK	24.4	1.87	26.1	1.87	27.2	1.84	25.8	1.99	26.1	1.86	25.7	1.84	25.5	1.91	26.0	1.82	26.9	1.89	27.8	1.83	27.7	1.87	27.9	1.91	26.6	1.90
	±0.7	±0.05	±0.5	±0.07	±0.5	±0.07	±0.6	±0.04	±0.4	±0.07	±0.2	±0.03	±0.6	±0.06	±0.2	±0.05	±0.4	±0.08	±0.3	±0.05	±0.2	±0.05	±0.4	±0.06	±0.3	±0.04
mclA	29.9	1.83	19.9	1.86	28.7	1.84	26.9	1.86	26.8	1.83	26.2	1.83	25.2	1.81	24.3	1.82	20.7	1.87	19.6	1.89	20.1	1.90	20.0	1.91	19.0	1.91
	±0.7	±0.05	±0.6	±0.04	±0.5	±0.06	±0.5	±0.03	±0.3	±0.04	±0.2	±0.04	±0.2	±0.02	±0.5	±0.06	±0.5	±0.01	±0.3	±0.07	±0.2	±0.02	±0.2	±0.05	±0.6	±0.03
mbrC	27.7 ±1.0	$\substack{1.81\\\pm0.04}$	17.5 ±0.4	1.98 ±0.06	27.1 ±0.7	1.80 ±0.05	26.0 ±0.2	1.83 ±0.06	26.3 ±0.9	1.84 ±0.03	25.4 ±0.3	1.87 ±0.05	24.4 ±0.5	1.80 ±0.05	24.2 ±0.1	1.78 ±0.06	19.3 ±0.5	1.80 ±0.06	16.8 ±0.2	1.94 ±0.04	17.0 ±0.3	1.90 ±0.03	16.9 ±0.2	$\substack{1.88\\\pm0.05}$	15.9 ±0.4	1.90 ±0.04
orf870	27.8	1.85	20.5	1.90	26.8	1.74	26.0	1.80	26.1	1.86	25.9	1.82	25.5	1.83	24.0	1.88	20.9	1.79	19.1	1.81	20.4	1.86	20.1	1.87	19.9	1.75
	±0.7	±0.07	±0.7	±0.05	±0.7	±0.06	±0.6	±0.05	±0.3	±0.06	±0.1	±0.04	±0.1	±0.06	±0.2	±0.05	±0.7	±0.06	±0.6	±0.07	±0.4	±0.05	±0.6	±0.04	±0.8	±0.04
pdhB	25.6	1.87	26.1	1.89	21.5	1.94	26.2	1.86	25.8	1.85	25.4	1.91	25.8	1.93	26.0	1.86	26.3	1.89	22.2	1.88	24.1	1.85	25.6	1.83	24.8	1.94
	±0.7	±0.06	±0.7	±0.06	±0.7	±0.07	±0.7	±0.04	±0.3	±0.06	±0.5	±0.04	±0.2	±0.06	±0.4	±0.06	±0.5	±0.04	±0.5	±0.02	±0.4	±0.06	±0.4	±0.07	±0.5	±0.04
maeB	25.9	1.84	26.1	1.85	22.3	1.84	24.8	1.83	25.1	1.85	24.7	1.86	24.5	1.79	24.3	1.83	24.0	1.96	23.2	1.85	23.1	1.86	24.2	1.87	22.7	1.88
	±0.8	±0.04	±1.0	±0.04	±0.8	±0.05	±0.8	±0.04	±0.4	±0.07	±0.3	±0.05	±0.6	±0.03	±0.3	±0.06	±0.8	±0.05	±0.4	±0.04	±0.6	±0.03	±0.6	±0.07	±0.4	±0.07
dctP	23.5	1.85	23.7	1.86	18.8	1.83	21.8	1.78	22.3	1.84	21.3	1.85	21.3	1.77	21.2	1.75	21.1	1.91	19.7	1.87	19.9	1.93	19.9	1.88	18.9	1.86
	±0.7	±0.04	±0.6	±0.06	±0.6	±0.05	±0.5	±0.05	±0.6	±0.06	±0.1	±0.05	±0.8	±0.04	±0.6	±0.06	±0.4	±0.06	±0.3	±0.03	±0.4	±0.06	±0.4	±0.06	±0.7	±0.04
dctM	27.6	1.76	27.7	1.79	23.1	1.84	25.9	1.84	26.2	1.83	25.8	1.81	25.5	1.76	25.3	1.87	24.9	1.77	23.9	1.78	24.5	1.84	24.7	1.83	23.5	1.71
	±0.5	±0.07	±0.6	±0.06	±0.5	±0.06	±0.5	±0.05	±0.1	±0.06	±0.2	±0.05	±0.2	±0.07	±0.4	±0.04	±0.6	±0.06	±0.3	±0.05	±0.3	±0.05	±0.4	±0.07	±0.5	±0.04
dctA	25.5	1.89	26.5	1.87	20.9	1.86	23.9	1.82	24.0	1.83	23.0	1.86	23.1	1.82	22.9	1.81	23.7	1.82	23.2	1.82	22.8	1.85	22.9	1.89	22.0	1.87
	±0.9	±0.05	±0.9	±0.03	±0.7	±0.06	±0.8	±0.06	±0.5	±0.07	±0.3	±0.06	±0.3	±0.05	±0.6	±0.07	±0.5	±0.05	±0.6	±0.06	±0.4	±0.05	±0.4	±0.04	±0.5	±0.03

<sup>a</sup> The lower value represents the standard deviation. The efficiencies were determined as described in the Methods section. The average efficiency per primer pair was used to calculate the ratios in Table S2.