

TABLE S1. Summary of performed physiological experiments with denitrifying strain EbN1. Tested concentrations of aromatic solvents and their effect on growth rate and maximum optical density in alkylbenzene- and succinate-utilizing cultures are displayed. Determined semi-inhibitory (about 50% growth inhibition) concentrations are highlighted in grey.

Solvent concentration [mM]	Alkylbenzene-utilizing cultures				Succinate-utilizing cultures after exposure to <sup>a</sup>									
	Ethylbenzene		Toluene		Ethylbenzene		Toluene		<i>p</i> -Cresol		Phenol		Solvent mixture	
	$\mu^b$ (h <sup>-1</sup> )	OD <sup>c</sup>	$\mu^b$ (h <sup>-1</sup> )	OD <sup>c</sup>	$\mu^b$ (h <sup>-1</sup> )	OD <sup>c</sup>	$\mu^b$ (h <sup>-1</sup> )	OD <sup>c</sup>	$\mu^b$ (h <sup>-1</sup> )	OD <sup>c</sup>	$\mu^b$ (h <sup>-1</sup> )	OD <sup>c</sup>	$\mu^b$ (h <sup>-1</sup> )	OD <sup>c</sup>
<u>Ethylbenzene<sup>d</sup></u>														
0.02	0.009	0.34												
0.08	0.017	0.35												
0.21	0.012	0.30												
<b>0.32</b>	<b>0.007</b>	<b>0.21</b>												
0.40	0.004	0.32												
0.48	0.001	0.11												
<u>Toluene<sup>d</sup></u>														
0.07			0.012	0.33										
0.24			0.015	0.32										
<b>0.74</b>			<b>0.003</b>	<b>0.35</b>										
0.86			0.001	0.10										
<u>Ethylbenzene<sup>e</sup></u>														
0.3					0.035	0.50								
0.4					0.038	0.45								
<b>0.5</b>					<b>0.016</b>	<b>0.33</b>								
0.6					0.004	0.21								
<u>Toluene<sup>e</sup></u>														
0.7							0.038	0.51						
1.0							0.025	0.34						
<b>1.2</b>							<b>0.014</b>	<b>0.31</b>						
1.5							0.008	0.26						
3.0							0.000	0.13						
<u><i>p</i>-Cresol<sup>e</sup></u>														
2.0									0.035	0.40				
2.5									0.031	0.39				
<b>3.0</b>									<b>0.026</b>	<b>0.33</b>				
3.5									0.015	0.24				
4.0									0.002	0.2				
<u>Phenol<sup>e</sup></u>														
5.0											0.034	0.57		
6.0											0.027	0.44		
<b>6.5</b>											<b>0.022</b>	<b>0.38</b>		
7.0											0.015	0.29		
8.0											0.003	0.21		
<u>Solvent mixture<sup>f</sup></u>														
1.9													0.025	0.39
2.5													0.019	0.35
2.6													0.017	0.33
<b>2.7</b>													<b>0.014</b>	<b>0.30</b>
2.9													0.003	0.24
3.0													0.001	0.19

<sup>a</sup> Succinate-adapted, actively growing cultures of strain EbN1 were shocked with ethylbenzene, toluene, *p*-cresol or phenol as single solvents and with a mixture of these four during early linear growth. The growth rate of untreated, succinate-utilizing cultures was essentially constant (0.053 h<sup>-1</sup>). The maximum optical density varied around an average of 0.51.

<sup>b</sup> The growth rate corresponds to the slope in the linear range of monitored time courses of growth.

<sup>c</sup> OD refers to the maximum optical density (at 660 nm) at the transition into stationary growth phase (alkylbenzene-utilizing cells) or at the end of the incubation time (succinate-utilizing cells).

<sup>d</sup> Equilibrium concentrations of the alkylbenzenes in the culture medium, while supplied in an HMN carrier phase. Ethylbenzene: 0.02 mM = 0.5% (v/v); 0.08mM = 2.0% (v/v); 0.21mM = 5.0% (v/v); 0.32 mM = 8.0% (v/v) and 0.48 mM = 12% (v/v). Toluene: 0.07 mM = 0.5% (v/v); 0.24 mM = 2.0% (v/v); 0.74 mM = 5.5 % (v/v) and 0.86 mM = 6.0% (v/v).

<sup>e</sup> Concentration of single solvents in the culture medium.

<sup>f</sup> The indicated concentrations refer to the total solvent concentration in the culture medium containing ethylbenzene, toluene, *p*-cresol and phenol.

TABLE S2. Fold changes in abundance of identified proteins during anaerobic growth of strain EbN1 with low and semi-inhibitory concentrations of ethylbenzene and toluene.

Protein name <sup>a</sup>	Predicted function <sup>a</sup>	Score <sup>b</sup>	Ethylbenzene [mM] <sup>c</sup>		Toluene [mM] <sup>c</sup>	
			0.08 <sup>d</sup>	0.32 <sup>d</sup>	0.07 <sup>e</sup>	0.74 <sup>e</sup>
<u>Denitrification/Respiration</u>						
NirS	Cytochrome <i>cdl</i> nitrite reductase precursor	328	-1.3	3.0	-2.3	3.2
FdhA (EbA2936)	Formate dehydrogenase, $\alpha$ -subunit	117	1.2	1.6	1.1	4.0
UbiE	Ubiquinone/menaquinone biosynthesis methyltransferase	90	2.5	1.1	1.2	1.4
<u>Ethylbenzene degradation pathway</u>						
EbdA	Ethylbenzene dehydrogenase, $\alpha$ -subunit	43	2.1	-1.3	1.1	2.7
EbdB	Ethylbenzene dehydrogenase, $\beta$ -subunit	285	1.4	-1.1	1.3	3.6
EbdC	Ethylbenzene dehydrogenase, $\gamma$ -subunit	89	1.2	1.1	-1.1	2.8
EbdD	Ethylbenzene dehydrogenase, chaperone	n.d.	-1.4	-1.8	-1.3	1.3
C1A68	Hypothetical protein, related to ethylbenzene degradation	292	1.2	-1.1	1.0	3.0
C1A84	Hypothetical protein, related to ethylbenzene degradation	n.d.	-2.1	1.6	-1.3	3.6
Bal	Benzoylacetate CoA-ligase	n.d.	1.1	-2.3	-1.1	1.8
Apc1	Acetophenone carboxylase, subunit 1	n.d.	-1.1	-2.4	-1.0	1.7
Apc3	Acetophenone carboxylase, subunit 3	n.d.	1.1	-1.9	-1.1	1.6
Apc4	Acetophenone carboxylase, subunit 4	n.d.	-1.1	-1.6	1.5	1.8
Apc5	Acetophenone carboxylase, subunit 5	n.d.	-1.6	-1.4	-1.2	2.0
<u>Toluene degradation pathway</u>						
BssA	Benzylsuccinate synthase, $\alpha$ -subunit	297	1.7	1.6	1.0	3.3
BssE	Benzylsuccinate synthase, chaperone	237	1.2	2.0	-1.4	3.1
C2A200	Hypothetical protein, related to toluene degradation	239	1.2	1.8	1.0	2.8
BbsH	Phenylitaconyl-CoA hydratase	n.d.	1.0	-1.3	-1.3	-1.1
BbsG	( <i>R</i> )-Benzylsuccinyl-CoA dehydrogenase	n.d.	1.2	-1.0	1.1	1.5
BbsF	Succinyl-CoA:( <i>R</i> )-benzylsuccinyl-CoA-transferase, subunit	n.d.	1.9	2.0	1.0	1.7
BbsD	2-[Hydroxy(phenyl)methyl]-succinyl-CoA dehydrogenase, subunit	n.d.	-1.1	-1.3	-1.3	-2.4
BbsC	2-[Hydroxy(phenyl)methyl]-succinyl-CoA dehydrogenase, subunit	n.d.	1.4	-1.7	-1.2	-2.2
BbsB	Benzoylsuccinyl-CoA thiolase, $\beta$ -subunit	n.d.	1.2	1.1	-1.3	-1.1
BbsA	Benzoylsuccinyl-CoA thiolase, $\alpha$ -subunit	n.d.	-1.1	1.0	-1.2	-1.1
EbA1932	Hypothetical protein, related to toluene degradation	n.d.	1.4	-1.0	-1.1	-1.1
EbA1936	Hypothetical protein, related to toluene degradation	n.d.	1.2	1.6	-1.5	1.2
BcrA	Benzoyl-CoA reductase, $\alpha$ -subunit	275	1.3	-1.7	-1.2	-3.7
BcrB	Benzoyl-CoA reductase, $\beta$ -subunit	369	1.5	-3.1	1.0	-2.9
<u>Polyhydroxyalkanoate synthesis</u>						
PhbB	Acetoacetyl-CoA reductase	128	1.3	1.3	1.2	3.1
PhaC	Putative poly(3-hydroxyalkanoate) synthase	121	1.1	1.0	1.5	3.5
EbA1323	Conserved hypothetical protein, predicted phasin family	155	-2.1	5.7	-1.5	5.5
EbA6852	Probable phasin	126	-7.6	4.0	-1.2	27.4
EbA5033	Hypothetical protein, probable phasin	87	2.4	5.4	2.6	6.6
<u>Stress-related proteins</u>						
SodB	Superoxide dismutase (Fe)	95	-2.2	1.6	1.1	2.8
EbA1861	Putative high affinity Fe <sup>2+</sup> transporter	71	1.3	-9.9	2.2	-37.3
EbA4918	Putative periplasmic iron binding protein of ABC Fe <sup>3+</sup> transporter	275	1.1	-2.6	1.3	-4.5
AcnA	Aconitase A	194	1.0	2.1	1.1	5.9
AcnA2	Aconitase A	222	-1.3	1.8	-1.1	4.5
BetB	Putative betaine aldehyde dehydrogenase (BADH) oxidoreductase protein	190	-2.0	2.3	-1.7	3.5
WrbA (EbA2303)	Flavoprotein, TrpR enhancer	85	-1.8	2.4	-1.1	5.8
AldB	Putative aldehyde dehydrogenase	73	1.1	-1.0	1.2	4.0
AdhB	Alcohol dehydrogenase II	96	1.0	1.1	-1.0	4.9
GabD	Succinate-semialdehyde dehydrogenase	146	-1.3	1.1	1.3	2.6
<u>Metabolism</u>						
AcsA	Acetyl-CoA synthase	299	1.4	2.0	-1.1	4.6
EbA6506	Acyl-CoA dehydrogenase	386	-1.3	1.4	-1.2	2.5
EbA6075	Predicted short chain dehydrogenase	176	-3.5	1.7	-2.8	2.4
EbA6736	Probable oxidoreductase	145	-1.3	2.4	-1.2	4.6
EbA2755	ABC-transporter, periplasmic component	145	1.1	-1.0	-1.1	-2.6
<u>Fatty acid synthesis</u>						
AccD	Putative acetyl-CoA carboxylase carboxyl transferase, $\beta$ -subunit	158	-1.1	-1.3	1.1	-2.5
BioB	Biotin synthase protein	107	1.0	-1.6	-1.1	-2.9
EbA4327	Acyl-CoA dehydrogenase	93	-1.2	2.9	-1.3	13.4

Amino acid synthesis

HisA	1-5-(Phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino]-imidazole-4-carboxamide isomerase	87	-1.1	1.8	1.2	2.5
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Transcription, translation, regulation

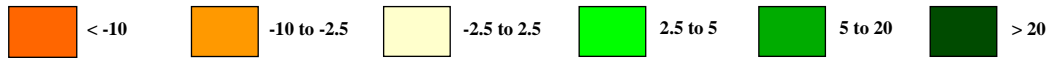
RplI	50S ribosomal protein L9	193	1.2	-1.6	-1.1	-2.7
RplY	50S ribosomal protein	89	-1.0	-1.8	-1.2	-2.7
RplA	50S ribosomal protein	276	2.6	-1.4	1.2	1.0
RplF	50S ribosomal protein	209	3.0	-1.2	1.3	-1.9
RpsB	30S ribosomal protein S2	154	1.3	-1.5	-1.1	-2.5
Tsf	Elongation factor Ts	288	-1.1	-1.7	-1.2	-3.1
PrkA	Protein kinase	254	-1.8	1.7	-1.1	3.4
GntR	Transcriptional regulator	84	-1.1	1.6	-1.1	3.5
Tnp17	Transposase IS4 family	81	2.2	-1.3	1.1	2.6
P2A352	Probable pyridoxamin 5'-phosphate oxidase	123	1.0	-1.4	1.1	-2.8

Unknown, conserved proteins

EbA1694	Conserved hypothetical protein	56	-2.2	5.1	-1.2	14.3
EbA5146	Conserved hypothetical protein	124	-2.5	3.6	-1.3	6.6
EbA3069	Conserved hypothetical protein	96	-1.5	2.1	-1.0	2.5
EbA6073	Conserved hypothetical protein	141	-2.6	1.5	-2.1	1.8
EbA5028	Conserved hypothetical protein	136	1.1	-1.8	-1.1	-2.5

Unknown, unique proteins

EbA7011	Hypothetical protein	107	-2.6	6.7	-1.0	66.4
EbA2155	Hypothetical protein	116	1.1	6.7	-1.0	16.0
EbA6953	Hypothetical protein, probable apolipoprotein A/E/C3	288	-1.6	7.4	-1.1	9.5
EbA3088	Hypothetical protein	108	-1.1	2.9	-1.1	4.4
EbA1597	Hypothetical protein	52	-1.8	1.7	1.1	2.8



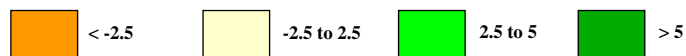
<sup>a</sup> Protein name and function as published by Rabus *et al.* (35). Proteins may fit in more than one group.

<sup>b</sup> Score of MS-based identification as calculated using Mascot. The Mascot score was averaged for proteins identified from several protein spots. n.d., not determined in this study. Protein assignment was based on co-detection of previously identified proteins (28).

<sup>c</sup> Fold changes in abundance were determined with 2D DIGE. Protein extracts from cultures growing with <sup>0</sup>0.21 mM ethylbenzene or <sup>0</sup>0.24 mM toluene served as reference state. Proteins with fold changes > 2.5 are green-colored and proteins with < -2.5 are red-colored, respectively.

TABLE S3. Fold changes in abundance of identified proteins from succinate-utilizing cells of strain EbN1 either shocked with ethylbenzene and toluene, or without addition of the alkylbenzenes.

Protein name <sup>a</sup>	Predicted function <sup>a</sup>	Score <sup>b</sup>	Succinate-utilizing cells shocked with							
			Ethylbenzene <sup>c</sup> (0.5 mM)			Toluene <sup>c</sup> (1.2 mM)			No-addition <sup>c</sup>	
			45 <sup>d</sup>	250 <sup>d</sup>	600 <sup>d</sup>	45 <sup>d</sup>	250 <sup>d</sup>	600 <sup>d</sup>	250 <sup>d</sup>	600 <sup>d</sup>
<u>Stress-related proteins</u>										
HtpG	Heat shock protein	142	2.3	2.6	2.3	2.2	1.9	1.9	-1.1	1.1
EbB88	Putative heat shock protein	103	2.8	3.3	3.0	2.8	2.1	2.4	1.4	1.7
ClpB	Chaperone	148	2.9	3.6	3.8	2.4	2.0	2.3	1.2	1.5
WbjB	Similar to polysaccharide biosynthesis protein	90	6.5	4.5	1.9	4.4	2.0	1.9	1.2	1.1
KatA	Catalase	150	1.8	2.5	2.5	1.7	1.4	1.8	4.0	3.9
<u>Denitrification</u>										
NirS	Cytochrome <i>cdI</i> nitrite reductase precursor	205	2.0	2.3	1.8	2.1	1.9	1.7	5.7	5.9
NosZ	Nitrous-oxide reductase precursor	270	4.1	5.1	11.2	4.8	5.2	7.5	-1.0	1.3
<u>Metabolism</u>										
AcsA	Acetyl-CoA synthetase	56	2.1	3.0	4.1	1.7	1.7	2.4	-1.6	-1.2
BcrA	Benzoyl-CoA reductase subunit	62	-1.4	-1.9	-2.2	-1.6	-3.0	-4.3	-1.6	-1.6
AceE	Pyruvate dehydrogenase multienzyme complex, E1 component	266	1.1	-1.1	-1.2	-1.0	-1.5	-1.4	-4.5	-1.9
BzdV	Subunit of oxidoreductase of unknown function, conserved in "Aromatoleum"-type benzoate degradation gene clusters	131	1.0	-1.1	-1.3	-1.1	-1.4	-1.2	-3.4	-1.8
SucB	2-Oxoglutarate dehydrogenase complex, dihydrolipoamide succinyltransferase	74	-2.4	-2.7	-2.8	-2.5	-2.6	-2.8	-2.5	-2.3
PpcK	Phosphoenolpyruvate carboxykinase	211	1.4	1.3	1.2	1.2	-1.2	-1.3	-3.4	-1.3
<u>Sulfur assimilation</u>										
CysP	Periplasmic thiosulfate-binding protein	118	-4.2	-4.2	-6.3	-4.2	-4.8	-7.3	-3.2	-3.2
CysD	Putative ATP sulfurylase small subunit	120	-3.3	-2.9	-2.7	-2.8	-3.3	-3.2	-3.0	-3.1
CysN	ATP sulfurylase large subunit	126	-3.8	-4.0	-4.5	-3.8	-4.5	-5.1	-4.5	-4.0
CysH	APS-reductase	179	-4.7	-5.2	-5.6	-4.0	-4.5	-4.8	-4.0	-3.9
<u>Proteins with unknown function</u>										
EbA2621	Conserved hypothetical protein	54	-1.6	-1.6	-1.7	-1.5	-1.6	-1.5	-2.9	-2.4
EbA3357	Hypothetical protein	54	1.1	1.7	1.7	1.2	1.0	1.2	3.1	3.4
EbA4221	Hypothetical protein	63	2.4	2.7	1.6	2.3	2.4	1.6	2.1	2.2



<sup>a</sup>Protein name and function as published by Rabus *et al.* (35). Proteins may fit in more than one group.

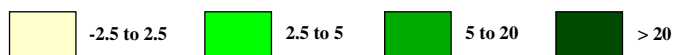
<sup>b</sup>Score of MS-based identification as calculated using Mascot. The Mascot score was averaged for proteins identified from several protein spots.

<sup>c</sup>Fold changes in abundance were determined with 2D DIGE. Protein extracts from succinate-utilizing cultures harvested 45 min after addition of the alkylbenzenes to the test cultures served as reference state. Proteins with fold changes > 2.5 are green-colored and proteins with < -2.5 are red-colored, respectively.

<sup>d</sup>Time after addition of the alkylbenzenes to the test cultures.

TABLE S4. Fold changes in abundance of identified proteins from succinate-utilizing cultures of strain EbN1 shocked with semi-inhibitory concentrations of *p*-cresol, phenol or the solvent mixture.

Protein name <sup>a</sup>	Predicted function <sup>a</sup>	Score <sup>b</sup>	Succinate-utilizing cells shocked with					
			<i>p</i> -Cresol <sup>c</sup> (3.0 mM)		Phenol <sup>c</sup> (6.5 mM)		Solvent mixture <sup>c</sup> (2.7 mM <sup>d</sup> )	
			45 <sup>e</sup>	250 <sup>e</sup>	45 <sup>e</sup>	250 <sup>e</sup>	45 <sup>e</sup>	250 <sup>e</sup>
<u>Denitrification</u>								
NirS	Cytochrome <i>cdI</i> nitrite reductase precursor	238	1.2	1.7	-1.4	1.1	1.7	3.0
NorQ	Putative chaperone required for nitric oxide reductase	90	-1.3	1.0	-1.6	1.0	1.6	2.6
NosZ	Nitrous oxide reductase precursor	95	1.1	1.9	1.6	2.0	2.1	2.6
<u>Ethylbenzene degradation</u>								
Apc1	Acetophenone carboxylase, subunit 1	193	-	-	-	-	1.7	5.9
Apc3	Acetophenone carboxylase, subunit 3	270	-	-	-	-	-1.5	2.6
Apc4	Acetophenone carboxylase, subunit 4	98	-	-	-	-	-1.3	6.1
Orf84	Putative methyltransferase	75	-	-	-	-	1.0	8.3
<u>Stress-related proteins</u>								
ClpB	Chaperone	361	1.2	2.2	1.8	4.1	2.4	3.4
EbB88	Putative heat shock protein	97	1.2	1.5	1.4	4.8	2.8	3.6
EbA2730	Heat shock protein (Hsp-20 family)	87	-	-	1.4	4.4	-	-
GrpE	Putative GrpE protein (Hsp-70 cofactor)	102	-	-	-	-	1.6	4.7
NorVW	Nitric oxide reductase (flavorubredoxin NorV) with its associated reductase (NorW)	176	-	-	-1.0	2.4	1.2	7.0
KatA	Catalase	179	1.0	2.0	-1.5	1.6	2.4	5.2
Dps	DNA-binding ferritin-like protein (oxidative damage protectant)	74	1.7	2.4	1.0	1.3	2.0	2.5
OmpC	Outer membrane protein	159	11.0	32.4	8.4	21.9	6.8	23.4
<u>Metabolism</u>								
AcsA	Acetyl-CoA synthetase	231	2.6	4.7	4.4	13.4	3.5	5.7
Icd	Isocitrate dehydrogenase	260	1.5	3.0	-1.4	2.1	2.2	4.3
GlcB	Malate synthase G	113	1.6	2.6	1.8	3.5	1.9	3.1
ThrC	Threonine synthase	230	4.3	1.5	-1.2	-1.3	1.6	1.4
BoxC	Enoyl-CoA-hydratase/isomerase	278	2.8	1.2	1.0	-1.1	1.3	1.0
DapA	Dihydrodipicolinate synthetase	61	-	-	1.1	1.3	1.2	10.4
GlnA	Glutamine synthetase	102	1.1	1.2	-	-	1.7	3.2
RpsB	30S ribosomal protein S2	120	-	-	-	-	1.9	3.3
KdsA	2-Dehydro-3-deoxyphosphooctonate aldolase (EC 2.5.1.55)	102	-	-	-1.2	1.2	1.5	2.8
RibH	6,7-Dimethyl-8-ribityllumazine synthase (riboflavin synthase)	80	-	-	-1.3	1.1	1.5	2.5
<u>Others</u>								
EbA1033	Putative exported solute binding protein	151	2.0	2.7	1.0	1.5	1.6	2.0
EbA3296	Zn-dependent hydrolase	126	-1.0	1.6	1.7	4.3	2.4	3.3
EbA6075	Predicted short chain dehydrogenase	136	-1.2	1.4	-1.8	1.1	1.8	3.9
<u>Hypothetical proteins</u>								
EbA1598	Hypothetical protein	111	-	-	3.1	3.9	-	-
EbA3069	Conserved hypothetical protein, probably involved in cell wall turnover	116	1.6	2.4	1.5	3.2	1.7	2.5
EbA5766	Conserved hypothetical protein	104	3.9	4.4	-	-	2.3	7.4
EbA6073	Conserved hypothetical protein	101	-1.1	1.3	-1.5	1.1	2.0	2.9
EbA6294	Conserved hypothetical protein	92	1.1	1.5	-1.3	1.2	1.5	3.1



<sup>a</sup> Protein name and function as published by Rabus *et al.* (35). Proteins may fit in more than one group.

<sup>b</sup> Score of MS-based identification as calculated using Mascot. The Mascot score was averaged for proteins identified from several protein spots.

<sup>c</sup> Fold changes in abundance were determined with 2D DIGE. Protein extracts from succinate-utilizing cultures harvested 45 min after addition of the aromatic stressors to the test cultures served as reference state. Proteins with fold changes > 2.5 are green-colored and proteins with < -2.5 are red-colored, respectively.

<sup>d</sup> Total solvent concentration of a mixture of ethylbenzene, toluene, *p*-cresol and phenol in the culture medium.

<sup>e</sup> Time after addition of *p*-cresol, phenol and the solvent mixture to the test cultures.