

Supplemental material

Multispecies diesel fuel biodegradation and niche formation are ignited by pioneer hydrocarbon-utilizing proteobacteria in a soil bacterial consortium

Jiro F. Mori and Robert A. Kanaly

Graduate School of Nanobiosciences, Yokohama City University, Yokohama, Japan

Table S1 Relative abundances of 16S rRNA gene amplicon OTUs detected from the consortium grown on different hydrocarbon sources.

Table S2 Statistics for metagenomes of the consortium grown on diesel fuel.

Table S3 Presence of aromatic hydrocarbon / alkane degradation genes detected in the bacterial MAGs.

Table S4 Results of LC/ESI(-)-MS/MS analyses of sample extracts to identify biotransformation products of different parent aromatic compounds.

Table S5 Presence of flagellar formation and chemotaxis genes in the bacterial MAGs.

Figure S1 Results of LC/ESI(-)-MS/MS analyses of extracts from SBM where *S. barthaii* KK22 was exposed to 50 mg/l of 1-hydroxy-2-naphthoic acid.

Figure S2 Results of LC/ESI(-)-MS/MS analyses of extracts from SBM where *S. barthaii* KK22 was exposed to 50 mg/l of toluene.

Figure S3 Results of LC/ESI(-)-MS/MS analyses of the deprotonated molecule, $[M - H]^- = 141$ for an authentic standard of catechol and for extracts from SBM where *Cupriavidus* strain KK10 was exposed to either 50 mg/l of salicyclic acid or 50 mg/l of benzoic acid.

Figure S4 Results of LC/ESI(-)-MS/MS analyses of extracts from SBM where *Achromobacter* strain KK8 was exposed to 50 mg/l of salicylic acid.

Table S1. Relative abundances of 16S rRNA gene amplicon OTUs detected from the consortium grown on different hydrocarbon sources.

OTU Taxonomy	Relative abundance (%)							
	Diesel-1	Diesel-2	Diesel avg.	NAPL	HEX	PHE	NAP	TOL
1 Gammaproteobacteria / Pseudomonadales / Pseudomonadaceae / Pseudomonas	74.21	67.20	70.71	79.20	84.22	21.21	22.28	2.77
2 Alphaproteobacteria / Sphingomonadales / Sphingomonadaceae / Sphingobium	8.57	16.11	12.34	10.14	0.10	53.36	42.61	53.46
3 Alphaproteobacteria / Rhizobiales / Brucellaceae / Ochrobactrum	5.77	6.47	6.12	0.63	3.24	4.35	5.90	3.61
4 Betaproteobacteria / Burkholderiales / Alcaligenaceae / Achromobacter	5.21	3.92	4.57	4.92	2.99	18.51	26.12	16.11
5 Betaproteobacteria / Burkholderiales / Oxalobacteraceae / Cupriavidus	2.06	2.32	2.19	0.55	0.07	0.79	0.86	18.85
6 Alphaproteobacteria / Rhizobiales / Hyphomicrobiaceae / Parvibaculum	1.16	1.00	1.08	0.01	0.01	0.14	0.05	0.08
7 Bacteroides / Shingobacteriia / Sphingobacteriales / Sphingobacteriaceae / Olivibacter	0.93	0.97	0.95	1.63	2.43	0.95	0.54	3.10
8 Alphaproteobacteria / Rhizobiales / Bradyrhizobiaceae / Bradyrhizobium	0.53	0.35	0.44	0.00	0.05	0.06	0.43	0.34
9 Gammaproteobacteria / Xanthomonadales / Xanthomonadaceae / Dokdonella	0.39	0.47	0.43	0.29	0.35	0.08	0.12	0.05
10 Gammaproteobacteria / Xanthomonadales / Xanthomonadaceae / Rhodanobacter	0.43	0.24	0.33	0.14	0.96	0.00	0.07	0.00
11 Alphaproteobacteria / Rhizobiales / Bradyrhizobiaceae / Bradyrhizobium	0.26	0.27	0.26	0.00	0.00	0.00	0.00	0.00
12 Alphaproteobacteria / Rhizobiales / Phyllobacteriaceae / Mesorhizobium	0.16	0.20	0.18	0.00	0.00	0.04	0.00	0.13
13 Gammaproteobacteria / Xanthomonadales / Xanthomonadaceae / Rhodanobacter	0.16	0.11	0.14	0.43	3.45	0.28	0.60	0.53
14 Alphaproteobacteria / Rhizobiales / Hyphomicrobiaceae / Parvibaculum	0.08	0.08	0.08	0.00	0.00	0.00	0.03	0.19
15 Alphaproteobacteria / Sphingomonadales / Sphingomonadaceae / Sphingomonas	0.05	0.05	0.05	0.11	0.22	0.04	0.00	0.02
16 Actinobacteria / Actinomycetales / Corynebacterineae / Mycobacteriaceae / Mycobacterium	0.00	0.00	0.00	1.75	1.69	0.00	0.00	0.00
Other (minor bacterial genera of Rhizobiales, Gemmatales, or Rhodospirillales)	0.04	0.23	0.14	0.17	0.23	0.20	0.39	0.76

Table S2. Statistics for metagenomes of the consortium grown on diesel fuel.

IMG genome ID	Paired reads	Quality paired reads	Contigs	Total bases	CDS	tRNA	tRNA
3300038749	23376413		21670846	65960	83291099	74942	706 53

Table S3. Presence of aromatic hydrocarbon / alkane degradation genes detected in the bacterial MAGs.

Gene	Pseudomonas	Sphingobium	Ochrobactrum	Achromobacter	Cupriavidus	Parvibaculum
Naphthalene & polycyclic aromatic hydrocarbon degradation						
Naphthalene 1, 2-dioxygenase (<i>nahAcAd</i>) [EC 1.14.12.12]					+	
Naphthalene dihydrodiol dehydrogenase (<i>nahB</i>) [EC 1.3.1.29] / Cis-2, 3-dihydrobiphenyl-2, 3-diol dehydrogenase (<i>bphB</i>) [EC 1.3.1.56]		+				+
1, 2-dihydroxynaphthalene dioxygenase (<i>nahC</i>) [EC 1.13.11.56] / Biphenyl-2, 3-diol 1, 2-dioxygenase (<i>bphC</i>) [EC 1.13.11.39]		+		+		+
2-hydroxychromene-2-carboxylase (<i>nahD</i>) [EC 5.99.1.4]	+	+		+	+	+
trans-o-hydroxybenzylidene pyruvate hydratase-alcoholase (<i>nahE</i>) [EC 4.1.2.45]		+		+		
Salicylaldehyde dehydrogenase (<i>nahF</i>) [EC 1.2.1.65]						
Salicylate 1-monoxygenase (<i>nahG</i>) [EC 1.14.13.1]				+	+	
2,6-dioxo-6-phenylhexa-3-enate hydrolase (<i>bphD</i>) [EC 3.7.1.8]	+			+		+
2-oxopent-4-anoate hydratase (<i>bphE</i>) [EC 4.2.1.80]		+			+	+
4-hydroxy-2-oxovalerate aldolase (<i>bphF</i>) [EC 4.1.3.39]		+			+	+
Single ring aromatic hydrocarbon degradation						
Toluene methyl monooxygenase (<i>xylM</i>) [EC 1.14.15.26]				+		
Aryl-alcohol dehydrogenase (<i>xylB</i>) [EC 1.1.1.90]		+				+
Benzaldehyde dehydrogenase (<i>xylC</i>) [EC 1.2.1.28]		+				+
Benzoate / toluate 1, 2-dioxygenase (<i>benAB</i> / <i>xylXY</i>) [EC 1.14.12.10]	+	+				+
Dihydroxycyclohexadiene carboxylate dehydrogenase (<i>benD</i> / <i>xylL</i>) [EC 1.3.1.25]	+	+		+	+	+
Benzene / toluene 1, 2-dioxygenase (<i>benZAB</i> / <i>todC1C2</i>) [EC 1.14.12.3]			+			
Phenol / toluene 2-monooxygenase (<i>poxABCDEF</i>) [EC 1.14.13.243, 1.14.13.244]			+			+
Catechol 1, 2-dioxygenase (<i>catA</i>) [EC 1.13.11.1]				+	+	
Muconate cycloisomerase (<i>catB</i>) [EC 5.5.1.1]		+			+	+
Muconolactone D-isomerase (<i>catC</i>) [EC 5.3.3.4]		+			+	
3-oxoadipate enol-lactonase (<i>catD</i> / <i>pcaD</i>) [EC 3.1.1.24]		+		+	+	+
3-oxoadipate CoA-transerase (<i>catJ</i> / <i>pcaJ</i>) [EC 2.8.3.6]		+		+	+	
3-oxoadipyl-CoA thiolase (<i>catF</i> / <i>pcaF</i>) [EC 2.3.1.16 / 2.3.1.174]		+		+	+	+
Protocatechuate 3, 4-dioxygenase (<i>pcaGH</i>) [EC 1.13.11.3]			+			
3-carboxy-cis, cis-muconate cycloisomerase (<i>pcaB</i>) [EC 5.5.1.2]			+			
4-carboxymuconolactone decarboxylase (<i>pcaC</i>) [EC 4.1.1.44]		+		+	+	
Catechol 2, 3-dioxygenase (<i>xylE</i>) [EC 1.13.11.2]				+	+	+
2-hydroxymuconate-semialdehyde hydrolase (<i>xylF</i>) [EC 3.7.1.9]				+		
2-hydroxymuconate-6-semialdehyde dehydrogenase (<i>xylG</i>) [EC 1.2.1.85]				+	+	+
4-oxalocrotonate tautomerase (<i>xylH</i>) [EC 5.3.2.6]				+	+	
2-oxo-3-hexenedioate decarboxylase (<i>xylI</i>) [EC 4.1.1.77]				+	+	
2-oxopent-4-enoate hydratase (<i>xylJ</i>) [EC 4.2.1.80]					+	+
4-hydroxy-2-oxovalerate aldolase (<i>xylK</i>) [EC 4.1.3.39]					+	+
Gentisate 1, 2-dioxygenase (<i>nagI</i>) [EC 1.13.11.4]				+	+	+
Maleylpyruvate isomerase (<i>nagL</i>) [EC 5.2.1.4]				+	+	+
Fumarylpyruvate hydrolase (<i>nagK</i>) [EC 3.7.1.20]				+	+	
Anthranilate 1, 2-dioxygenase (<i>antAB</i>) [EC 1.14.12.1]				+		
3-phenylpropanoate dioxygenase (<i>hcaEF</i>) [EC 1.14.12.19]				+		+
Alkane degradation						
Alkane 1-monooxygenase (<i>alkB</i>) [EC 1.14.15.3]		+				
(Putative) Cytochrome P450 CYP153 family alkane monooxygenase			+			+
(Putative) Flavin-binging alkane monooxygenase (<i>almA</i>)		+			+	+
Alcohol dehydrogenase (<i>adh</i>) [EC 1.1.1.1]		+		+	+	+
Aldehyde dehydrogenase (<i>aldh</i>) [EC 1.2.1.3]		+		+	+	+
Acyl-CoA synthetase (<i>acs</i>) [EC 6.2.1.3]		+		+	+	+

Table S4. Results of LC/ESI(-)-MS/MS analyses of sample extracts to identify biotransformation products of different parent aromatic compounds discussed in Figure 5.

Product detected	Parent compound ^a	Strain	Detected [M – H] ⁻	t _R ^b (min)	CID ^c (eV)	Diagnostic fragments from product ion scan analyses (% relative intensity)
salicylic acid	phenanthrene	KK22	137	3.4	8	137 (M ⁻ , 100), 93 (M ⁻ – CO ₂ , 50)
1,2-dihydroxynaphthalene	phenanthrene	KK22	159	6.3	8	159 (M ⁻ , 100), 141 (M ⁻ – H ₂ O, 3), 131 (M ⁻ – CO, 1), 127 (C ₁₀ H ₇ ⁻ , 2), 115 (C ₉ H ₇ ⁻ , 5), 113 (M ⁻ – CO – H ₂ O, 6), 101 (C ₈ H ₅ ⁻ , 2), 97 (2), 87 (1), 85 (1), 83 (1)
salicylic acid	naphthalene	KK22	137	3.5	8	137 (M ⁻ , 100), 93 (M ⁻ – CO ₂ , 29)
1,2-dihydroxynaphthalene	naphthalene	KK22	159	6.3	8	159 (M ⁻ , 100), 141 (M ⁻ – H ₂ O, 5), 127 (C ₁₀ H ₇ ⁻ , 1), 115 (C ₉ H ₇ ⁻ , 14), 113 (M ⁻ – CO – H ₂ O, 3), 101 (C ₈ H ₅ ⁻ , 5), 97 (14), 87 (1), 85 (1), 83 (1)
salicylic acid	1-hydroxy-2-naphthoic acid	KK22	137	3.5	8	137 (M ⁻ , 100), 93 (M ⁻ – CO ₂ , 27)
1,2-dihydroxynaphthalene	1-hydroxy-2-naphthoic acid	KK22	159	6.2	8	159 (M ⁻ , 100), 141 (M ⁻ – H ₂ O, 2), 131 (M ⁻ – CO, 1), 127 (C ₁₀ H ₇ ⁻ , 1), 115 (C ₉ H ₇ ⁻ , 4), 113 (M ⁻ – CO – H ₂ O, 5), 101 (C ₈ H ₅ ⁻ , 1), 97 (3), 87 (1), 85 (1), 83 (2)
benzoic acid	toluene	KK22	121	6.0	8	121 (M ⁻ , 100), 77 (M ⁻ – CO ₂ , 41)
catechol ^d	benzoic acid	KK22	141	5.8	8	141 (M ⁻ , 14), 123 (M ⁻ – H ₂ O, 20), 109 (M ⁻ – CH ₃ OH, 100), 97 (C ₅ H ₅ O ₂ ⁻ , 6), 91 (M ⁻ – CH ₃ OH – H ₂ O, 5), 79 (C ₅ H ₅ O ₂ ⁻ – H ₂ O, 13), 77 (C ₆ H ₅ ⁻ , 14)
catechol ^d	salicylic acid	KK22	141	5.9	8	141 (M ⁻ , 26), 123 (M ⁻ – H ₂ O, 11), 109 (M ⁻ – CH ₃ OH, 100), 97 (C ₅ H ₅ O ₂ ⁻ , 17), 91 (M ⁻ – CH ₃ OH – H ₂ O, 7), 77 (C ₆ H ₅ ⁻ , 1)
catechol ^d	benzoic acid	KK10	141	5.8	8	141 (M ⁻ , 48), 123 (M ⁻ – H ₂ O, 41), 109 (M ⁻ – CH ₃ OH, 100), 97 (C ₅ H ₅ O ₂ ⁻ , 43), 91 (M ⁻ – CH ₃ OH – H ₂ O, 13), 79 (C ₅ H ₅ O ₂ ⁻ – H ₂ O, 15), 77 (C ₆ H ₅ ⁻ , 17), 69 (3)
catechol ^d	salicylic acid	KK10	141	5.9	8	141 (M ⁻ , 93), 123 (M ⁻ – H ₂ O, 44), 109 (M ⁻ – CH ₃ OH, 100), 97 (C ₅ H ₅ O ₂ ⁻ , 19), 91 (M ⁻ – CH ₃ OH – H ₂ O, 9), 79 (C ₅ H ₅ O ₂ ⁻ – H ₂ O, 41), 77 (C ₆ H ₅ ⁻ , 6), 69 (1)
catechol ^d	benzoic acid	KK8	141	5.9	8	141 (M ⁻ , 25), 123 (M ⁻ – H ₂ O, 45), 109 (M ⁻ – CH ₃ OH, 100), 97 (C ₅ H ₅ O ₂ ⁻ , 17), 91 (M ⁻ – CH ₃ OH – H ₂ O, 12), 79 (C ₅ H ₅ O ₂ ⁻ – H ₂ O, 21), 77 (C ₆ H ₅ ⁻ , 12), 69 (3)
catechol ^d	salicylic acid	KK8	141	5.8	8	141 (M ⁻ , 45), 123 (M ⁻ – H ₂ O, 12), 109 (M ⁻ – CH ₃ OH, 100), 97 (C ₅ H ₅ O ₂ ⁻ , 8), 91 (M ⁻ – CH ₃ OH – H ₂ O, 9), 79 (C ₅ H ₅ O ₂ ⁻ – H ₂ O, 10), 77 (C ₆ H ₅ ⁻ , 10), 69 (3)
catechol ^d	benzoic acid	KK6	141	5.9	8	141 (M ⁻ , 29), 123 (M ⁻ – H ₂ O, 31), 109 (M ⁻ – CH ₃ OH, 100), 97 (C ₅ H ₅ O ₂ ⁻ , 36), 91 (M ⁻ – CH ₃ OH – H ₂ O, 11), 79 (C ₅ H ₅ O ₂ ⁻ – H ₂ O, 4), 77 (C ₆ H ₅ ⁻ , 10), 69 (1)
gentisic acid	salicylic acid	KK8	153	3.6	20	153 (M ⁻ , 30), 135 (M ⁻ – H ₂ O, 14), 109 (M ⁻ – CO ₂ , 100), 108 (73), 91 (M ⁻ – CO ₂ , – H ₂ O, 10), 81 (M ⁻ – CO ₂ , – CO, 11)

^a Parent compounds were administered as the sole source of carbon and energy at a concentration of 50 mg/l each except for 1,2-dihydroxynaphthalene which was administered at 25 mg/l.

^b t_R, retention time.

^c Collision induced dissociation energy.

^d Catechol was detected as its methanol adduct in ESI negative ionization mode, [M – H]⁻ = 141.

Table S5. Presence of flagellar formation and chemotaxis genes in the bacterial MAGs.

	<i>Pseudomonas</i>	<i>Sphingobium</i>	<i>Ochrobactrum</i>	<i>Achromobacter</i>	<i>Cupriavidus</i>	<i>Parvibaculum</i>
flagella basal-body P-ring protein FlgA	+	+	+	+	+	+
flagellar basal-body rod protein FlgB	+	+	+	+	+	+
flagellar basal-body rod protein FlgC	+	+	+	+	+	+
flagellar basal-body rod modification protein FlgD	+	+	+	+	+	+
flagellar hook protein FlgE	+	+	+	+	+	+
flagellar basal-body rod protein FlgF	+	+	+	+	+	+
flagellar basal-body rod protein FlgG	+	+	+	+	+	+
flagellar L-ring protein precursor FlgH	+	+	+	+	+	+
flagellar P-ring protein precursor FlgI	+	+	+	+	+	+
flagellar protein FlgJ				+	+	
flagellar hook-associated protein 1 FlgK	+	+	+	+	+	+
flagellar hook-associated protein 3 FlgL	+	+	+	+	+	
negative regulator of flagellin synthesis FlgM	+	+		+	+	
flagella synthesis protein FlgN				+	+	
flagellar biosynthesis protein FlhA	+	+	+	+	+	+
flagellar biosynthetic protein FlhB	+	+	+	+	+	+
flagellar transcriptional activator FlhC				+	+	
flagellar transcriptional activator FlhD				+	+	
flagellar biosynthesis protein FlhF						
flagellin (FlxC)	+					
flagellar hook-associated protein 2 (FlxD)	+	+	+	+	+	
flagellar hook-basal body complex protein FlxE	+	+		+	+	+
flagellar M-ring protein FlxF	+	+	+	+	+	+
flagellar motor switch protein FlxG	+	+	+	+	+	+
flagellar assembly protein FlxH	+	+		+	+	+
flagellum-specific ATP synthase (Flil)	+	+	+	+	+	+
flagellar FlilJ protein	+			+	+	
flagellar hook-length control protein FlxK	+			+	+	
flagellar FlilL protein	+			+	+	
flagellar motor switch protein FlxM	+	+	+	+	+	+
flagellar motor switch protein FlxN/FlxY	+	+	+	+	+	+
flagellar protein FlxO/FlxZ	+	+		+	+	+
flagellar biosynthetic protein FlxP	+	+	+	+	+	+
flagellar biosynthetic protein FlxQ	+	+	+	+	+	+
flagellar biosynthetic protein FlxR	+	+	+	+	+	+
flagellar protein FlxS	+	+		+	+	
flagellar protein FlxT				+	+	
chemotaxis protein MotA	+	+	+	+	+	+
chemotaxis protein MotB	+	+	+	+	+	+
two-component system, chemotaxis family, sensor kinase CheA [EC:2.7.13.3]	+	+		+	+	
two-component system, chemotaxis family, response regulator CheB [EC:3.1.1.61]	+	+	+	+	+	+
chemotaxis protein CheC		+				
chemotaxis protein CheD [EC:3.5.1.44]	+			+	+	
chemotaxis protein methyltransferase CheR [EC:2.1.1.80]	+	+	+	+	+	+
two-component system, chemotaxis family, response regulator CheV	+				+	
purine-binding chemotaxis protein CheW	+	+		+	+	+
chemotaxis protein CheX						
two-component system, chemotaxis family, response regulator CheY	+	+	+	+	+	+
chemotaxis protein CheZ	+			+	+	
aerotaxis receptor	+			+	+	+
methyl-accepting chemotaxis protein	+	+		+	+	+

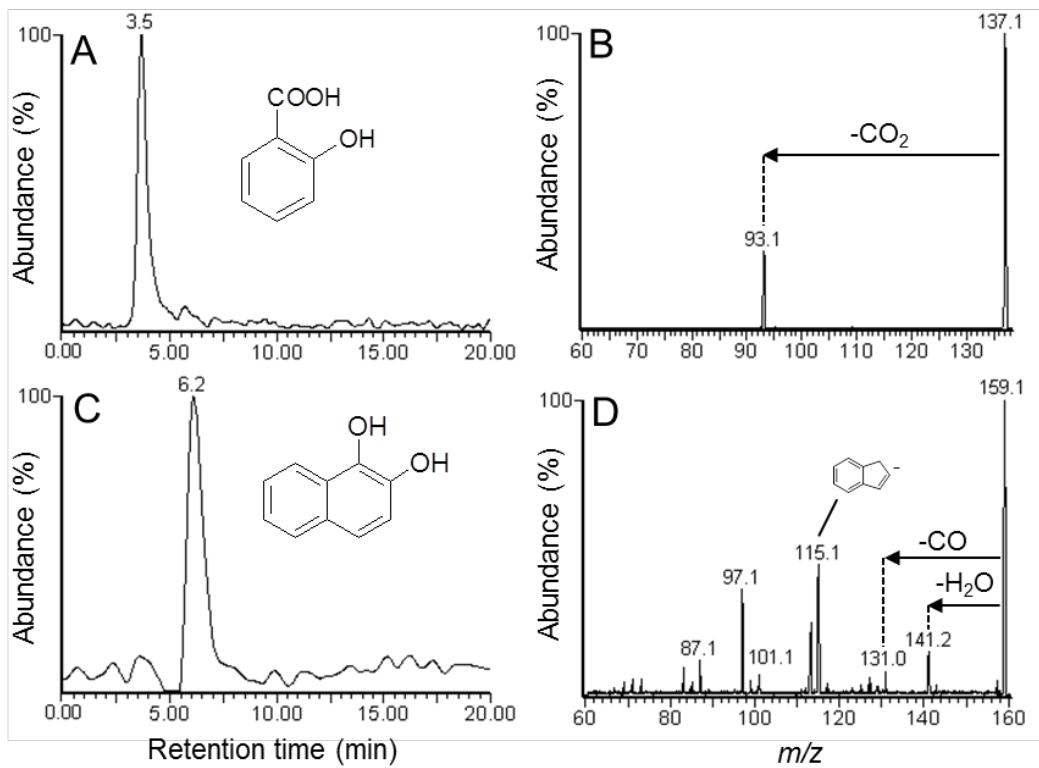


Figure S1. Results of LC/ESI(-)-MS/MS analyses of extracts from SBM where *S. barthai KK22* was exposed to 50 mg/l of 1-hydroxy-2-naphthoic acid. (A) Extracted ion chromatogram of a metabolite of 1-hydroxy-2-naphthoic acid that corresponded to $[M - H]^- = 137$; identified as salicylic acid (shown); (B) Results of product ion scan analysis of the metabolite that corresponded to $[M - H]^- = 137$; (C) Extracted ion chromatogram of metabolite of 1-hydroxy-2-naphthoic acid that corresponded to $[M - H]^- = 159$; identified as 1,2-dihydroxynaphthalene (shown); (D) Results of product ion scan analysis of the metabolite that corresponded to $[M - H]^- = 159$. The baseline from m/z 60 to m/z 158 was magnified 5 fold.

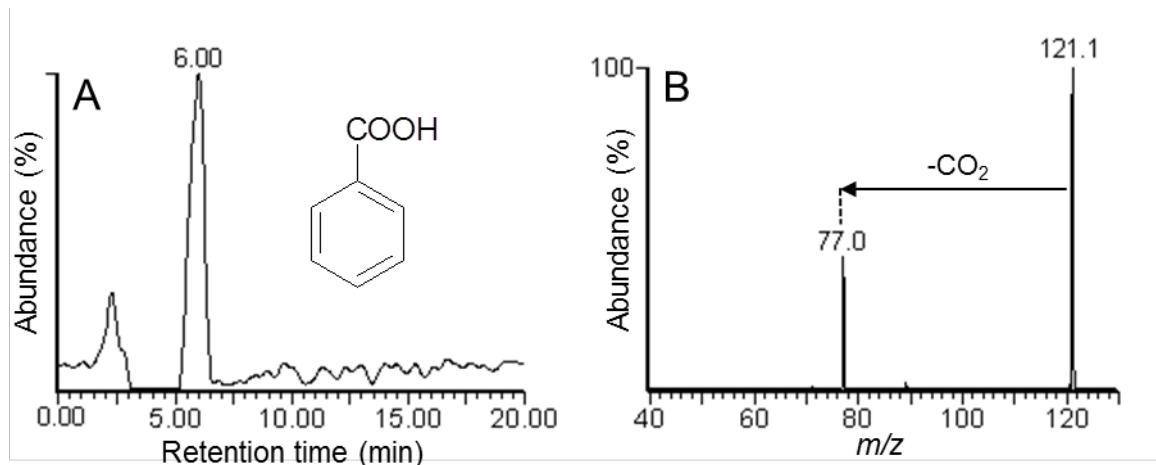


Figure S2. Results of LC/ESI(-)-MS/MS analyses of extracts from SBM where *S. barthai KK22* was exposed to 50 mg/l of toluene. (A) Extracted ion chromatogram of a metabolite of toluene that corresponded to $[M - H]^- = 121$; identified as benzoic acid (shown); (B) Results of product ion scan analysis of the metabolite that corresponded to $[M - H]^- = 121$.

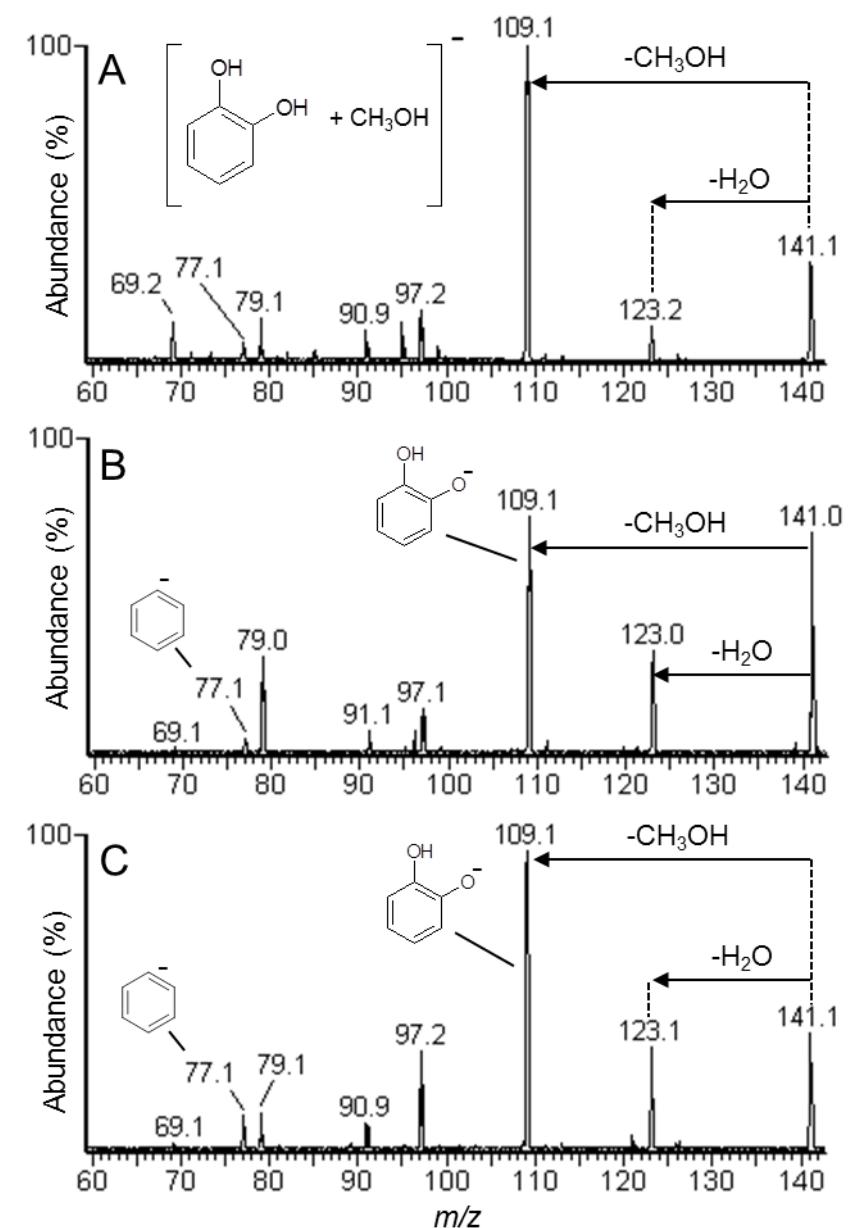


Figure S3. Results of LC/ESI(-)-MS/MS analyses of the deprotonated molecule, $[M - H]^- = 141$ for (A) an authentic standard of catechol; (B) for a metabolite detected in extracts from SBM where *Cupriavidus* strain KK10 was exposed to 50 mg/l of salicyclic acid; (C) for a metabolite detected in extracts from SBM where *Cupriavidus* strain KK10 was exposed to 50 mg/l of benzoic acid. Catechol was detected as its methanol adduct as shown in (A) under negative ionization mode conditions. The baselines from m/z 60 to m/z 108 was magnified 5 fold in all spectra.

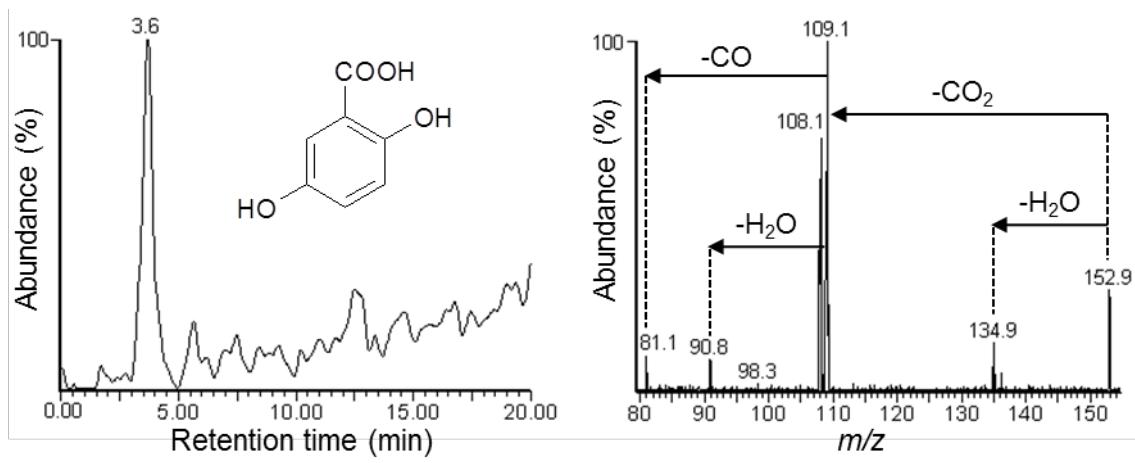


Figure S4. Results of LC/ESI(-)-MS/MS analyses of extracts from SBM where *Achromobacter* strain KK8 was exposed to 50 mg/l of salicylic acid. (A) Extracted ion chromatogram of a metabolite of salicylic acid that corresponded to $[M - H]^- = 153$; identified as gentisic acid (shown); (B) Results of product ion scan analysis of the metabolite that corresponded to $[M - H]^- = 153$.