

1 Methanogenic complete degradation of lignin-derived
2 monoaromatic compounds by microbial enrichments from rice
3 paddy field soil

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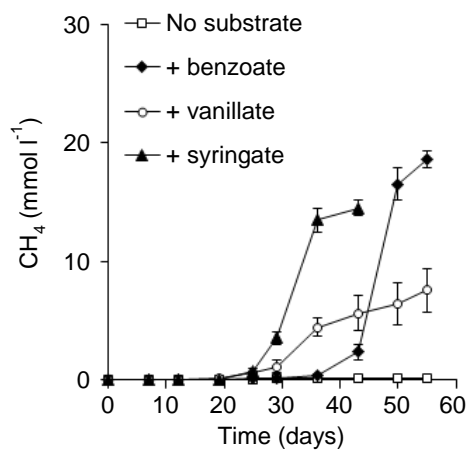
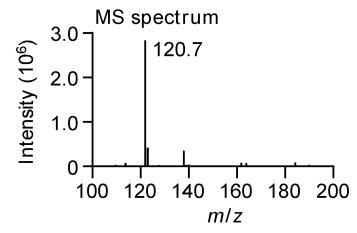
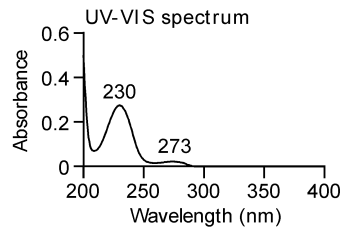
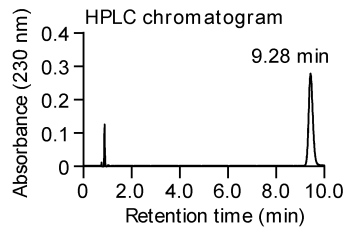
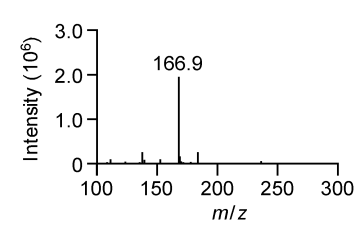
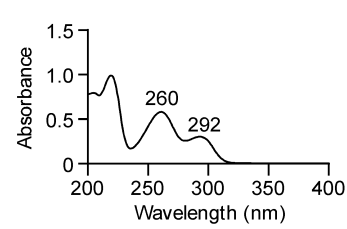
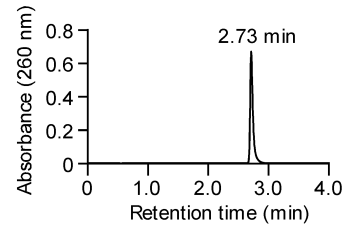


Fig. S1. Methane production by the first enrichment cultures. Production of CH₄ was monitored during the incubation of respective cultures inoculated with rice paddy field soil and supplemented with 5 mM of benzoate (closed diamonds), vanillate (open circles), or syringate (closed triangles). The data for no-amendment control (open squares) are also presented. Data are presented as the means of three independent cultures, and error bars represent standard deviations.

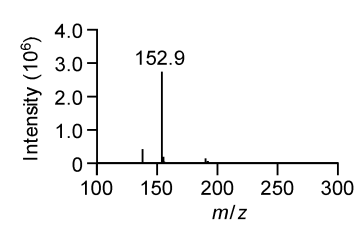
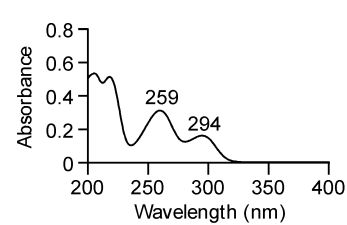
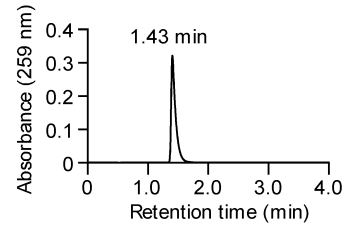
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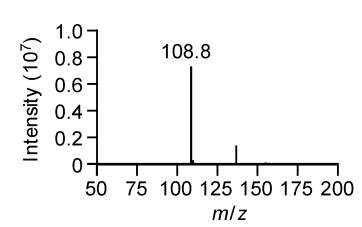
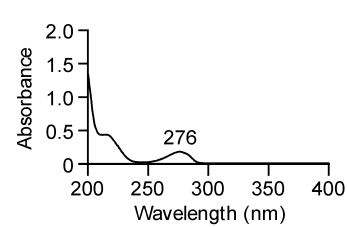
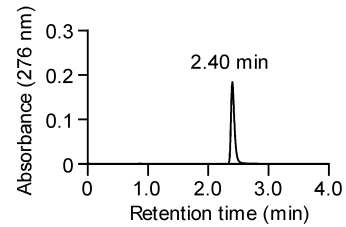
B. vanillate



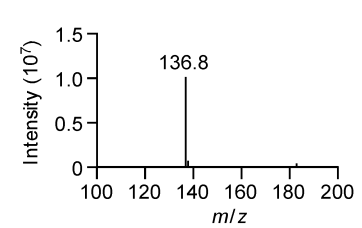
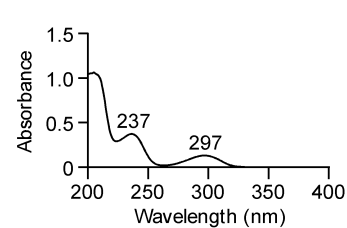
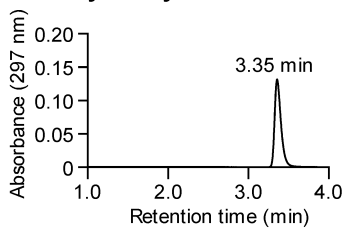
C. protocatechuate



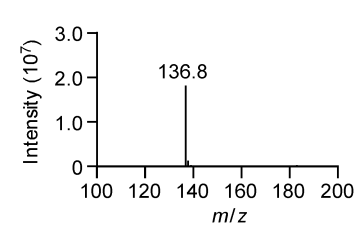
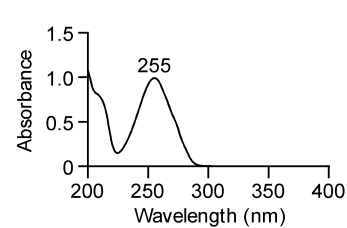
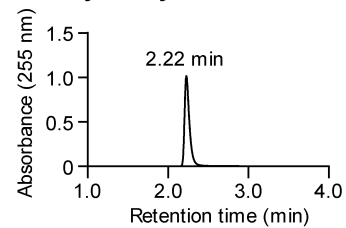
D. catechol



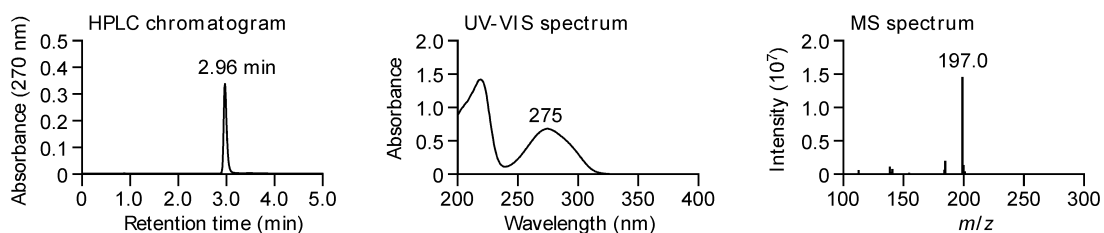
E. 3-hydroxybenzoate



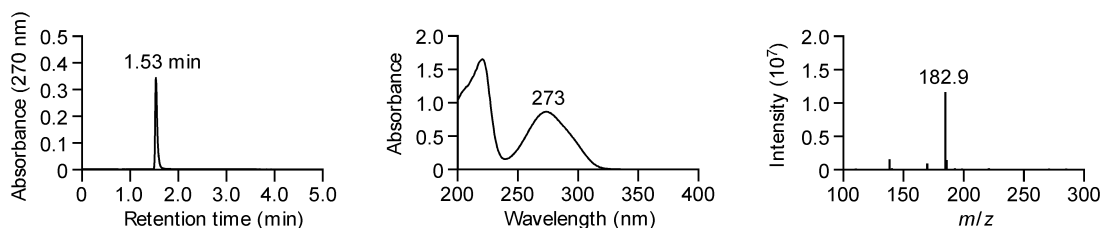
F. 4-hydroxybenzoate



G syringate



H. 3-O-methyl gallate



I. gallate

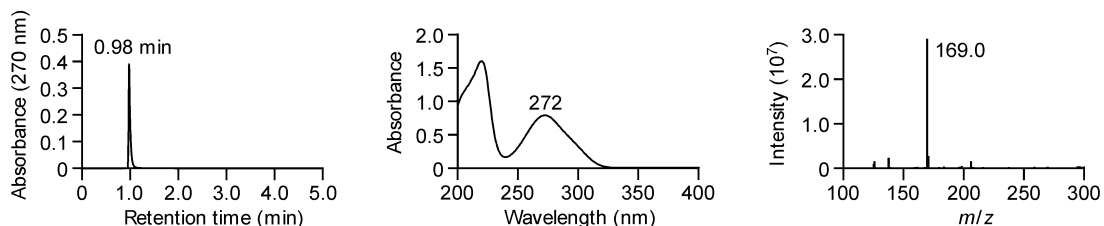
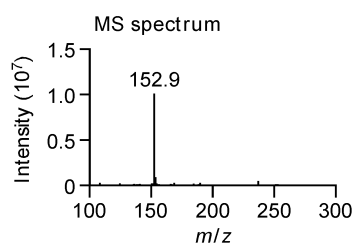
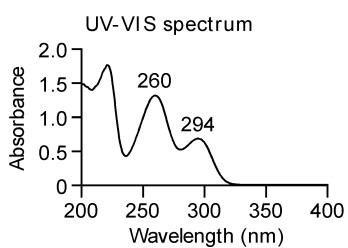
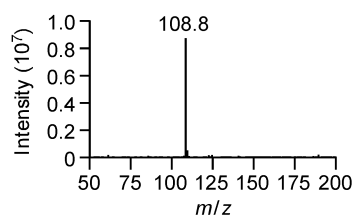
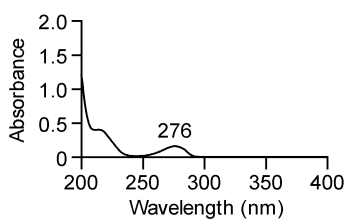


Fig. S2. LC-MS analysis of authentic benzoate (A), vanillate (B), protocatechuate (C), catechol (D), 3-hydroxybenzoate (E), 4-hydroxybenzoate (F), syringate (G), 3-*O*-methyl gallate (H), and gallate (I). HPLC chromatogram (left), UV-VIS spectra (center), and negative-ion ESI-MS spectra (right) are shown. In the HPLC analysis, compounds were detected at the following wavelength: benzoate, 230 nm; vanillate, 260 nm; PCA, 259 nm; catechol, 276 nm; 3-hydroxybenzoate, 297 nm; 4-hydroxybenzoate, 255 nm; and syringate, 3-MGA, and gallate, 270 nm. The retention times of benzoate, vanillate, PCA, catechol, 3-hydroxybenzoate, 4-hydroxybenzoate, syringate, 3-*O*-methyl gallate, and gallate were 9.28, 2.73, 1.43, 2.40, 3.35, 2.22, 2.96, 1.53, and 0.98 min, respectively.

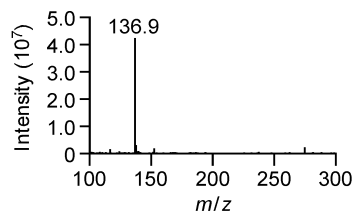
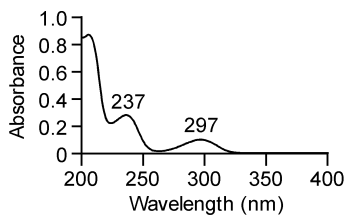
A. compound 1



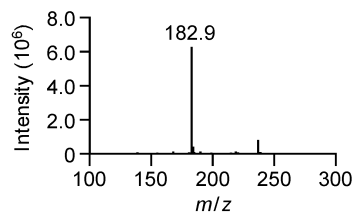
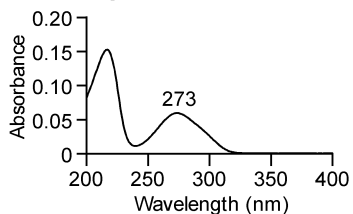
B. compound 2



C. compound 3



D. compound 4



E. compound 5

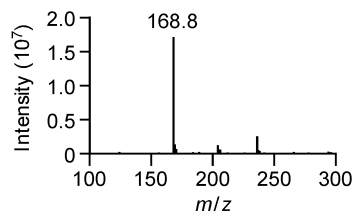
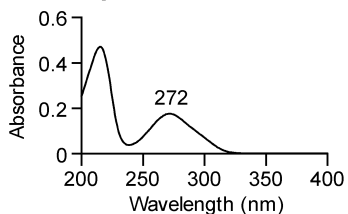


Fig. S3. UV/VIS and mass spectrum of compound I to V produced in the enrichment of vanillate and syringate. UV/VIS spectrum (left) and negative-ion ESI-MS spectrum (right) are shown.

Table S1. The archaeal phylotypes detected from the aromatics-degrading enrichment cultures.

Phylogenetic group (Family)	Phylotype	Closest relative (identity, %)	Number of clones ^a					
			BA	VA	PCA	Cat	SA	GA
<i>Methanosarcinaceae</i>	ArA-01	NR_104728 <i>Methanosarcina vacuolata</i> Z-761 (98.9)	6	13	18	17	18	24
<i>Methanomicrobiaceae</i>	ArA-02	NR_028253 <i>Methanoculleus palmolei</i> DSM 4273 (97.9)	3	8	5	6	11	3
<i>Methanocellaceae</i>	ArA-03	AM114193 <i>Methanocella arvoryzae</i> MRE50 (97.5)	14	3				3
<i>Thermoplasmatales_in certae_sedis</i>	ArA-04	CP005934 <i>Candidatus</i> “ <i>Methanomassiliicoccus intestinalis</i> ” Issoire-Mx1 (96.8)	1					
Total			24	24	23	23	29	30

^a BA; benzoate, VA; vanillate, PCA; protocatechuate, Cat; catechol, SA; syringate, GA; gallate

Table S2. The bacterial phylotypes detected from the aromatics-degrading enrichment cultures.

Phylogenetic group		Phylo- type	Closest relative (identity, %)	Number of clones ^a						
Phylum	Family			BA	VA	PCA	Cat	SA	GA	
<i>Firmicutes</i>	<i>Veillonellaceae</i>	ArB-01	NR_117663 <i>Sporomusa sphaeroides</i> DSM2875 (99.6)		8			22		
		ArB-02	NR_104801 <i>Sporomusa paucivorans</i> DSM3697 (89.8)					5		
		ArB-03	NR_114884 <i>Propionispora hippei</i> KS (94.1)		1				3	
	<i>Peptococcaceae</i>	ArB-04	NR_119207 <i>Pelotomaculum schinkii</i> HH (96.8)					22		
		ArB-05	NR_025551 <i>Desulfosporosinus auripigmenti</i> OREX-4 (97.9)		10			12		
		ArB-06	NR_025757 <i>Cryptanaerobacter phenolicus</i> LR7.2 (98.4)		6	30		2		
		ArB-07	AJ229190 Unidentified eubacterium from anoxic bulk soil, clone BSV28 (97.2)					1	3	
	<i>Ruminococcaceae</i>	ArB-08	AB596889 <i>Acetivibrio</i> sp. 6-13 (92.0)						4	22
		ArB-09	JX101685 <i>Intestinimonas butyriciproducens</i> AP4 (97.1)		3				4	13
		ArB-10	NR_044972 <i>Sporobacter termitidis</i> SYR (96.9)					4		
		ArB-11	AF550610 <i>Lachnospiraceae</i> bacterium 19gly4 (96.8)					2	2	
		ArB-12	DQ002932 <i>Anaerotruncus colihominis</i> HKU19 (94.1)			2				
		ArB-13	NR_025025 <i>Papillibacter cinnamivorans</i> CIN1 (89.5)						1	
	<i>Gracilibacteraceae</i>	ArB-14	NR_115692 <i>Gracilibacter thermotolerans</i> JW/YJL-S1 (97.7)	3	2				1	
		ArB-15	NR_041236 <i>Lutispora thermophila</i> EBR46 (97.3)		1				2	
	Clostridiales_incertae_sedis	ArB-16	NR_044093 <i>Proteiniborus ethanoliigenes</i> GW (94.7)		1	1			2	
		ArB-17	X76161 <i>Clostridium aminobutyricum</i> DSM2634 (95.7)	1		1			1	1
		ArB-18	AY571338 <i>Sedimentibacter hongkongensis</i> KI (97.6)		2					

Table S2. Continued.

Phylogenetic group		Phylo- type	Closest relative (identity, %)	Number of clones ^a					
Phylum	Family			BA	VA	PCA	Cat	SA	GA
<i>Firmicutes</i>	<i>Clostridiaceae</i>	ArB-19	NR_112900 <i>Christensenella minuta</i> YIT12065 (90.7)		1			2	
		ArB-20	NR_026322 <i>Clostridium pascui</i> DSM10365 (99.6)			3			
		ArB-21	AB971811 <i>Clostridium sporogenes</i> JCM7849 (87.5)					1	
		ArB-22	NR_113027 <i>Clostridium subterminale</i> JCM1417 (96.7)					1	
	<i>Erysipelotrichaceae</i>	ArB-23	NR_028816 <i>Turicibacter sanguinis</i> MOL361 (88.4)		1				
	<i>Syntrophomonadaceae</i>	ArB-24	NR_115800 <i>Syntrophomonas wolfei</i> 4J5 (95.1)		1				
<i>Proteobacteria</i>	<i>Syntrophaceae</i>	ArB-25	NR_102776 <i>Syntrophus aciditrophicus</i> SB (99.0)	33					
	<i>Desulfovibrionaceae</i>	ArB-26	AJ582758 <i>Desulfovibrio idahonensis</i> CY2 (99.6)					4	
	<i>Campylobacteraceae</i>	ArB-27	CP007201 <i>Sulfurospirillum multivorans</i> DSM12446 (99.2)	2					
<i>Bacteroidetes</i>	unclassified	ArB-28	AB623230 <i>Bacteroidetes</i> bacterium 4F6B (89.8)	3	3	4	4		1
	<i>Sunxiuqinia</i>	ArB-29	AB921558 <i>Bacteroidales</i> bacterium Fu11-5 (91.7)				4		
	<i>Bacteroidaceae</i>	ArB-30	NR_125463 <i>Bacteroides luti</i> UasXn-3 (98.5)	2	1	1			
	<i>Porphyromonadaceae</i>	ArB-31	AB910740 <i>Paludibacter propionicigenes</i> (97.7)		1				
<i>Ignavibacteriae</i>	unclassified	ArB-32	NR_074698 <i>Ignavibacterium album</i> JCM16511 (85.0)		1				
<i>Spirochaetes</i>	<i>Spirochaetaceae</i>	ArB-33	AY695841 <i>Spirochaetes</i> bacterium SA-10 (93.2)					1	
<i>Verrucomicrobia</i>	unclassified	ArB-34	AY960781 <i>Bacterium</i> Ellin518 (97.0)					1	
Total				44	45	40	60	48	40

^a BA; benzoate, VA; vanillate, PCA; protocatechuate, Cat; catechol, SA; syringate, GA; gallate