Supporting Information

Transformation of lignin-derived aromatics into non-aromatic polymeric substances with fluorescent activities (NAPSFA) by *Pseudomonas* sp. ITH-SA-1

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Culture conditions, determination of pigment production, and fluorescence emission

Syringaldehyde (SYAL), vanillin (VNL), or *p*-hydroxybenzaldehyde (PHBA) was added to Marine Broth 2216 (Difco) (MB) or Bacto Marine Agar 2216 (Difco) (MA) at a final concentration of 1 mg/mL. In the case of liquid cultures, bacterial strains were cultured in MB-SYAL, MB-VNL, or MB-PHBA at room temperature with shaking at 110 rpm. Samples were periodically removed from the cultures and centrifuged, and the resulting supernatants were used for evaluation of pigment production and fluorescence emission. The emission of fluorescence was determined by the naked eye under UV illumination (254 nm and 365 nm) of the sample. In the case of plate cultures, bacterial strains were streaked on MA-SYAL, MA-VNL, or MA-PHBA, and pigment production in the area surrounding individual colonies was determined by the naked eye.

Extraction of total DNA and sequencing of 16S rRNA genes

Total DNA was extracted from pure cultures of environmental isolates using Insta Gene Matrix (BioRad), and 16S rRNA gene was amplified from total DNA using PCR. The 16S rRNA genes were amplified using a pair of universal primers: 27f (5'-AGAGTTTGATCMTGGCTCAG-3', positions 8-27 of *Escherichia coli* 16S rRNA gene) and 1525r (5'-AAGGAGGTGATCCAGCC-3', positions 1541-1525 of *E. coli* 16S rRNA gene). The PCR products were purified from agarose gels, and the nucleotide sequence of purified DNA fragments was determined in both orientations using a Dye Terminator Cycle Sequencing Kit (Perkin-Elmer) and an ABI-Prism model 3100 automatic sequencer (Perkin-Elmer). The resulting sequences were searched for homology with sequences deposited in DNA databases using BLASTN.

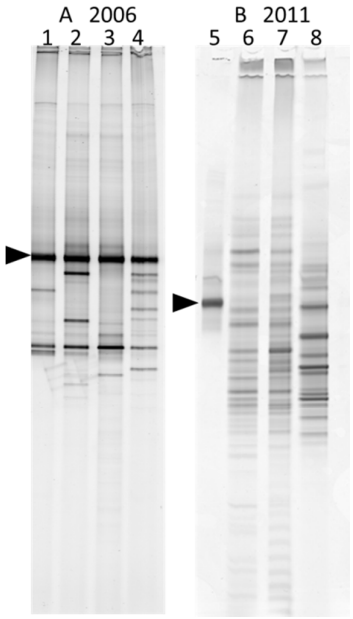

Figure S1. Comparison of adaptive changes in the structures of biphenyl (BPH)-degrading bacterial communities in seawater samples taken from Heita Bay in Iwate Prefecture. A and B indicate typical profiles of 16S rRNA gene–based PCR-denaturing gradient gel electrophoresis (DGGE) performed in the years 2006 and 2011, respectively. Lanes 1-4 and 6-8 show the structures of bacterial communities in BPH-containing enrichment cultures, and lane 5 shows the PCR product for DGGE derived from the total DNA of *Cycloclasticus pugetti* PS-1, shown as positive control for *Cycloclasticus*. Black arrowheads indicate DGGE bands closely related to *C. pugetti* PS-1. In both experiments, the construction of enrichment cultures containing BPH, cultivations, extraction of total DNA, and PCR-DGGE analyses were performed as described previously.[13] The DGGE profiles for enrichment cultures performed in the year 2006 were used as representative DGGE results for prior to March 11, 2011.

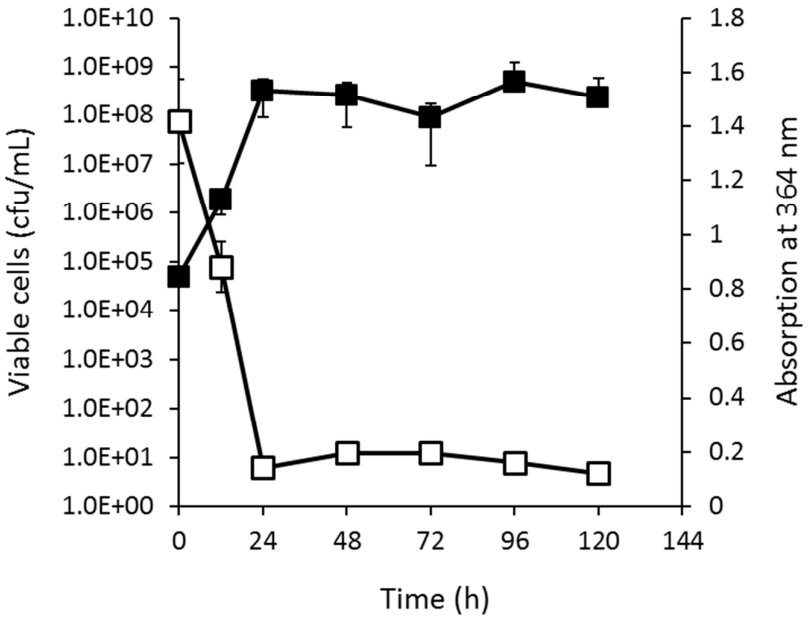

Figure S2. Relationship between cell growth and SYAL concentration. ■, growth of *Pseudomonas* sp. ITH-SA-1 in MB-SYAL; □, absorption at 364 nm. Each value is the average of at least 3 replicates from at least 3 independent experiments.

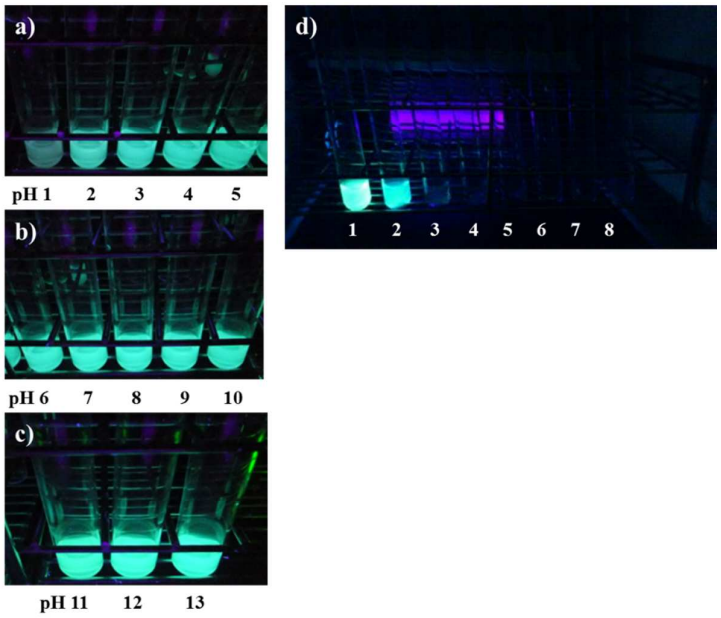

Figure S3. Fluorescence of MEFA under various conditions. a)-c), Fluorescence of MEFA at pH 2 through 13. d), Fluorescence of MEFA dissolved in various solvents. 1, Milli-Q water; 2, methanol; 3, ethanol; 4, 1-propanol; 5, 2-propanol; 6, acetonitrile; 7, acetone; 8, DMSO. Fluorescence of MEFA was recorded photographically under UV-illumination (365 nm). At least three independent experiments were conducted, and representative results are shown here.

Table S1 Pigment-producing activity and emission of fluorescence of isolated strains cultured in MB medium containing SYAL, VNL, or PHBA.

		SYAL		VNL		PHBA	
Strain	Genus	Pigment	Fluorescent	Pigment	Fluorescent	Pigment	Fluorescent
ITH-SA-1	*Pseudomonas*	+++	+++	-	-	-	-
ITH-V-1-1	*Altererythrobacter*	+++	++	-	+	-	+
ITH-V-2-1	*Altererythrobacter*	-	-	-	+	-	-
ITH-V-2-2	*Altererythrobacter*	-	-	-	+	-	-
ITH-V-3-2	*Kocuria*	+	++	+	+	+	-
ITH-V-3-5	*Kocuria*	+	++	+	+	+	-
ITH-P-1	*Microbacterium*	+	+	+	-	++	-
ITH-P-2	*Microbacterium*	N.D.	+	+	-	++	N.D.
ITH-P-3	*Microbacterium*	+	N.D.	+	-	++	-
ITH-P-4	*Microbacterium*	+	++	+	-	++	+
ITH-P-5	*Microbacterium*	+	++	+	-	N.D.	+
ITH-P-7	*Microbacterium*	+	+	++	-	++	-
ITH-P-8	*Brevibacterium*	+	++	+	+	+	N.D.
ITH-P-9	*Brevibacterium*	+	+	+	-	+	N.D.
ITH-D-01	*Glaciecola*	+	++	+	-	N.D.	N.D.
ITH-D-03	*Alteromonas*	+	++	-	-	-	-
ITH-D-05	*Alteromonas*	+	++	+	+	-	-
ITH-D-06	*Alteromonas*	N.D.	++	N.D.	+	-	-

Pigment production was classified roughly by the naked eye. +++, ++, and + indicate strong, moderate, and weak production, respectively. Fluorescence was also classified roughly by the naked eye under a black light. +++, ++, and + indicate that greenish fluorescence was strong, moderate, and weak, respectively. – indicates that pigments production was not observed or weakly cyanic fluorescence potentially due to autofluorescence of the MB broth was observed. N.D. indicates that pigment production or fluorescence of the tested strain was not reproducible from experiment to experiment, and therefore could not be evaluated. At least 3 independent experiments were performed, and the degree of decolorizing activity, pigment production, and fluorescence was determined by a majority of observers.

Table S2 Production of fluorescent substances and growth of *Pseudomonas* sp. ITH-SA-1 in different media amended with lignin-derived aromatics or major intermediates in the pathway for the metabolism of SYAL.

Substrate	MB		MM		LB		TS		NB	
	Fluorescent	Growth	Fluorescent	Growth	Fluorescent	Growth	Fluorescent	Growth	Fluorescent	Growth
SYAL	+	+	-	-	+	+	+	+	+	+
SYAC	+	+	-	-	+	+	+	+	+	+
3MGA	+	+	-	-	+	+	+	+	+	+
VNL	-	+	-	-	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
PHBA	-	+	-	-	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
GA	-	+	-	-	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
PDC	-	+	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
Pyruvate	-	+	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
Oxaloacetate	-	+	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.

+, fluorescent substances were produced in the supernatants or the cultures became turbid; -, fluorescent substances were not produced in the supernatants or the cultures did not become turbid; N.T. not tested. The substrates except PHBA were added to a medium at a final concentration of 1 mg/mL, and PHBA was added to a medium at a final concentration of 0.5 mg/mL. MB, Marine broth; MM, minimum medium; LB, Luria broth; TS, Trypto-soy broth; NB, nutrient broth.