Supplemental content

Characterization of the catabolic pathway for a phencycoumaran-type lignin-derived biaryl in *Sphingobium* sp. strain SYK-6

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**Supplementary tables:** Tables S1 and S2
**Supplementary methods**
**Supplementary figures:** Figs. S1-S8
Table S1. Strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Strain/Plasmid</th>
<th>Relevant characteristic(s)</th>
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<td>Katayama et al. 1987</td>
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* NaI', Smr', Ap', Km', and Te', resistance to nalidixic acid, streptomycin, ampicillin, kanamycin, and tetracycline, respectively.

References


Figurski DH, Helinski DR (1979) Replication of an origin-containing derivative of plasmid RK2 dependent on a plasmid function provided in *trans*. Proc Natl Acad Sci USA 76:1648-1652


http://voice.nagaokaut.ac.jp/transactions-on-gigaku/
Table S2. Primer sequences used in this study

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Supplemental methods

General experimental conditions for chemical synthesis

$^1$H and $^{13}$C NMR spectra were recorded on a JNM Lambda 400 NMR spectrometer (JEOL). Chemical shifts are shown as $\delta$-values from TMS as the internal reference. Column chromatography was carried out on columns of silica gel 60N (63–210 mesh, Kanto Chemical Co., Inc.).

Chemical synthesis

($\pm$)-dehydrodiconiferyl alcohol (DCA) was prepared by oxidative coupling of coniferyl alcohol (2), and similarly, ($\pm$)-dicarboxylic acid derivative of DCA (DCA-CC) was prepared by oxidative coupling of ethyl ferulate (3) followed by saponification. ($\pm$)-monocarboxylic acid derivative of DCA (DCA-C) was prepared by few step syntheses from above ($\pm$)-dehydrodiconiferyl alcohol (DCA). 5-formylferulic acid was synthesized from 5-bromo-2-hydroxy-3-methoxybenzaldehyde (8) through Heck reaction with ethyl acrylate. A stilbene derivative (DCA-S) was prepared by few step syntheses from 5-formylferulic acid.

Reagents and conditions

(a) NaBH$_4$; (b) HRP/H$_2$O$_2$ 23% (2 steps); (c) HRP/H$_2$O$_2$ 37%; (d) 2N NaOH/dioxane 52%; (e) TBSCl, imidazole 95%; (f) DDQ 89%; (g) pinnic oxidation 98%; (h) TBAF 78%
Reagents and conditions
(i) PivCl, pyridine 94%; (j) Heck reaction, Ethyl acrylate 37%; (k) 2N NaOH/dioxane 42%; (l) Wittig reaction 61%; (m) 2N NaOH/dioxane 31%; (n) PivCl, pyridine; (o) NaBH₄ 56% (2 steps); (p) NBS, PPh₃ 90%; (q) PPh₃, benzene reflux 81%

Synthesis of (±)-DCA
To a solution of coniferyl aldehyde (1) (5.0 g, 28 mmol; Sigma-Aldrich) in methanol (100 ml), NaBH₄ (2.25 g, 60 mmol) was added at 0°C. The solution was stirred at this temperature for 1.5 h, and then saturated NH₄Cl solution was added. The mixture was extracted with ethyl acetate (EtOAc), and the extract was washed with brine, dried over MgSO₄, filtered, and concentrated under reduced pressure. The crude coniferyl alcohol (2) (5.0 g) was dissolved in acetone/water (1/3) (320 ml), and horse radish peroxidase (3 mg) was added before dropwise addition of 3% H₂O₂ (10 ml). The mixture was stirred 10 min, and the reaction was stopped by the addition of 2N HCl. The reaction mixture was extracted by EtOAc, and the extracts were washed with brine and dried over MgSO₄. Concentration in vacuo and purification by silica gel flash chromatography (hexane/EtOAc: 1/2) gave 1.20 g (23% 2steps) of (±)-DCA as a white solid.

¹H NMR (400 MHz, CD₃OD): δ 6.96(1H, bs), 6.94(2H, bs), 6.81(1H, dd, J=8.0, 1.7Hz), 6.76(H, d, J=8.0Hz), 6.53(1H, d, J=15.9Hz), 6.22(1H, dt, J=15.9, 5.9Hz), 5.51(1H, d, J=6.4Hz), 4.19(2H, dd, J=5.9, 1.2Hz), 3.86(3H, s), 3.86-3.74(2H, m), 3.80(3H,s), 3.48(1H, m). ¹³C NMR (100 MHz, CD₃OD): δ 149.3, 149.1, 147.6, 145.5, 134.5, 132.6, 132.0, 130.3, 127.5, 119.8, 116.5, 116.2, 112.1, 110.5, 89.3, 64.8, 63.9, 56.7, 56.4, 55.1 ppm.

Synthesis of (±)-DCA-CC
To a stirred solution of ethyl ferulate (3) (10 g, 45 mmol; Tokyo Chemical Industry Co., Ltd.) and Horse radish peroxidase (10 mg) in acetone/water (2/3) (250 ml), 3% H₂O₂ (20 ml) was added. The mixture was stirred for 30 min, and the reaction was stopped by the addition of 2N HCl. The reaction mixture was extracted by EtOAc, and the extracts were washed with brine and dried over MgSO₄. Concentration in vacuo and purification by silica gel flash chromatography gave 3.71g (37%) of diethyl ester (±)-4 as a white solid. ¹H
NMR (400 MHz, CDCl₃): δ 7.64(1H, d, J=16.0Hz), 7.619(1H, bs), 7.03(1H, d, bd, J=1.2Hz), 6.95-6.85(3H, m), 6.31(1H, d, J=16.0Hz), 6.11(1H, d, J=8.3Hz), 5.64(1H, bs), 4.35-4.20(5H, m), 3.92(3H, s), 3.88(3H, s), 1.34(6H, t, J=7.1Hz). ¹³C NMR (100 MHz, CDCl₃): δ 170.3, 167.2, 150.0, 146.7, 146.1, 144.8, 144.6, 131.5, 128.7, 125.9, 119.5, 117.9, 116.0, 114.5, 112.0, 108.8, 87.5, 61.9, 60.4, 45.2, 56.1, 56.0, 55.6, 14.4, 14.3 ppm.

To a solution of diethyl ester (±)-4 (22.8 mg, 0.0515 mmol) in dioxane (1 ml), 2N NaOH (2.5 ml) was added dropwise at 0°C. After stirring for 10 min at room temperature, the reaction was acidified with 1N HCl. The reaction was extracted by EtOAc and washed with brine. The organic layer was then dried (MgSO₄) and concentrated under reduced pressure. The crude residue was dissolved in CHCl₃ and left to stand overnight at room temperature. The formed precipitate was collected by filtration, washed with cold CHCl₃ and dried in vacuo to give (±)-DCA-CC (10.3 mg, 52%) as a white solid.

¹H NMR (400 MHz, Acetone-d₆): δ 11.08(1H, bs), 7.74(1H, bs), 7.65(1H, d, J=16.0Hz), 7.34(2H, bs), 7.11(1H, d, J=2.0Hz), 6.93(1H, dd, J=8.3, 2.0Hz), 6.85(1H, d, J=8.3Hz), 6.43(1H, d, J=16.0Hz), 6.05(1H, d, J=7.8Hz), 4.46(1H, d, J=7.8Hz), 4.39(3H, s), 3.85(3H, s). ¹³C NMR (100 MHz, Acetone-d₆): δ 171.9, 168.0, 151.0, 148.6, 148.0, 145.8, 145.6, 132.2, 129.5, 127.6, 120.1, 118.9, 116.7, 115.8, 113.4, 110.7, 88.4, 56.5, 56.3, 55.8 ppm.

**tri-TBS ether (±)-5**

To a stirred solution of (±)-DCA (156 mg, 0.436 mmol) and imidazole (150 mg, 2.2 mmol) in dry THF (10 ml) at room temperature, i-butylidimethylsilyl chloride (240 mg, 1.6 mmol) was added. After being stirred at room temperature for 12 h, the reaction mixture was concentrated in vacuo and the residue was purified by a silica gel column chromatography (hexane/EtOAc: 5/1) to give the tri-TBS ether (±)-5 (290 mg, 95%) as a colorless oil.

¹H NMR (400 MHz, CDCl₃): δ 6.90-6.80(3H, m), 6.81(1H, d, J=2.0Hz), 6.77(1H, d, J=8.1Hz), 6.51(1H, d, J=15.8Hz), 6.13(1H, d, J=15.8Hz), 5.52(1H, d, J=6.1Hz), 4.33(2H, dd, J=5.4, 1.7Hz), 3.91(3H, s), 3.91-3.75(2H, m), 3.76(3H, s), 3.58(1H, m), 0.98(9H, s), 0.94(9H, s), 0.88(9H, s), 0.13(6H, s), 0.11(6H, s), 0.05(3H, s), 0.03(3H, s). ¹³C NMR (100 MHz, CDCl₃): δ 150.9, 147.9, 144.8, 144.2, 135.1, 130.9, 129.7, 128.7, 126.8, 120.7, 118.5, 115.4, 110.1, 110.0, 88.3, 65.5, 64.0, 56.0, 55.5, 53.8, 26.0, 25.9, 25.7, 18.5, 18.4, 18.2, -4.67, -5.13, -5.37, -5.46 ppm.

**Aldehyde (±)-6**

To a stirred solution of (±)-5 (290 mg, 0.414 mmol) in CH₂Cl₂ (5 ml) at room temperature, 2,3-dichloro-5,6-dicyano-p-benzoquinone (136 mg, 0.6 mmol) was added. After being stirred at room temperature for 3 h, 4-(dimethylamino)pyridine (86 mg, 0.7 mmol) was added. After stirring at room temperature for 30 min, the reaction mixture was concentrated in vacuo and the residue was purified by a silica gel column chromatography (hexane/EtOAc: 2/1) to give aldehyde (±)-6 (214.7 mg, 89%) as a pale yellow oil.

¹H NMR (400 MHz, CDCl₃): δ 9.65(1H, d, J=7.6Hz), 7.40(1H, d, J=15.6Hz), 7.11(1H, bs), 7.02(1H, bs), 6.90-6.75(3H, m), 6.59(1H, dd, J=15.6, 7.6Hz), 5.60(1H, d, J=6.1Hz), 3.94(3H, s), 3.91(1H, m), 3.82(1H, dd, J=10.0, 7.6Hz), 3.77(3H, s), 3.64(1H, m), 0.98(9H, s), 0.88(9H, s), 0.13(6H, s), 0.05(3H, s), 0.03(3H, s). ¹³C NMR (100 MHz, CDCl₃): δ 193.6, 153.2, 151.5, 151.1, 145.1, 144.7, 134.2, 129.7, 127.8, 126.3, 120.9, 118.7, 118.5, 112.1, 110.0, 89.1, 65.1, 56.1, 55.5, 53.3, 25.8, 25.7, 18.4, 18.2, -4.66, -5.39, -5.49 ppm.
Carboxylic acid (±)-7
To a stirred solution of aldehyde (±)-6 (75.5 mg, 0.13 mmol) and 2,3-dimethylbutene (700 μl, 6.59 mmol) in t-BuOH:H₂O (2:1, 3.9 ml) at room temperature, sodium dihydrogen phosphate (115 mg, 0.95 mmol) was added followed by the addition of sodium chloride (109 mg, 1.2 mmol). After stirred at this temperature for 12 h, the reaction mixture was quenched with brine and extracted with EtOAc. The organic phases were dried with Na₂SO₄ and concentrated in vacuo. Purification of the crude residue by silica gel column chromatography (hexane/EtOAc: 1/1) gave (±)-7 (75.8 mg, 98%) as a colorless oil. (400 MHz, CDCl₃): δ 7.72(1H, d, J=15.9Hz), 7.08(1H, bs), 7.00(1H, bs), 6.85(1H, bs), 6.82(1H, m), 6.78(1H, d, J=8.3Hz), 6.29(1H, d, J=15.9Hz), 5.60(1H, d, J=5.8Hz), 3.93(3H, s), 3.89(1H, m), 3.81(1H, dd, J=9.8, 7.6Hz), 3.77(3H, s), 3.63(1H, m), 0.98(9H, s), 0.88(9H, s), 0.13(6H, s), 0.05(3H, s), 0.03(3H, s). ¹³C NMR (100 MHz, CDCl₃): δ 171.8, 151.0, 147.3, 145.0, 144.5, 134.4, 129.4, 120.8, 118.5, 118.3, 114.1, 112.0, 110.0, 89.0, 65.2, 60.4, 56.1, 55.5, 53.4, 25.8, 25.7, 18.4, 18.2, -4.66, -5.38, -5.47 ppm.

Synthesis of (±)-DCA-C
To a stirred solution of (±)-7 (75.8 mg, 0.126 mmol) in THF (5 ml) at room temperature, tetrabutylammonium fluoride solution (1.0 M in THF, 300 μl, 0.3 mmol) was added. After being stirred at room temperature for 30 min, the reaction was acidified with 1N HCl. The reaction mixture was extracted by EtOAc and washed with brine. The organic layer was then dried (MgSO₄) and concentrated under reduced pressure. Purification of the crude residue by silica gel column chromatography (hexane/EtOAc: 1/3) gave (±)-DCA-C (36.8 mg, 78%) as a pale yellow solid. ¹H NMR (400 MHz, CD₂OD): δ 7.62(1H, d, J=15.9Hz), 7.18(1H, bs), 7.14(1H, d, J=1.2Hz), 6.94(1H, d, J=2.0Hz), 6.82(1H, dd, J=8.1, 2.0Hz), 6.77(H, d, J=8.1Hz), 6.34(1H, d, J=15.9Hz), 5.58(1H, d, J=6.4Hz), 3.90(3H, s), 3.90-3.75(2H, m), 3.81(3H, s), 3.53(1H, m). ¹³C NMR (100 MHz, CD₂OD): δ 171.0, 151.9, 149.2, 147.8, 146.7, 145.9, 134.2, 131.0, 129.8, 119.8, 119.0, 116.6, 116.2, 113.7, 110.6, 89.8, 64.7, 56.8, 56.4, 54.8 ppm.

Pivaloylate 9
To a solution of 5-bromo-2-hydroxy-3-methoxybenzaldehyde 8 (1.0 g, 4.33 mmol; WAKO Chemical Co., Ltd.) and pyridine (0.63 ml, 7.8mmol) in dry CH₂Cl₂ (8 ml) at 0°C, pivaloyl chloride (0.64 ml, 5.2 mmol) was slowly added. After being stirred at room temperature for 12 h, the reaction mixture was concentrated in vacuo and the residue was purified by a silica gel column chromatography (hexane/EtOAc: 3/1) to give the pivaloylate 9 (1.28 g, 94%) as a white solid. ¹H NMR (400 MHz, CDCl₃): δ 10.04(1H, s), 7.57(1H, d, J=2.2Hz), 7.25(1H, d, J=2.2Hz), 3.83(3H, s), 1.38(9H,s). ¹³C NMR (100 MHz, CDCl₃): δ 186.9, 175.9, 152.6, 142.1, 130.2, 122.7, 120.7, 119.6, 56.6, 39.4, 27.1 ppm.

Ethyl ester 10
A solution of 9 (940 mg, 2.98 mmol), ethyl acrylate (3 ml, 27.6 mmol), Et₃N (600 μl, 4.3 mmol), Pd(OAc)₂ (13.5 mg, 0.06 mmol), and triphenylphosphine (31.5 mg, 0.12 mmol) in N,N-dimethylformamide (DMF) (8 ml) was heated at 90°C for 6 h. After cooling, water was added and extracted by EtOAc. The organic layer was evaporated under reduced pressure to give a residue, which was purified by silica gel column chromatography (hexane/EtOAc: 2/1) to give 10 (360 mg, 37%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃): δ 10.11(1H, s), 7.63(H, d, J=15.8Hz), 7.60(1H, d, J=2.0Hz), 7.27(H, d, J=2.0Hz), 6.43(1H, d, J=15.8Hz), 4.25(2H, q, J=7.2Hz), 3.86(3H, s), 1.39(3H, s), 1.32(3H, s), 1.27(2H, t, J=7.2Hz). ¹³C NMR (100 MHz, CDCl₃): δ 187.7, 176.0, 166.4, 152.1, 142.7, 133.1, 129.6, 120.1, 119.9, 115.8, 60.7, 56.4, 39.5, 27.1, 14.3 ppm.
5-Formylferulic acid
To a solution of 10 (34 mg, 0.1 mmol) in dioxane (1 ml), 2N NaOH (1 ml) was added dropwise at 0°C. The reaction mixture was stirred at room temperature overnight, and then acidified with 1N HCl. The reaction was extracted by EtOAc and washed with brine. The organic layer was then dried (MgSO₄) and concentrated under reduced pressure. Purification of the crude residue by silica gel column chromatography (hexane/EtOAc: 1/5) gave 5-formylferulic acid (9.5 mg, 43%) as a pale yellow solid.

H NMR (400 MHz, Acetone-d₆): δ 10.85(1H, bs), 10.17(1H, bs), 7.66(1H, d, J=16.1Hz), 7.65(2H, bs), 6.52(1H, d, J=16.1Hz), 3.99(3H, s). ¹³C NMR (100 MHz, Acetone-d₆): δ 196.1, 167.7, 154.0, 149.8, 144.3, 127.5, 125.3, 122.2, 118.1, 116.5, 56.7 ppm.

Stilbene 15
To a stirred suspension of 10 (160 mg, 0.48 mmol) and phosphonium salt 14 (325 mg, 0.576 mmol) in THF (5 ml) at 0°C, potassium tert-butoxide (65 mg, 0.576 mmol) was added. The reaction mixture was stirred at room temperature for 1.5 h. The formed precipitate was isolated by filtration, and the solvent was evaporated, and the product was purified by silica gel column chromatography (hexane/EtOAc: 3/1) to give 15 (38.8 mg, 16%) as a pale yellow oil.

(HOAc, 5 ml) at 0°C, 14 (325 mg, 0.576 mmol) in THF (5 ml), tert-butoxide (65 mg, 0.576 mmol) was added. The reaction mixture was stirred at room temperature for 1.5 h. The formed precipitate was isolated by filtration, and the solvent was evaporated, and the product was purified by silica gel column chromatography (hexane/EtOAc: 3/1) to give 15 (38.8 mg, 16%) as a pale yellow oil.

DCA-S
To a solution of 15 (28.7 mg, 0.053 mmol) in dioxane (1 ml), 2N NaOH (1 ml) was added dropwise at 0°C. The reaction mixture was stirred at room temperature overnight, and then acidified with 1N HCl. The reaction was extracted by EtOAc and washed with brine. The organic layer was then dried (MgSO₄) and concentrated under reduced pressure. Purification of the crude residue by silica gel column chromatography (hexane/EtOAc: 0/1) gave DCA-S (5.7 mg, 31%) as a pale yellow solid.

H NMR (400 MHz, Acetone-d₆): δ 10.58(1H, bs), 8.29(1H, bs), 7.71(1H, bs), 7.64(1H, d, J=16.0Hz), 7.56(1H, d, J=1.7Hz), 7.36(1H, d, J=16.6Hz), 7.31(1H, d, J=16.6Hz), 7.25(1H, d, J=2.0Hz), 7.24(1H, d, J=2.0Hz), 7.07(1H, dd, J=8.3, 2.0Hz), 6.84(1H, d, J=8.3Hz), 6.45(1H, d, J=16.0Hz), 3.96(3H, s), 3.92(3H, s). ¹³C NMR (100 MHz, Acetone-d₆): δ 176.7, 175.9, 166.8, 151.9, 151.4, 144.2, 140.3, 139.5, 135.7, 132.6, 131.5, 131.2, 123.0, 121.1, 119.4, 118.5, 118.3, 110.1, 109.6, 60.6, 56.1, 55.8, 39.3, 39.1, 27.2, 14.3 ppm.

Alcohol 12
To a solution of vanillin 8 (1.52 g, 10.0 mmol; Kanto Chemical Co., Inc.) and pyridine (1.7 ml, 21 mmol) in dry CH₂Cl₂ (20 ml) at 0°C, pivaloyl chloride (1.68 ml, 13.65 mmol) was slowly added. After being stirred at room temperature for 12 h, saturated NH₄Cl solution was added. The mixture was extracted with EtOAc and the extract was washed with brine, dried over MgSO₄, filtered, and concentrated under reduced pressure. The crude 11 was used in the next reaction without further purification. To a solution of crude 11 (10 mmol) in methanol (20 ml), NaBH₄ (1.9 g, 50 mmol) was added at 0°C. The solution was stirred at room temperature for 45 min, and then saturated NH₄Cl solution was added. The mixture was extracted with EtOAc and the extract was washed with brine, dried over MgSO₄, filtered, and concentrated under reduced pressure. Purification of the crude residue by silica gel column chromatography (hexane/EtOAc: 2/1) gave 12 (1.35 g, 5.68 mmol, 56%) as a colorless oil.

H NMR (400 MHz, CDCl₃): δ 6.95(1H, d, J=1.2Hz), 6.94(1H, d, J=8.1Hz), 6.86(H, d, J=1.2Hz), 4.61(2H, s), 2.72(3H, s).
3.77(3H, s), 1.34(9H, s). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 190.9, 176.8, 151.3, 139.6, 139.5, 122.5, 118.9, 111.0, 65.0, 56.1, 55.8, 39.0, 27.2, 14.1 ppm.

Bromide 13
To a solution of 12 (1.33 g, 5.58 mmol) and triphenylphosphine (1.537 g, 5.86 mmol) in dry THF (15 ml) at 0°C, N-bromosuccinimide (1.043 g, 5.86 mmol) was added. After being stirred at room temperature for 1.5 h, the reaction mixture was concentrated in vacuo and the residue was purified by a silica gel column chromatography (hexane/EtOAc: 4/1) to give 13 (1.50 g, 90%) as a pale yellow solid.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 6.96-6.91(3H, m), 4.45(2H, s), 3.79(3H, s), 1.34(9H, s).

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 176.5, 151.3, 140.3, 136.2, 122.8, 121.3, 113.1, 55.9, 39.0, 33.3, 27.2, 14.2 ppm.

Phosphonium salt 14
A solution of 13 (1.49 g, 4.95 mmol) and triphenylphosphine (1.36 g, 5.2 mmol) in dry benzene (40 ml) was refluxed for 4 h. After cooling to room temperature, the reaction mixture left to stand overnight. Formed white precipitate was isolated by filtration and dried under reduced pressure to give 14 (2.26 g, 81%) as a white solid.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.80-7.50(15H, m), 6.98(1H, bs), 6.70(1H, d, J=8.1Hz), 6.49(1H, dt, J=8.1, 2.3Hz), 5.27(2H, d, J=13.9Hz), 3.44(3H, s), 1.30(9H, s). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 176.8, 151.4, 151.3, 140.3, 140.2, 135.0, 134.9, 134.5, 134.4, 130.2, 130.1, 125.7, 125.6, 123.1, 123.0, 122.8, 122.7, 118.0, 117.1, 116.4, 116.3, 56.2, 39.1, 30.7, 30.2, 27.1 ppm.

Preparation of DCA-L
E. coli BL21(DE3) harboring pET24930 was grown in LB containing ampicillin at 30°C. Expression of SLG_24930 was induced for 24 h at 16°C by adding 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) when the optical density at 600 nm (OD$_{600}$) of the culture reached 0.5. Cells were centrifuged at 5,000 × g for 15 min at 4°C. A cell pellet was washed twice with 50 mM Tris-HCl buffer (pH 7.5; buffer A) and suspended in the same buffer to an OD$_{600}$ of 5.0. A 100 mM solution of DCA dissolved in dimethyl sulfoxide was added into 50 ml of the cell suspension to a final concentration of 2 mM. After incubation with shaking for 36 h at 30°C, the culture was centrifuged and the supernatant was collected. To obtain DCA-L, the supernatant was extracted with ethyl acetate, and compounds were separated by thin-layer chromatography using Silica Gel 60 F$_{254}$ (Merck Millipore) with the solvent mixture of 58% benzene, 25% ethyl acetate, and 17% methanol. Compounds were visualized under UV light at 254 nm. DCA-L has an $R_f$ of 0.73 in this system and was extracted with ethyl acetate. The resultant compound was confirmed by LC-MS analysis.
Construction of expression plasmids
DNA fragments carrying SLG_04410, SLG_05620, and SLG_07280 were amplified by PCR using SYK-6 total DNA as a template and primer sets shown in Table S2. The amplified fragments were ligated in pT7Blue, and then the NdeI-BamHI fragments of the resultant plasmids were inserted in pCold-TF to generate pCTF04410, pCTF05620, and pCTF07280. For creation of pCTF12260, the 0.8-kb BamHI digest of the PCR product of SLG_12260 (carrying the downstream region of SLG_12260) was ligated into pT7Blue to obtain pT12260S. The 1.3-kb NdeI-BamHI fragment of the same PCR product (carrying the upstream region of SLG_12260) was ligated into pT7Blue to obtain pT12260L. The 1.3-kb NdeI-BamHI of pT12260L and the 0.8-kb BamHI fragment of pT12260S were ligated into pCold-TF to obtain pCTF12260. DNA fragments carrying SLG_09420, SLG_09790, SLG_24930, and SLG_24940 were amplified by PCR using SYK-6 total DNA as a template and primer sets shown in Table S2. The PCR amplified fragments were ligated in pT7Blue, and the NdeI-BamHI fragments of the resulting plasmids were cloned in pET-16b to obtain pET09420, pET09790, pET24930, and pET24940. Nucleotide sequences of the inserts in these plasmids were confirmed by nucleotide sequencing.
Fig. S1. LC-MS analysis of authentic DCA (a and e), DCA-C (b and f), DCA-CC (c and g), and DCA-S (d and h). HPLC chromatograms detected at 230 nm (a and b) and 290 nm (c and d), and negative-ion ESI-MS spectra (e-h) are shown. The retention times of DCA, DCA-C, DCA-CC, and DCA-S were 2.0, 2.3, 3.0, and 7.3 min, respectively.
Fig. S2. LC-MS analysis of authentic vanillate (a and e), ferulate (b and f), vanillin (c and g), and 5-formylferulate (d and h). HPLC chromatograms detected at 290 nm (a-d), and negative-ion ESI-MS spectra (e-h) are shown. The retention times of vanillate, ferulate, vanillin, and 5-formylferulate were 1.1, 1.4, 1.4, and 2.0 min, respectively.
**Fig. S3.** Phylogenetic tree of putative SYK-6 ADHs with known quinohemoprotein ADHs of other strains. Enzymes: DHG_ECOLI, quinoprotein glucose dehydrogenase of *E. coli* K-12; DHM1_METEA, methanol dehydrogenase of *Methylobacterium extorquens* AM1; QGDA_PSEPU, quinohemoprotein alcohol dehydrogenase ADH II G of *P. putida* HK5; QHED_COMTE, quinohemoprotein ethanol dehydrogenase of *Comamonas testosteroni* ATCC 15667; and QHED_PSEPU, quinohemoprotein alcohol dehydrogenase ADH IIB of *P. putida* HK5.
Fig. S4. Phylogenetic tree of putative SYK-6 ADHs with known aryl ADHs of other strains. Enzymes: XYLBPSEPU, benzyl alcohol dehydrogenase of P. putida mt-2; GEOA_CASDE, geraniol dehydrogenase of Castellaniella defragrans 65Phen; CAD_STRMY, coniferyl alcohol dehydrogenase II of Streptomyces sp. NL15-2K; ADH_THEBR, isopropanol dehydrogenase of Thermoanaerobacter brockii HTD4; and ADH1_GEOSE, alcohol dehydrogenase of Geobacillus stearothermophilus NCA1503.
**Fig. S5.** Expression of putative SYK-6 ADH genes in *E. coli* demonstrated on SDS-PAGE. M, molecular size markers. (a) Cell extracts of *E. coli* NEB10-beta harboring pCold-TF (lane 1), pCTF04410 (lane 2), pCTF05620 (lane 3), pCTF07280 (lane 4), and pCTF12260 (lane 5). (b) Cell extracts of *E. coli* BL21(DE3) harboring pET-16b (lane 1), pET09420 (lane 2), pET09790 (lane 3), pET24930 (lane 4), and pET24940 (lane 5).
**Fig. S6.** Phylogenetic tree of putative SYK-6 ALDHs with known ALDHs of other strains. Enzymes: VDH_PSHR199, vanillin dehydrogenase of *Pseudomonas* sp. HR199; XYLC_PPMT53, benzaldehyde dehydrogenase of *P. putida* MT53; MMSA_PAPAO1, methylmalonate-semialdehyde dehydrogenase of *P. aeruginosa* PAO1; KGSDH_ACADP1 α-ketoglutaric semialdehyde dehydrogenase of *Acinetobacter baylai* ADP1; CALB_PSHR199, coniferyl aldehyde dehydrogenase of *Pseudomonas* sp. HR199; CYMC_PPFI cuminaldehyde dehydrogenase of *P. putida* F1; PUUC_ECK12, 3-hydroxypropionaldehyde dehydrogenase of *E. coli* K12; GABD_ECK12, succinate-semialdehyde dehydrogenase of *E. coli* K12; and SLG_07060_LigV, vanillin dehydrogenase of SYK-6.
Fig. S7. Expression of putative ALDH genes of SYK-6 in *E. coli* BL21(DE3). Cell extracts of *E. coli* harboring pET-21a(+) and pET-21a(+) with ALDH genes were separated on SDS-12% polyacrylamide gels. Lanes: M, molecular size markers; 1, pET-21a(+); 2, SLG_31150; 3, SLG_12190; 4, SLG_12800; 5, SLG_34940; 6, SLG_27910; 7, SLG_07790; 8, SLG_07610; 9, SLG_28320; 10, SLG_27920; 11, SLG_07270; 12, SLG_12020; 13, SLG_38120; 14, SLG_11410; 15, SLG_09510; 16, SLG_27210; 17, SLG_p_00680; 18, SLG_20400; 19, SLG_18210; 20, SLG_09400; 21, SLG_28150; 22, SLG_09920; 23, SLG_32240; and 24, SLG_07060.
Fig. S8. Conversion of DCA-L by resting cells of SYK-6 (circles) and SME044 (triangles). The data are averages ± standard deviations of three independent experiments.