Supplemental figures

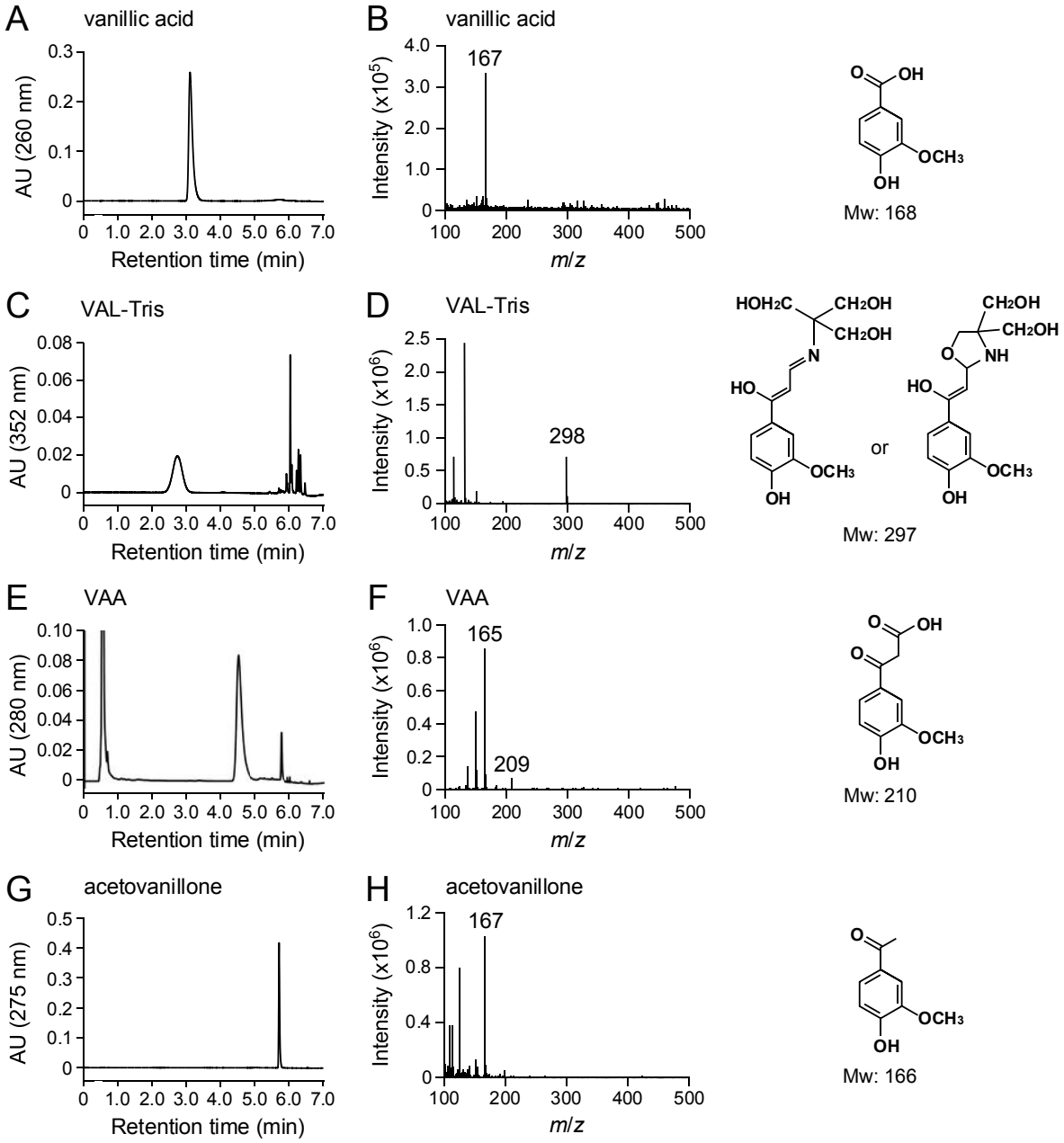


FIG S1. HPLC-MS analysis of authentic compounds.

(A, C, E, G, I, K, and M) HPLC chromatograms of vanillic acid (retention time, 3.1 min), VAL-Tris (2.9 min), VAA (4.7 min), acetovanillone (5.8 min), syringic acid (2.6 min), HPV (4.1 min), and HPS (2.9 min). (B, D, F, H, J, L, and N) ESI-MS spectra of vanillic acid (negative mode), VAL-Tris (positive mode), VAA (negative mode), acetovanillone (positive mode), syringic acid (negative mode), HPV (positive mode), and HPS (negative mode).

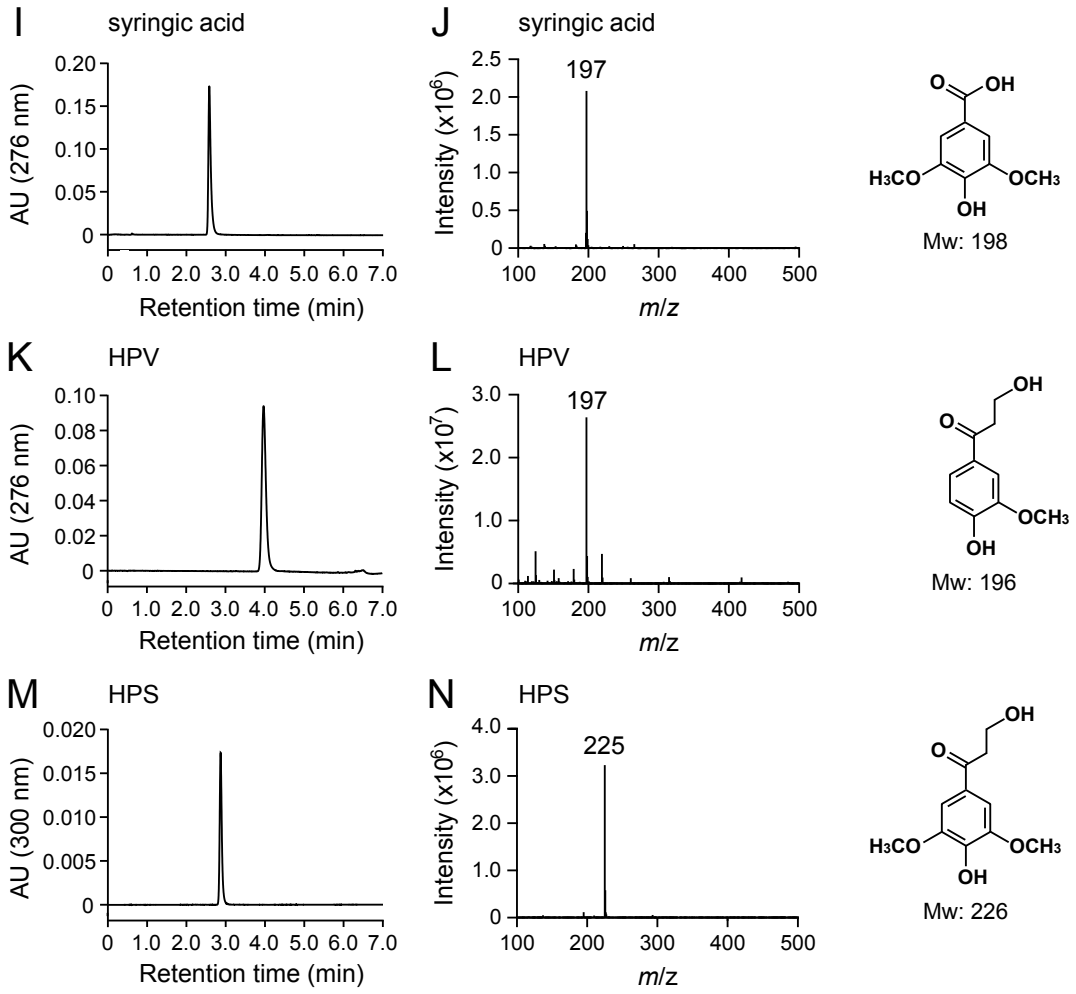


FIG S1—*Continued.*

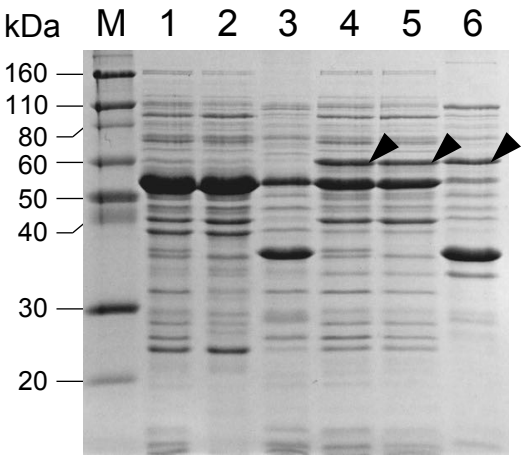


FIG S2. Expression of *hpvZ* in *E. coli*.
Proteins (10 μg) were separated on SDS-12% polyacrylamide gels and stained with Coomassie Brilliant Blue. Lanes: 1–3, *E. coli* BL21(DE3) harboring pCold I (vector) and pTf16; 4–6, *E. coli* BL21(DE3) harboring pCold12830 and pTf16; 1 and 4, cell extracts; 2 and 5, soluble fractions; 3 and 6, membrane fractions; M, molecular mass markers.

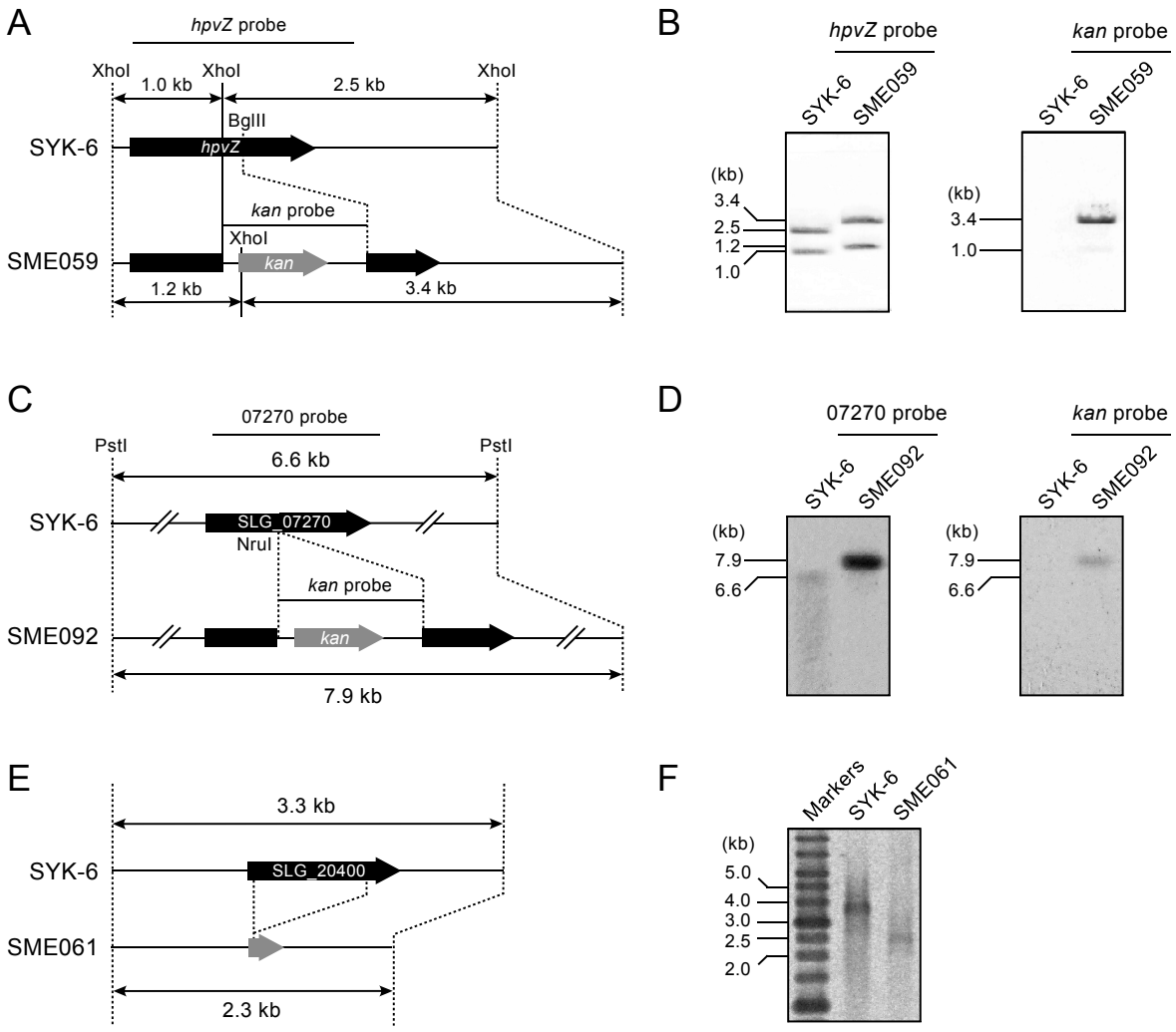


FIG S3. Disruption of SLG_12830, SLG_07270, and SLG_20400 in SYK-6.
Schematic representations of the disruptions of SLG_12830 (*hpvZ*) (A) and SLG_07270 (C) by the insertion of a kanamycin resistance gene (*kan*) and the disruption of SLG_20400 (E) by deletion. (B) Southern hybridization analysis of SME059. Total DNA of SYK-6 and SME059 digested with XhoI were hybridized with a *hpvZ* probe and a *kan* probe. (D) Southern hybridization analysis of SME092. Total DNA of SYK-6 and SME092 digested with PstI were hybridized with a 07270 probe and a *kan* probe. (F) Colony PCR analysis of SME061 using primers shown in Table 3.

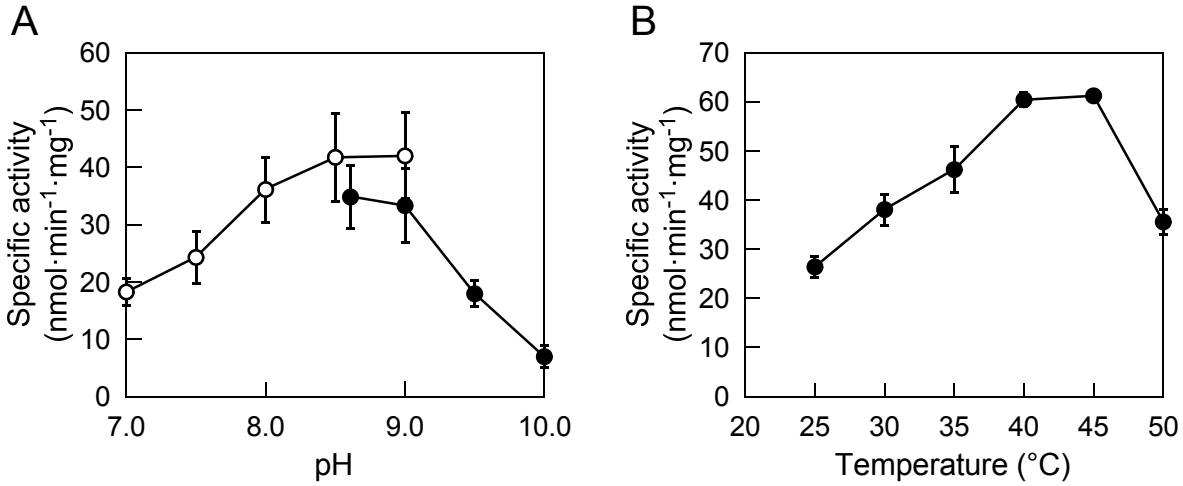


FIG S4. Optimum pH (A) and temperature (B) for HpvZ.

(A) The HPV-oxidizing activity of HpvZ was determined using 50 mM GTA buffer (pH 7.0 to 9.0; open circles) and 50 mM CHES (*N*-cyclohexyl-2-aminoethanesulfonic acid) buffer (pH 8.6 to 10.0; closed circles) at 30°C. (B) The HPV-oxidizing activity of HpvZ was determined using 50 mM Tris-HCl buffer (pH 7.5) at 25 to 50°C. The data represent the averages ± standard deviations of three independent experiments.

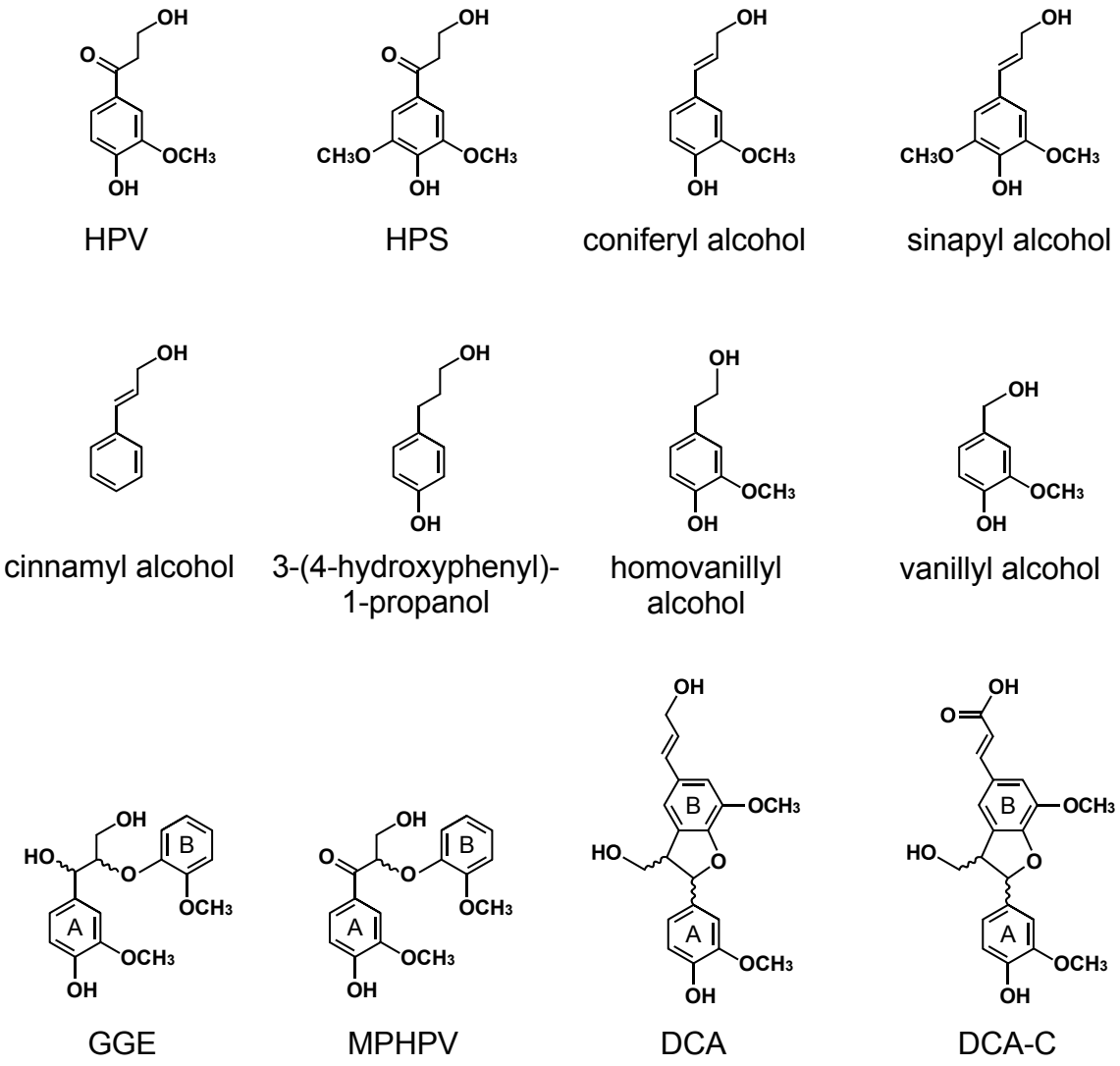
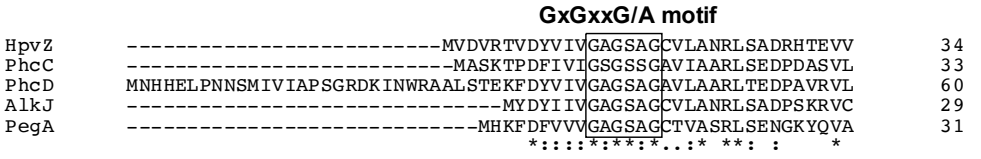


FIG S5. Chemical structures of substrates used to examine the substrate range of HpvZ. Abbreviations: HPV, β-hydroxypropiovanillone; HPS, β-hydroxypropiosyringone; GGE, guaiacylglycerol-β-guaiacyl ether; MPHPV, α-(2-methoxyphenoxy)-β-hydroxypropiovanillone; DCA, dehydrodiconiferyl alcohol; DCA-C, 3-(2-(4-hydroxy-3-methoxyphenyl)-3-(hydroxymethyl)-7-methoxy-2,3-dihydrobenzofuran-5-yl)acrylic acid.

A



B

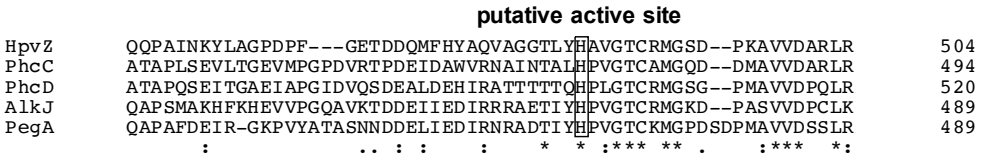


FIG S6. Comparison of the amino acid sequence of HpvZ with those of other GMC oxidoreductase family enzymes.

Enzymes: HpvZ (SLG_12830), HPV oxidase; PhcC (SLG_09480) and PhcD (SLG_09500), DCA-C oxidases of *Sphingobium* sp. SYK-6; AlkJ (CAB51051), alcohol dehydrogenase of *Pseudomonas putida* GPo1; PegA (BAE96591), polyethylene glycol dehydrogenase of *Sphingopyxis terrae*. The GxGxxG/A motif located at N-terminal region (A) and the putative active-site histidine located at C-terminal region (B) are shown.

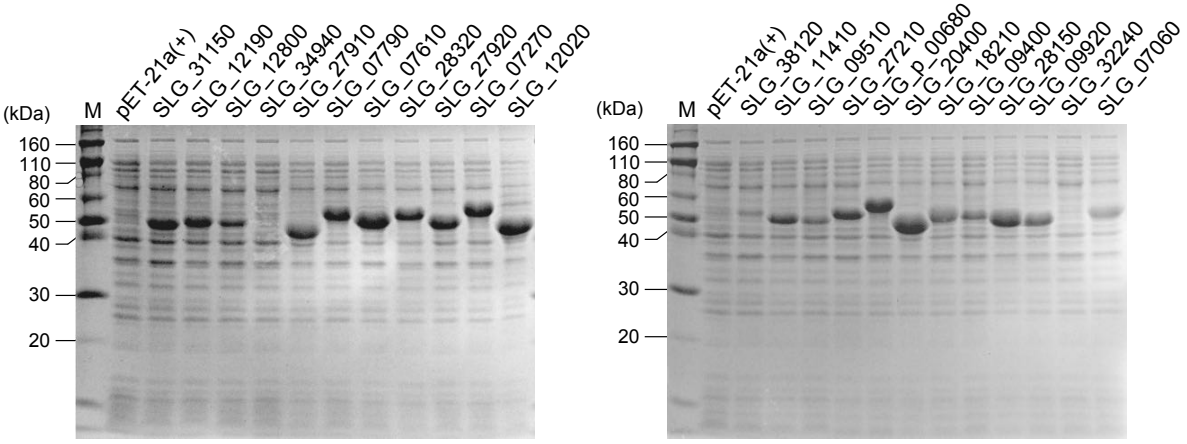


FIG S7. Expression of the ALDH genes of SYK-6 in *E. coli*.

Cell extracts (10 μg) of *E. coli* BL21(DE3) carrying each of the 23 ALDH genes were separated by SDS-12% polyacrylamide gels and stained with Coomassie Brilliant Blue. Lane M, molecular mass markers.

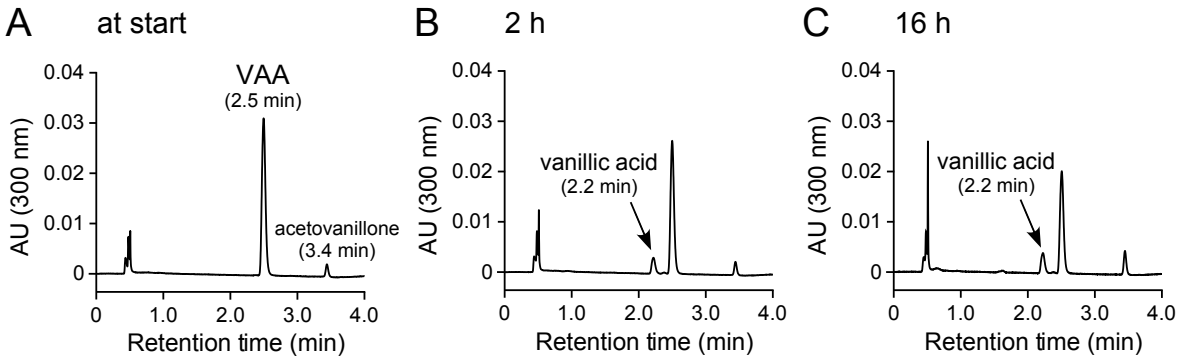


FIG S8. Conversion of VAA by cell extract of *desV ligV* double mutant (SME077). A SME077 cell extract (>10 kDa; 500 µg of protein/ml) was incubated with 100 µM VAA in the presence of CoA, $MgSO_4$, and ATP. Portions of the reaction mixtures were collected at the start (A), and after 2 h (B) and 16 h (C) of incubation, and analyzed by HPLC.

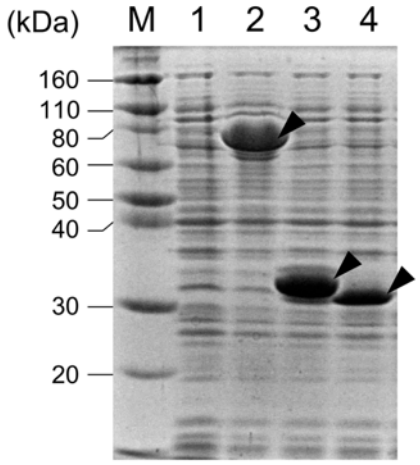


FIG S9. Expression of *ferA, ferB,* and *ferB2* in *E. coli*.
Proteins (10 μg) were separated on SDS-12% polyacrylamide gels and stained with Coomassie Brilliant Blue. Lanes: M, molecular mass markers; 1–4, cell extracts of *E. coli* BL21(DE3) harboring pET-16b (lane 1), pE16FA (lane 2), pE16FB (lane 3), and pE16FB2 (lane 4).

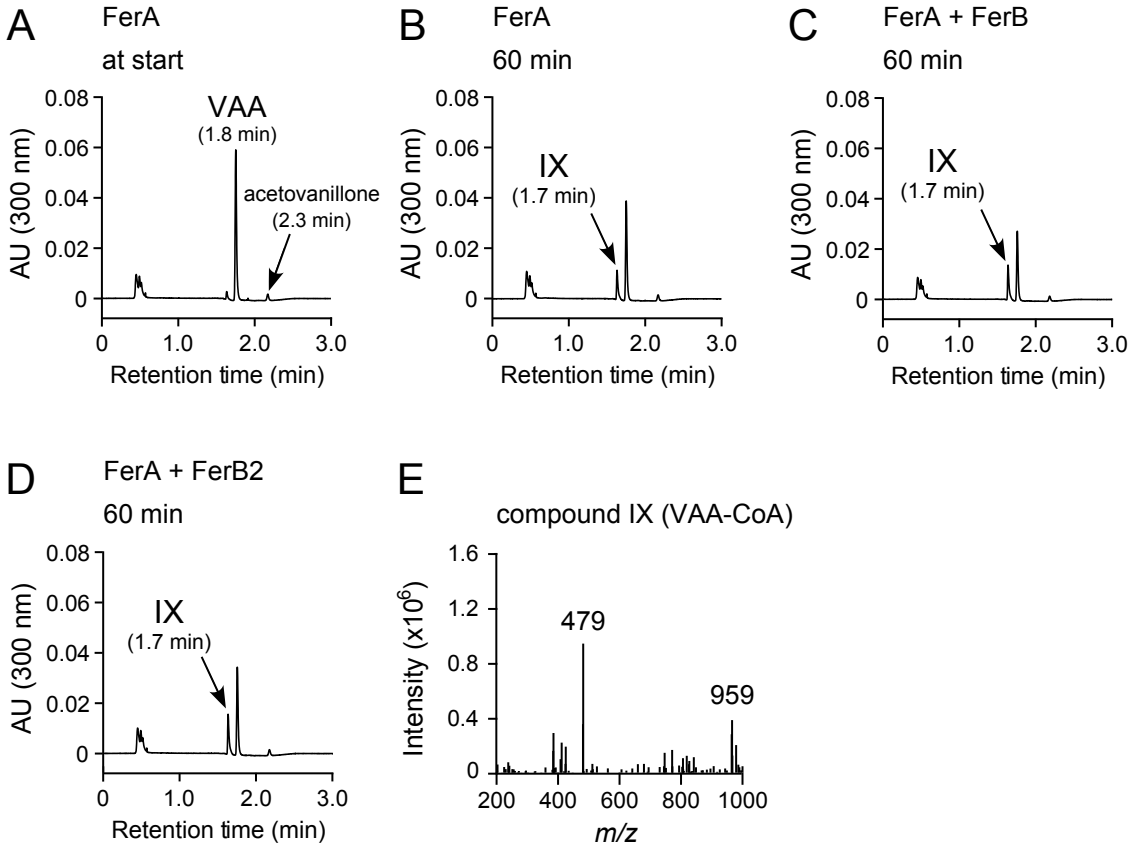


FIG S10. Conversion of VAA by enzymes for the ferulate catabolism.
VAA (100 μM) was incubated with cell extracts of *E. coli* BL21(DE3) harboring pE16FA (A and B), cell extracts of *E. coli* BL21(DE3) harboring pE16FA and *E. coli* BL21(DE3) harboring pE16FB (C), and cell extracts of *E. coli* BL21(DE3) harboring pE16FA and *E. coli* BL21(DE3) harboring pE16FB2 (D) in the presence of CoA, $MgSO_4$, and ATP. Portions of the reaction mixtures were collected at the start (A) and after 60 min (B–D) of incubation, and analyzed by HPLC-MS. Negative-ion ESI-MS spectrum of compound IX is shown in panel E.

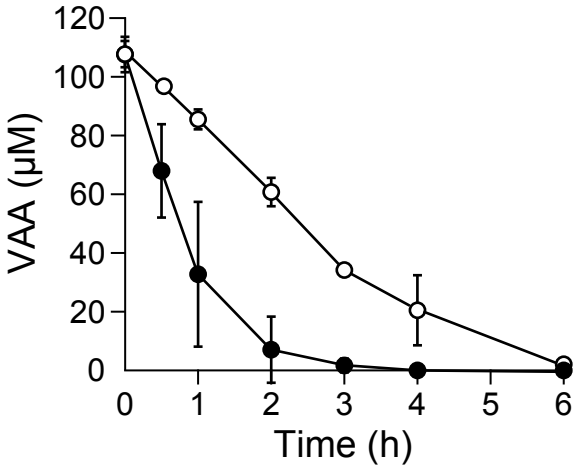


FIG S11. Conversion of VAA by *ferA* mutant (SME009).
Resting cells (OD_{600} of 1.0) of SYK-6 (open circles) and SME009 (closed circles) were incubated with 100 μM VAA. Portions of the reaction mixtures were collected, and the amount of VAA was determined by HPLC. All the experiments were performed in triplicate, and the data represent the averages ± standard deviations.