Supplemental material

Biotechnological Production of Caffeic Acid by Bacterial Cytochrome P450 CYP199A2

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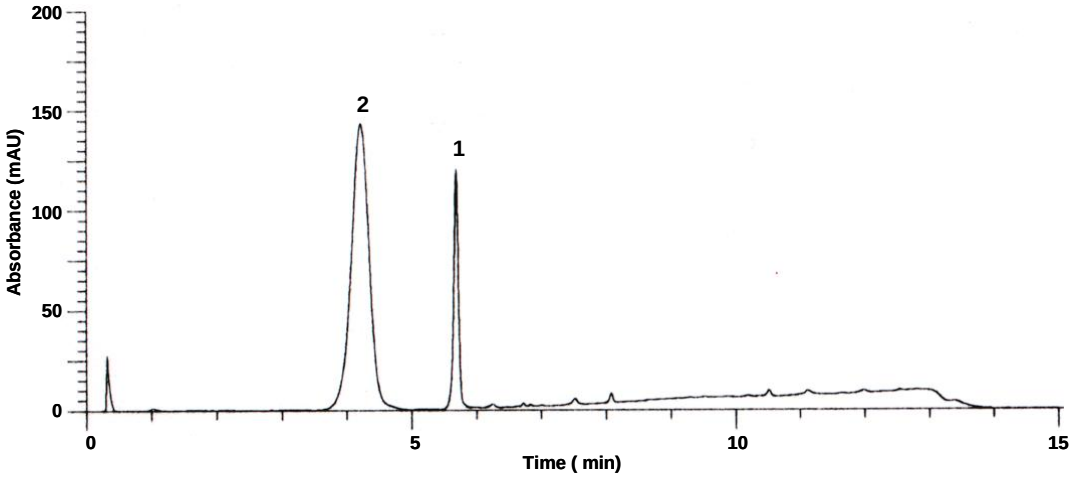
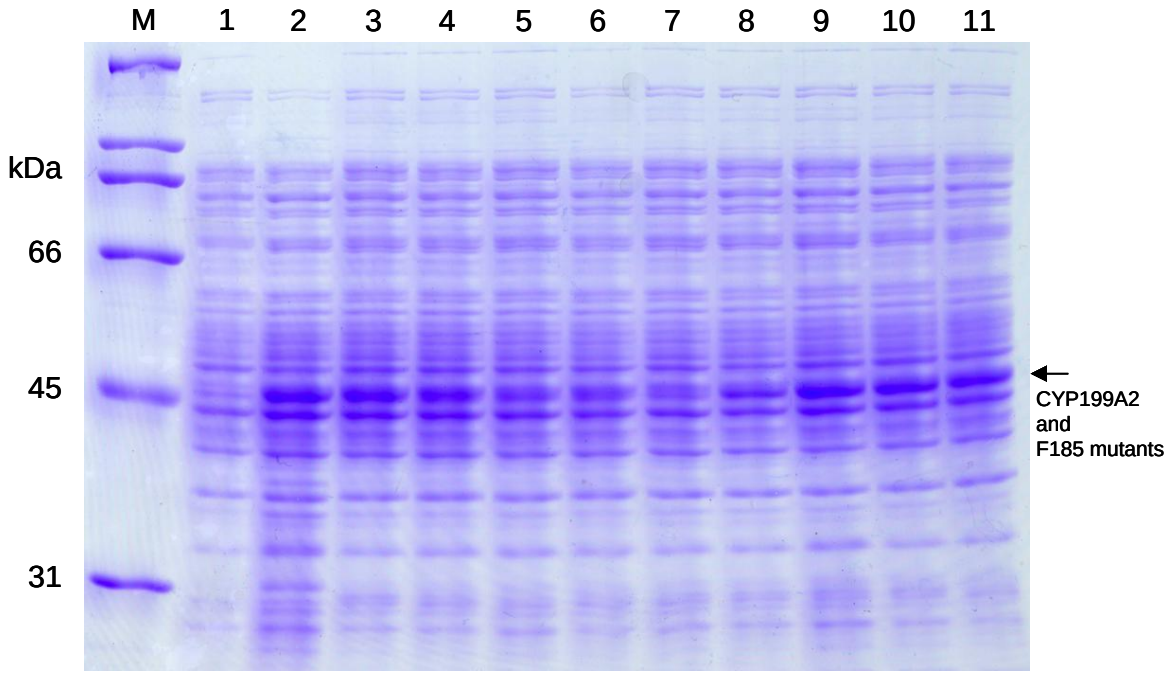


FIG. S1. HPLC chromatogram of the reaction of CYP199A2 with *p*-coumaric acid. Peaks 1 and 2 were found to correspond to *p*-coumaric and caffeic acid, respectively.



Lane M, molecular mass markers
Lane 1, *E coli* cells containing pET21a
Lane 2, *E coli* cells containing pET21a carrying *CYP199A2* gene
Lanes 3-11, *E coli* cells containing pET21a carrying F185 mutant genes
Lane 3, F185Y; Lane 4, F185W; Lane 5, F185V; Lane 6, F185L; Lane 7, F185I;
Lane 8, F185G; Lane 9, F185A; Lane 10, F185S; Lane 11, F185T

FIG. S2. SDS-PAGE profiles of the expression of the wild-type CYP199A2 and its F185 mutants in cell-free extracts of recombinant *E. coli* strains. *E. coli* strains expressing only the wild-type or mutant CYP199A2 were analyzed, and not those co-expressing putidaredoxin reductase and palustrisredoxin, because the protein band corresponding to CYP199A2 in SDS-PAGE analysis is very close to that corresponding to putidaredoxin reductase. The expression levels of putidaredoxin reductase and palustrisredoxin have been previously reported (T. Furuya and K. Kino, ChemSusChem, 2, 645-649 (2009); T. Furuya and K. Kino, Appl. Microbiol. Biotechnol, 85, 1861-1868 (2010)).

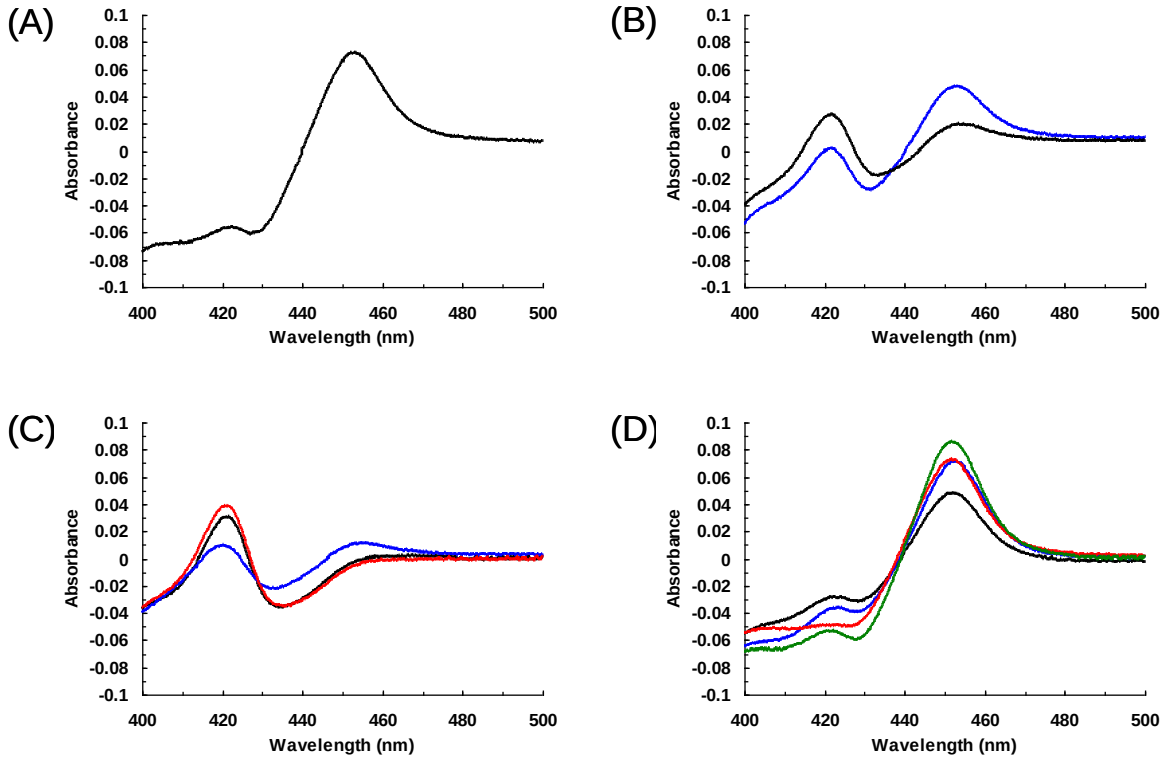


FIG. S3. CO-reduced difference spectra of the wild-type CYP199A2 and its F185 mutants in cell-free extracts of recombinant *E. coli* strains. (A) Black line, wild-type CYP199A2. (B-D) F185 mutants. (B) Black line, F185Y; blue line, F185W. (C) Black line, F185V; blue line, F185L; red line, F185I. (D) Black line, F185G; blue line, F185A; red line, F185S; green line, F185T.

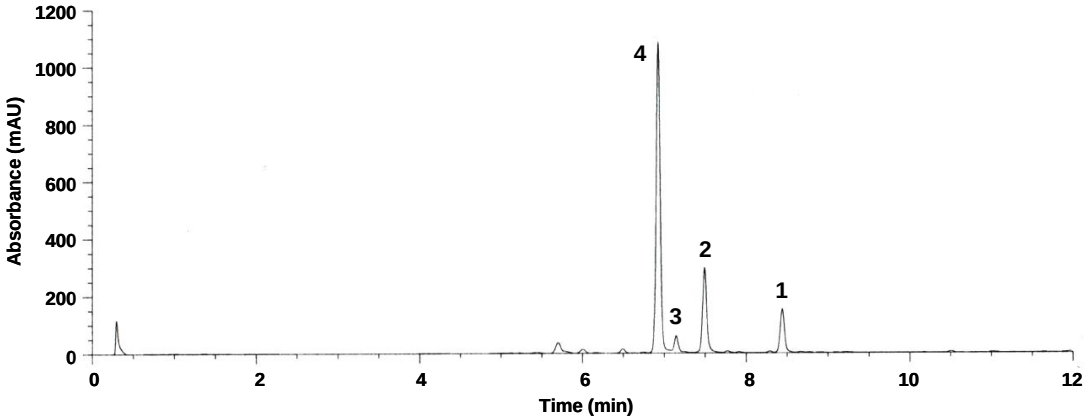


FIG. S4. HPLC chromatogram of the reaction of the CYP199A2 F185L mutant with 2-naphthoic acid. Peaks 1, 2, 3, and 4 were found to correspond to 2-naphthoic acid and 8-, 7-, and 5-hydroxy-2-naphthoic acid, respectively.

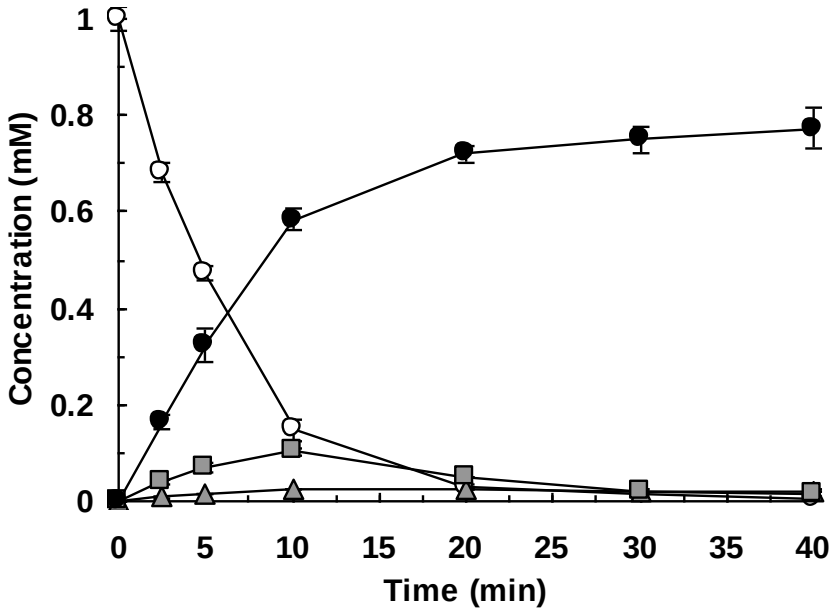


FIG. S5. 2-Naphthoic acid hydroxylation by *E. coli* cells expressing the CYP199A2 F185L mutant. The time course of 2-naphthoic acid consumption (white circles) and of 7-, 8-, and 5-hydroxy-2-naphthoic acid production (gray triangles, gray squares, and black circles, respectively) are shown. Plots indicate the average values of three independent experiments, and error bars indicate standard deviations from the means.

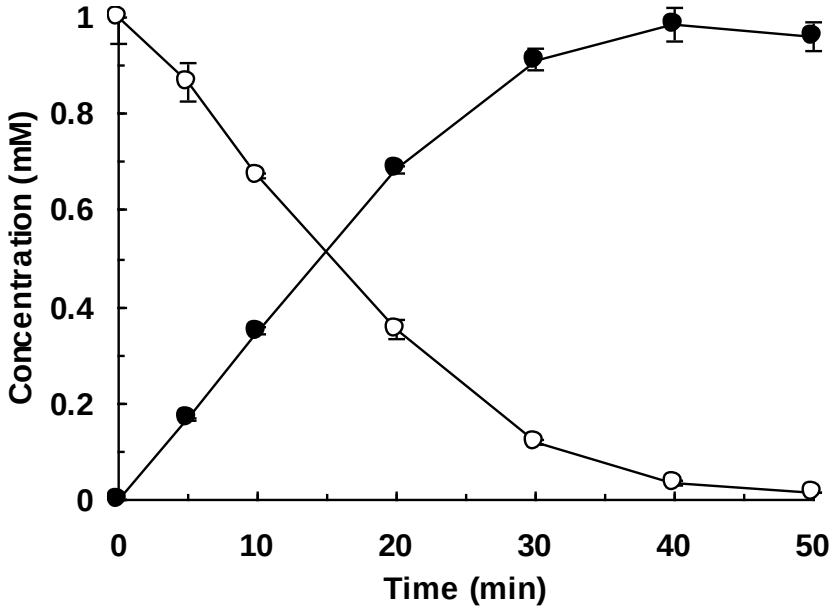


FIG. S6. *p*-Coumaric acid hydroxylation by *E. coli* cells expressing the CYP199A2 F185L mutant. The time courses of *p*-coumaric acid consumption (white circles) and of caffeic acid production (black circles) are shown. Plots indicate the average values of three independent experiments, and error bars indicate standard deviations from the means.

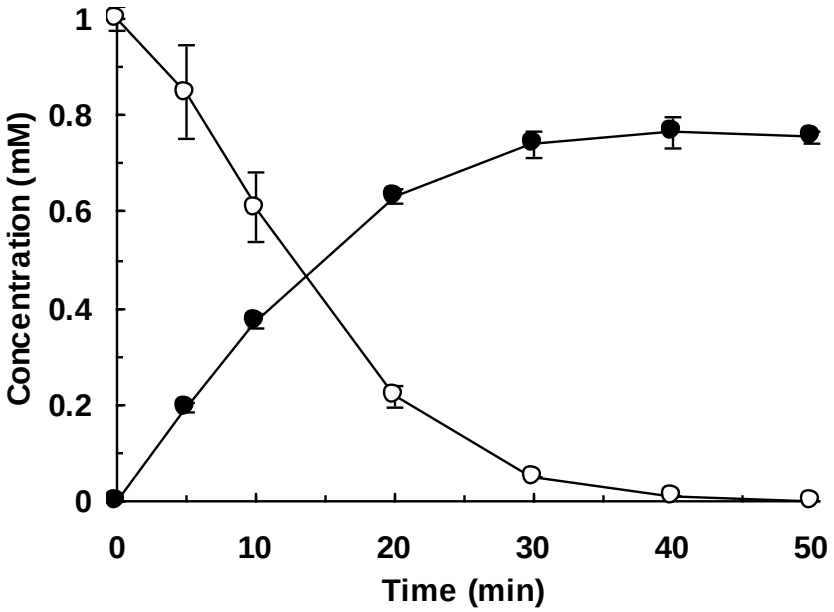


FIG. S7. *m*-Coumaric acid hydroxylation by *E. coli* cells expressing the CYP199A2 F185L mutant. The time courses of *m*-coumaric acid consumption (white circles) and of caffeic acid production (black circles) are shown. Plots indicate the average values of three independent experiments, and error bars indicate standard deviations from the means.

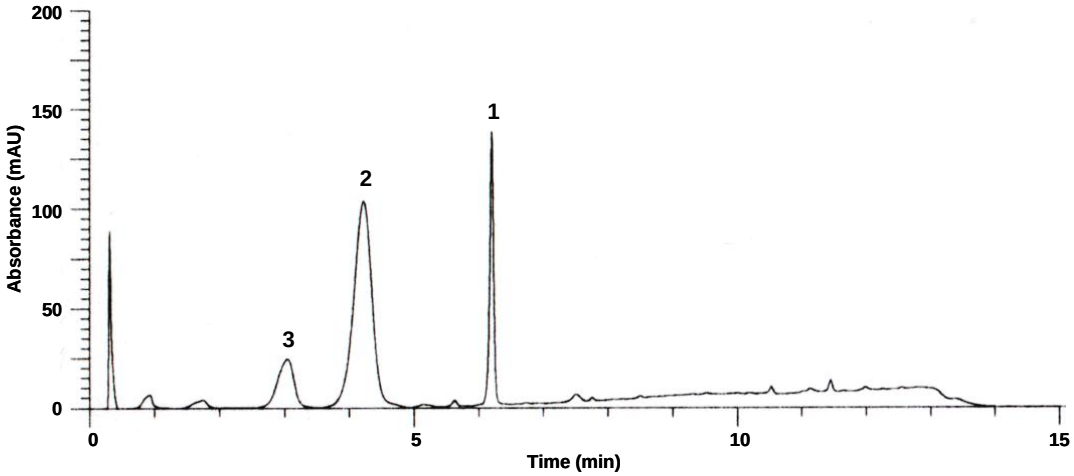


FIG. S8. HPLC chromatogram of the reaction of the CYP199A2 F185 mutant with *m*-coumaric acid. Peaks 1 and 2 were found to correspond to *p*-coumaric and caffeic acid, respectively. Peak 3 was tentatively identified as 3,5-dihydroxycinnamic acid.