Ancestral Bacterial Laccase Enables Efficient Biodetoxification of Inhibitory Wheat Straw Hydrolysate for Sustainable Microbial Protein Production

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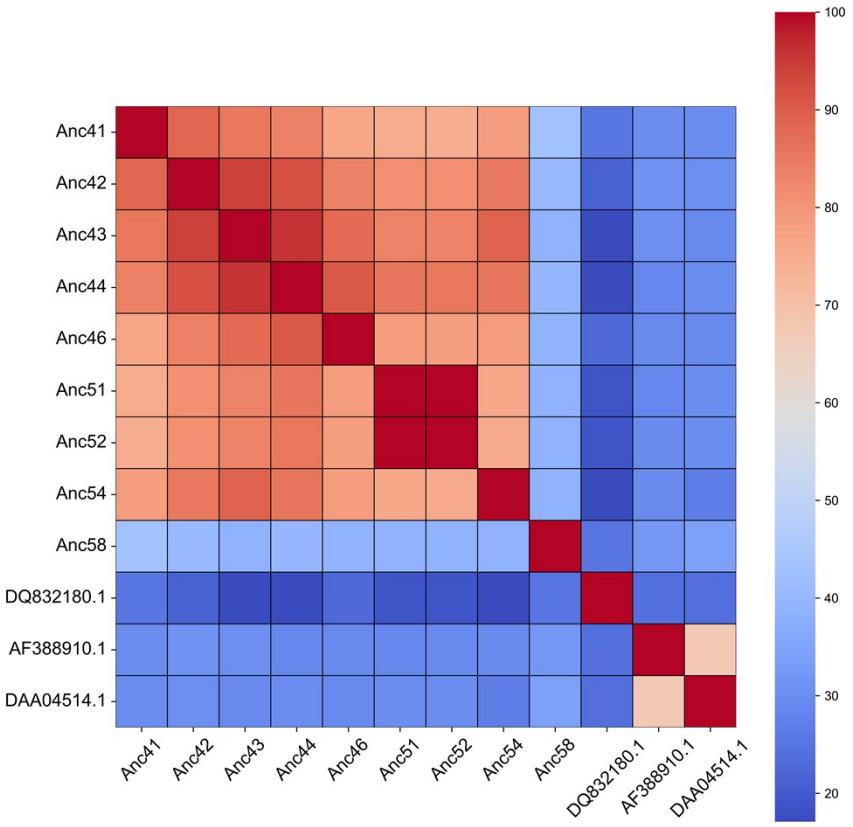


Fig. S1. Heatmap showing sequence similarity between ancestral laccases and previously reported laccases used for hydrolysate detoxification.

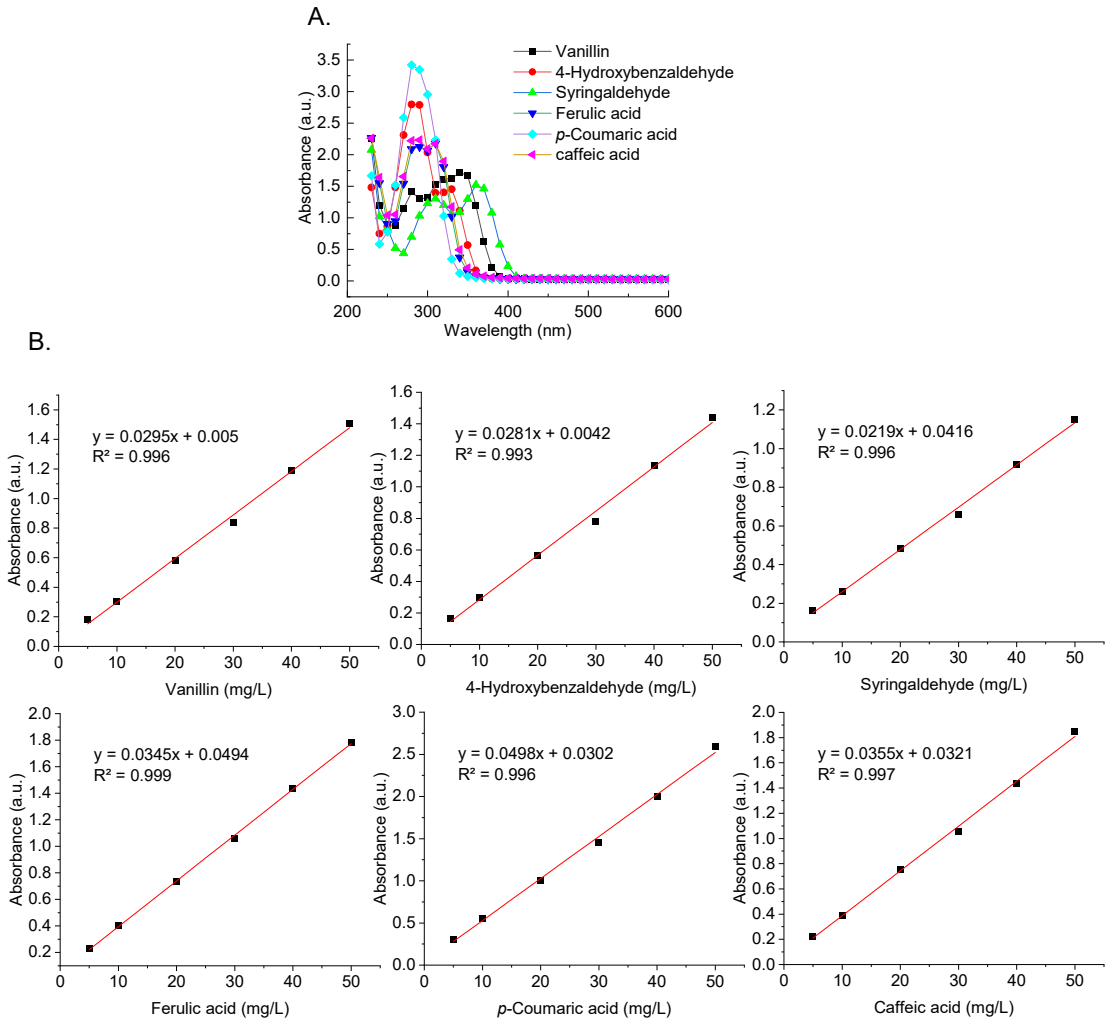


Fig. S2. Spectrophotometric analysis of phenolic compounds. (A). Maximum absorption wavelengths of phenolic compounds. (B) Standard curves of six phenolic compounds (5–50 mg/L).

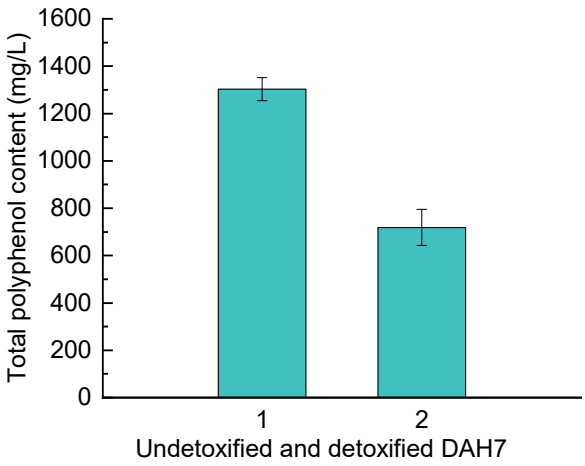


Fig. S3. Total phenolic content of detoxified and non-detoxified DAH7. 1. DAH7; 2. DAH7 was detoxified by Anc44. Treatment was carried out at 60°C for 24 h, with cell density OD_{600} = 30.

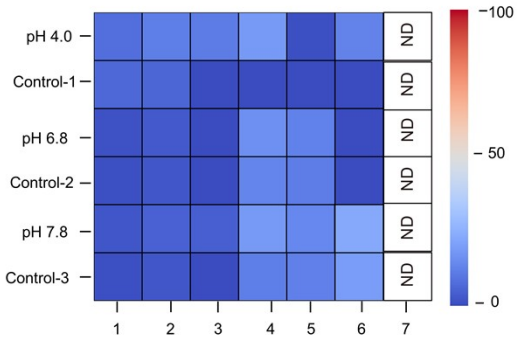


Fig. S4. pH-dependent degradation of seven phenolic inhibitors by *T. versicolor* laccase. Heat-map shows residual substrate (%) after 3 h reaction (at 60°C) with *T. versicolor* laccase. Substrates (50 mg/L each): 1, vanillin; 2, 4-hydroxybenzaldehyde; 3, syringaldehyde; 4, ferulic acid; 5, *p*-coumaric acid; 6, caffeic acid; 7, 4-hydroxybenzoic acid.

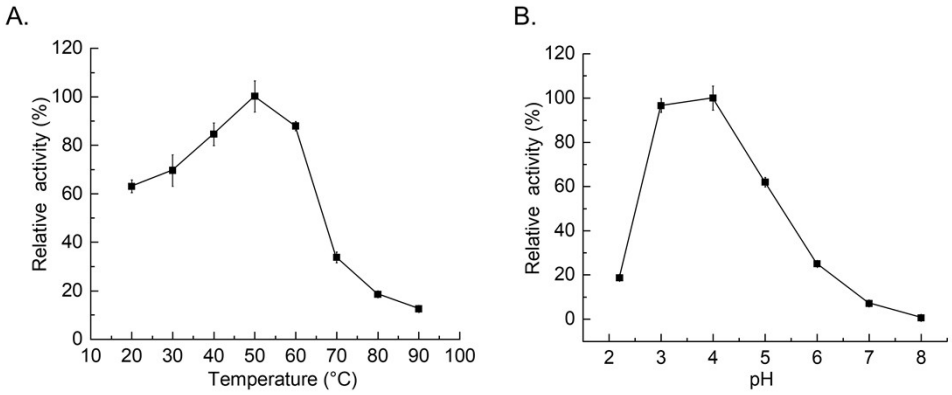


Fig. S5. Enzymatic characterization of the commercial laccase from *T. versicolor*. (A) Optimal temperature. (B) Optimal pH. The enzymatic activity was assayed using ABTS as the substrate.

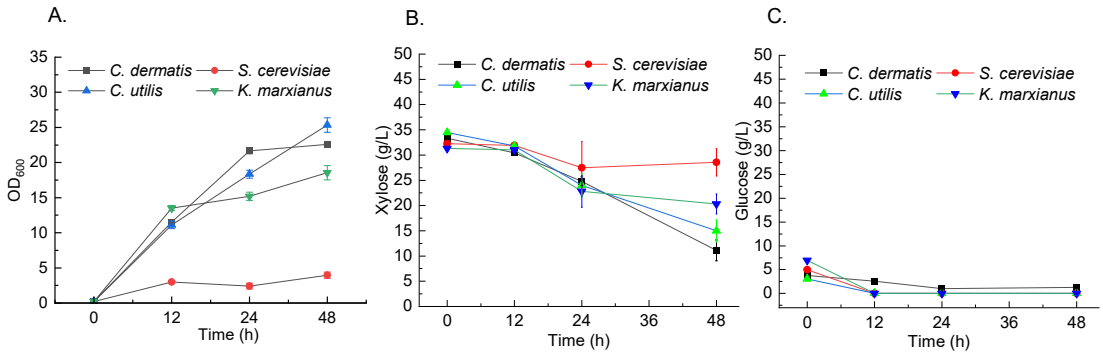


Fig. S6. Growth and sugar consumption profiles of yeasts in YPDX-2 media (glucose : xylose, w/w = 1:5).

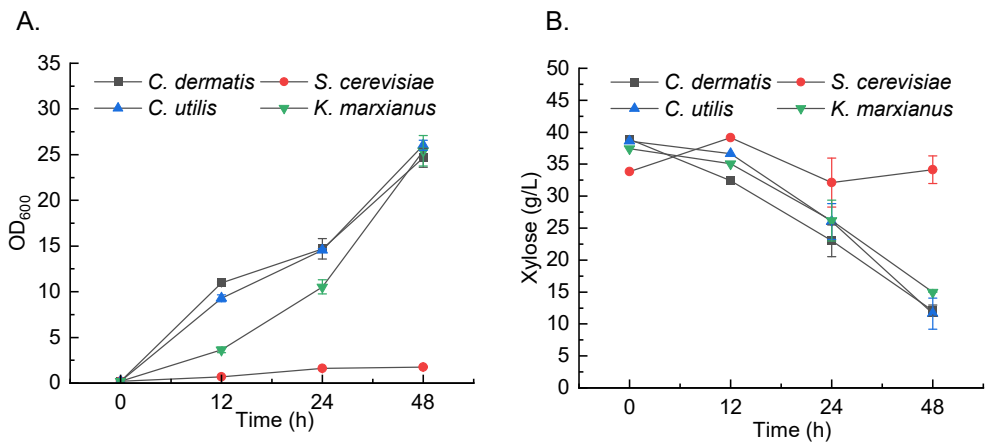
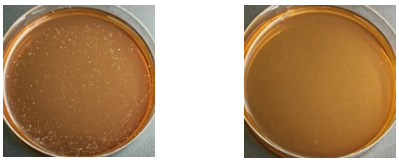


Fig. S7. Growth and xylose utilization profiles of yeast strains in YPDX-3 medium (xylose).



C. utilis ATCC 22023 *C. dermatis* ZZ-46

Fig. S8. Growth of *C. utilis* and *C. dermatis* on YPDX-1 plates containing 30% DAH7 treated by *E. coli* BL21 cells carrying pET-28a(+). Detoxification was carried out using the *E. coli* at a cell density of OD_{600} = 5 for 24 h at 60°C.

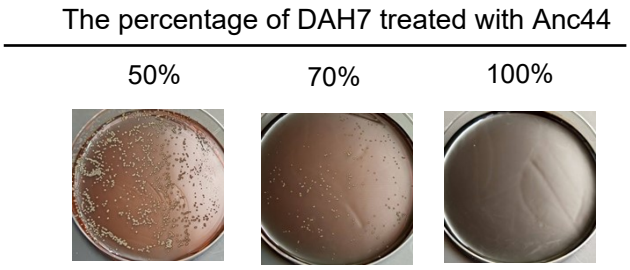


Fig. S9. Growth of *C. utilis* ATCC 22023 on YPDX-1 plates containing a high concentration of DAH7 detoxified by Anc44 at a cell density of OD_{600} = 30 for 24 h at 60°C.

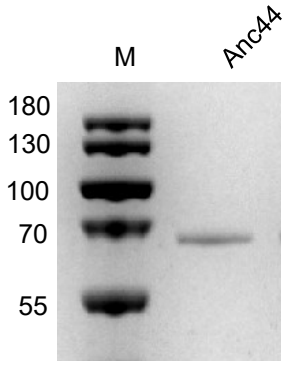


Fig. S10. SDS-PAGE analysis of Anc44 purified by Ni^{2+}-affinity chromatography.

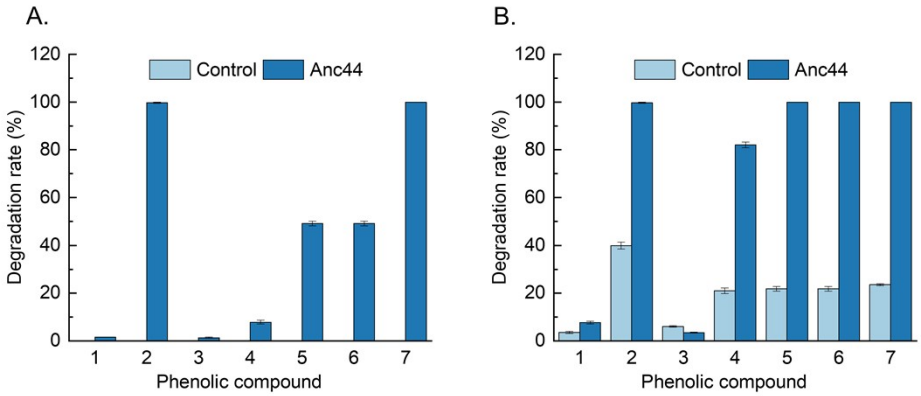


Fig. S11. Degradation of mixed phenolic compounds by Anc44. (A) Degradation efficiency at 0.5 h; (B) Degradation efficiency at 12 h. The phenolic compounds 1–7: 1, 4-hydroxybenzoic acid; 2, caffeic acid; 3, 4-hydroxybenzaldehyde; 4, vanillin; 5, syringaldehyde; 6, *p*-coumaric acid; 7, ferulic acid. The reaction system contained 50 mmol/L NaH_2PO_4-$C_6H_8O_7$ buffer (pH 6.8), Anc44 (0.05 mg/L), and a mixture of seven phenolic compounds (200 mg/L each). All stated concentrations are final. Reactions were incubated at 60°C with shaking at 400 rpm for 0.5 and 12 h. Blank controls were prepared by replacing the enzyme with an equal volume of NaH_2PO_4-$C_6H_8O_7$ buffer.

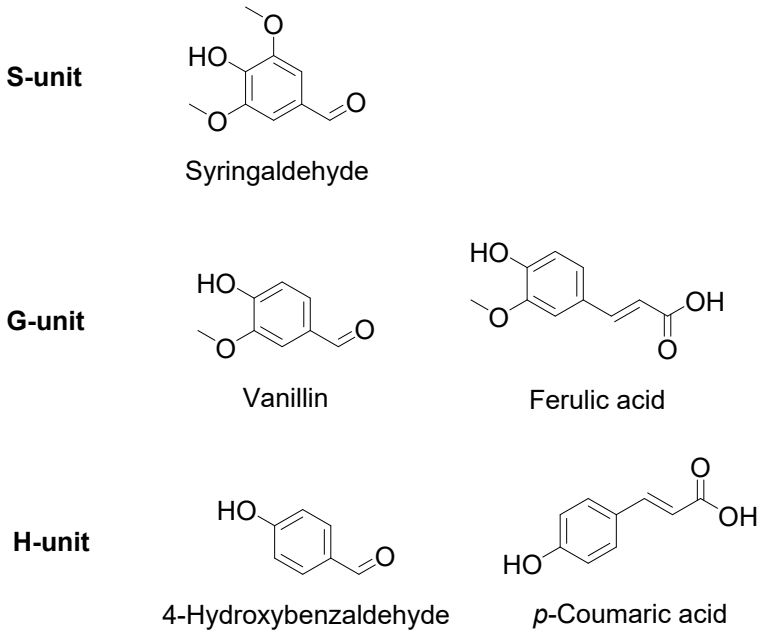


Fig. S12. Structures of the five phenolic compounds identified in DAH7.

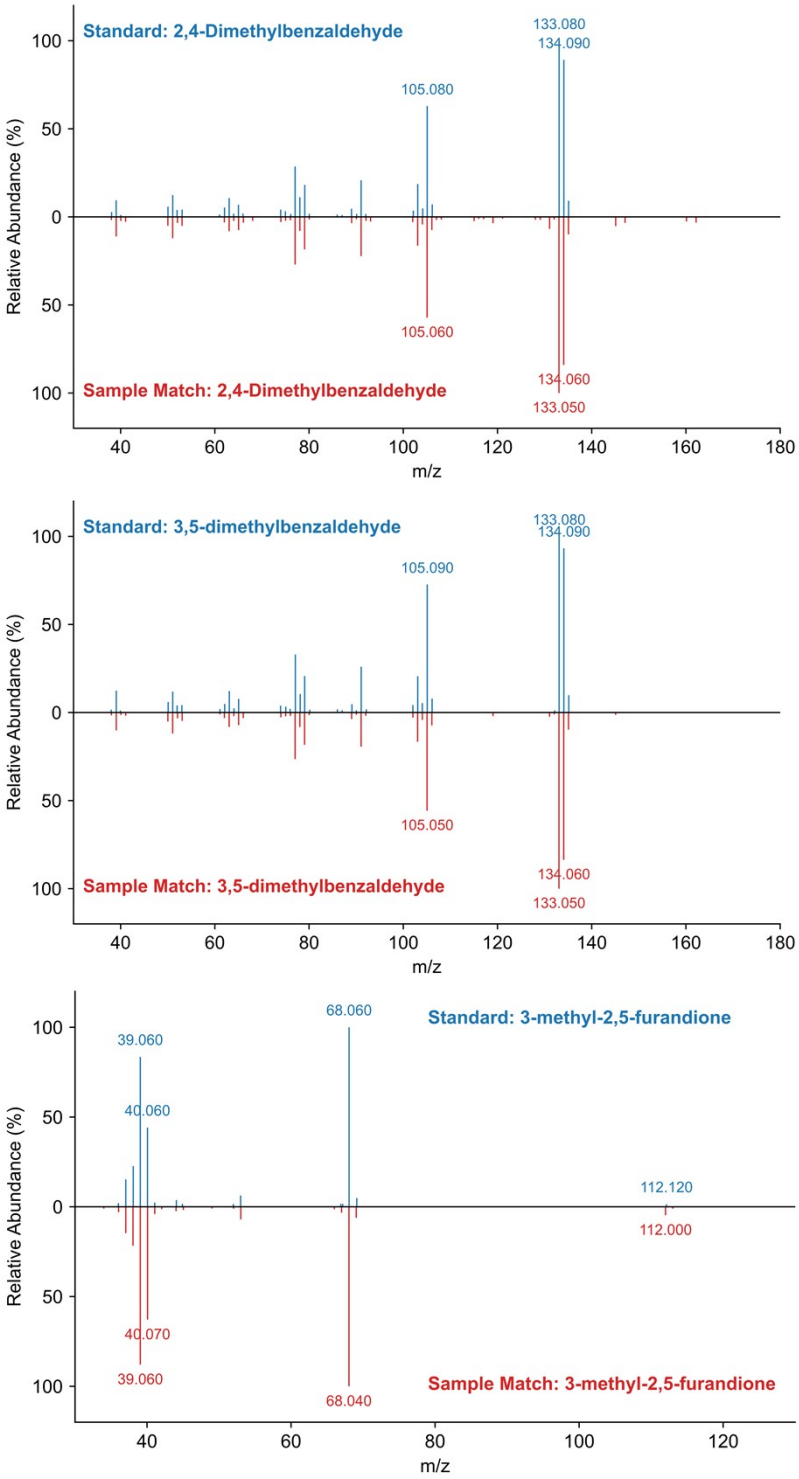


Fig. S13. Identification of degradation products by GC-MS with authentic standards. Head-to-tail mass spectral comparisons between detected products (bottom, red) and corresponding authentic standards (top, blue).

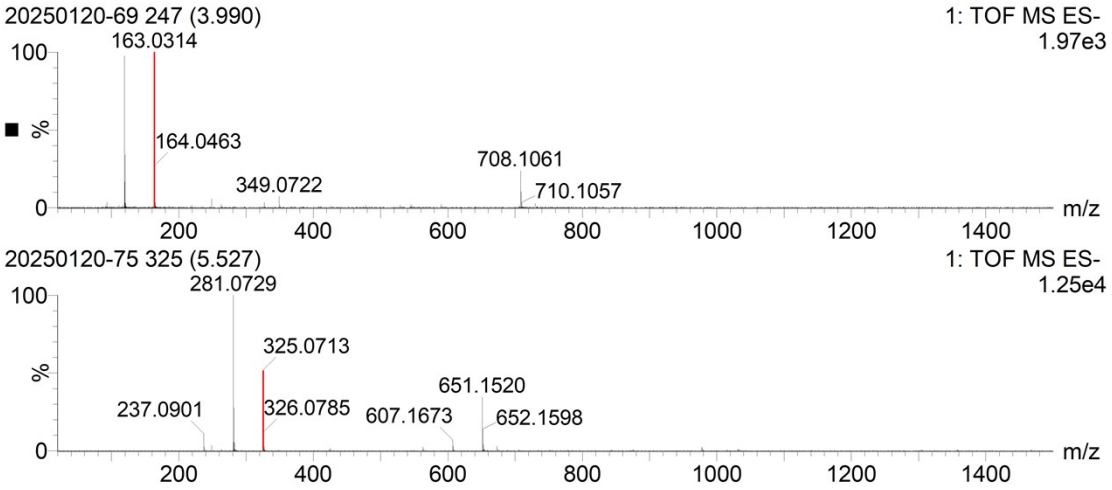


Fig. S14. Mass spectrometry analysis of *p*-coumaric acid degradation products catalyzed by the ancestral laccase Anc44.

Table S1. Mass balance and product distribution of syringaldehyde and ferulic acid degradation by Anc44.

Substrate	Product identity	Formula	Mass (mg)	Amount (μmol)	Molar yield (%)[a]
Syringaldehyde	3-methyl-2,5-furandione	$C_5H_4O_3$	0.749	6.68	46.25
	3,5-dimethylbenzaldehyde	$C_9H_{10}O$	0.141	1.05	7.37
	Unidentified [b]		-	-	46.48
Ferulic Acid	3-methyl-2,5-furandione	$C_5H_4O_3$	0.681	6.08	39.33
	2,4-dimethylbenzaldehyde	$C_9H_{10}O$	0.148	1.1	7.14
	Unidentified [b]	-	-	-	53.53

Note: Reaction conditions: Substrate (3.0 mg), 60°C, 0.5 h. Identification and quantification: Products were identified via comparison with authentic standards (MS/retention time) and quantified using external calibration curves (R^2> 0.99).[a] Molar yield calculated based on consumed substrate. [b] Unidentified fraction corresponds to GC-MS undetected products, presumably non-volatile or high-molecular-weight species.