**Electronic Supplementary Information (ESI) for:**

# A biocatalytic hydrogenation of carboxylic acids

Yan Ni, Peter-Leon Hagedoorn,* Jian-He Xu, Isabel Arends, Frank Hollmann*

## 1. General

*Chemicals*

All the carboxylic acids, aldehydes and alcohols were obtained from Sigma-Aldrich or Alfa Aesar. Other chemicals and solvents were of the highest grade available.

*Preparation of P. furiosus cells*

*P. furiosus* (DSM 3638) was grown in a 100 L fermenter at 90°C, under anaerobic conditions with potato starch as carbon source as previously described.[1] Cells were harvested by crossflow filtration and centrifugation and stored at -80°C for further use.

*General reaction conditions*

Hydrogenation experiments were carried out in 16 ml autoclave reactors (Figure S1). Unless indicated otherwise, a typical *P. furiosus*-mediated hydrogenation reaction was performed in sodium phosphate buffer (100 mM, pH 6.5) containing 10 mM starting material and 150 g $L^{-1}$ frozen cells of *P. furiosus*. The reaction mixtures were flushed with $N_2$ followed with $H_2$ and incubated for 24h at 40$^{o}$C under $H_2$ atmosphere (5 bar) with gentle magnetic mixing. The reaction mixture was acidified to pH 2.0 with 5 *N* HCl, extracted twice with ethyl acetate containing 1-octanol or *n*-decane as an internal standard, and analyzed by GC. For vanillic acid, the samples were centrifuged for 15 min at 13,000 rpm after adding equal volumes of acetonitrile, and the supernatant was analyzed by HPLC. In case of non-aqueous system, the reactions were performed in organic solvents containing 10 mM starting material and 150 g $L^{-1}$ frozen cells of *P. furiosus* for 24 h at 40$^{o}$C under 5 bar $H_2$. After centrifugation, the organic phase was analyzed by GC.

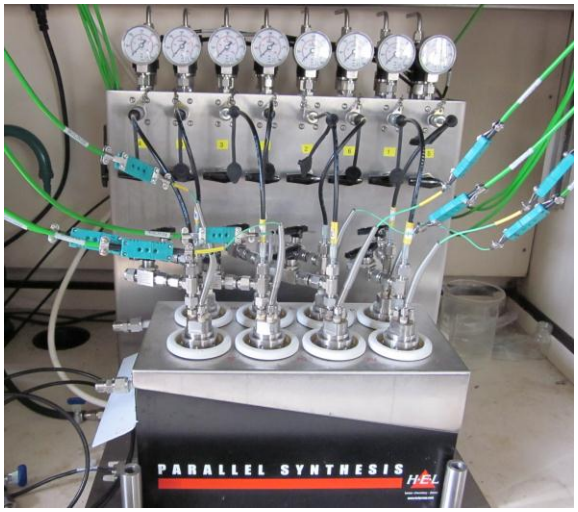
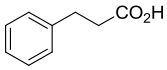
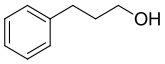
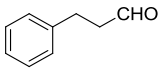
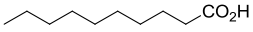
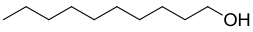
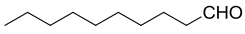
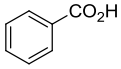
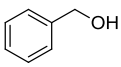


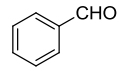
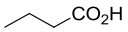
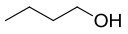
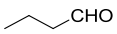
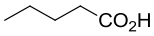
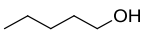
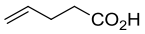
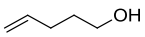
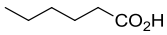
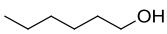
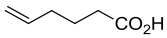
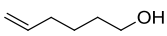
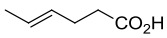
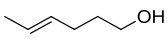
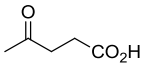
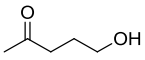
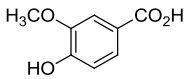
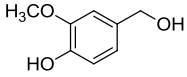
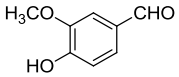

**Figure S1.** Experimental setup of the biocatalytic hydrogenation for carboxylic acids.

*Analytical procedures*

The reaction progress was determined using GC analysis or HPLC. GC was performed using a CP-Sil 5 CB column (50 m × 0.53 mm × 1.0 µm) or a CP-Wax 52 CB column (50 m × 0.53 mm × 2.0 µm) with $N_2$ as carrier gas and flame ionization detector. HPLC analysis was carried out with a Waters Xterra column (C18, 5 µm, 4.6 × 150 mm) with $CH_3CN$:$H_2O$:HCOOH (20:80:1, v/v/v) as eluent. The samples were isocratically eluted at a flow rate of 1 ml/min. The identity of products was confirmed with authentic standards.

**Table S1**. Details of GC and HPLC analysis

| Compound | Retention time (min) | Method [a] |
|---|---|---|
|  | 5.82 | A |
|  | 5.19 | A |
|  | 4.66 | A |
|  | 6.08 | A |
|  | 5.44 | A |
|  | 4.99 | A |
|  | 6.18 | B |
|  | 5.00 | B |

| | | |
|---|---|---|
|  | 4.34 | B |
|  | 4.82 | C |
|  | 3.34 | C |
|  | 2.69 | C |
|  | 15.01 | D |
|  | 7.11 | D |
|  | 15.67 | D |
|  | 8.00 | D |
|  | 14.21 | E |
|  | 9.07 | E |
|  | 14.59 | E |
|  | 10.35 | E |
|  | 14.69 | E |
|  | 10.41 | E |
|  | 9.95 | F |
|  | 16.29 | F |
|  | 2.90 | G |
|  | 2.31 | G |
|  | 3.31 | G |

[a] (A) GC (CP-Sil 5 CB column), 120°C for 3 min, 50°C/min to 195°C, 195°C for 2.7 min, 50°C/min to 300°C; (B) GC (CP-Sil 5 CB column), 100°C for 3 min, 25°C/min to 275°C; (C) GC (CP-Sil 5 CB column), 60°C for 3.5 min, 50°C/min to 200°C, 200°C for 1 min; (D) GC (CP-Wax 52 CB column), 60°C for 5 min, 50°C/min to 100°C, 100°C for 5 min, 50°C/min to 150°C, 150°C for 3 min, 50°C/min to 200°C, 200°C for 2 min; (E) GC (CP-Wax 52 CB column), 60°C for 5 min, 50°C/min to 100°C, 100°C for 4 min, 50°C/min to 150°C, 150°C for 2 min, 50°C/min to 200°C, 200°C for 4 min; (F) GC (CP-Wax 52 CB column), 100°C for 6 min, 50°C/min to 150°C, 150°C for 6 min, 50°C/min to 230°C, 230°C for 5 min; (G) HPLC, flow rate 1 ml/min, $CH_3CN$:$H_2O$:HCOOH=20:80:1.

## 2. Experimental details not included in the manuscript

*First hydrogenation of 3-phenylpropionic acid*

For proof of principle demonstration, *P. furiosus* frozen cells was evaluated in hydrogenation of 3-phenylpropionic acid. When the reaction was carried out in 100 ml autoclave under $H_2$ ($p$=2 bar) with a substrate concentration of 2.0 mM for 26 h at 50°C, 0.80 mM 3-phenyl-1-propanol was obtained (40% yield) (Figure S2). No 3-phenylpropanal was detectable during the reaction.

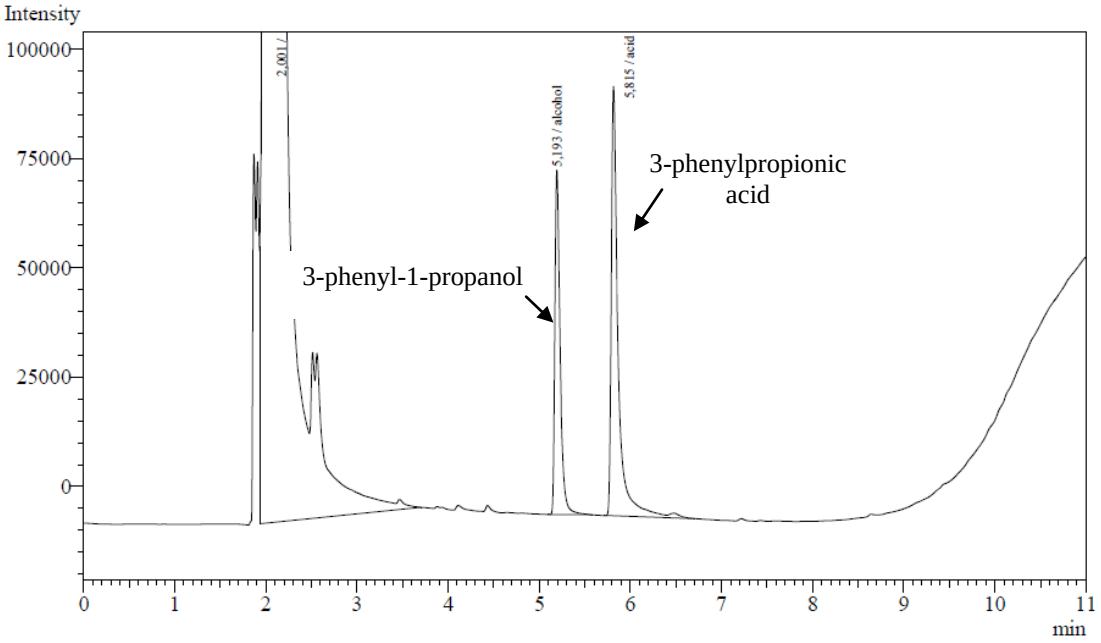



**Figure S2.** GC analysis for hydrogenation of 3-phenylpropionic acid catalyzed by *P. furiosus*. Reaction conditions: *P. furiosus* frozen cells (100 g $L^{-1}$), 3-phenylpropionic acid (2.0 mM), $H_2$ ($p$=2 bar), sodium phosphate buffer (100 mM, pH 7.0), 50°C for 26 h.

*Effect of hydrogen pressure on the hydrogenation of 3-phenylpropionic acid*

When the reaction was carried out in a simple setup consisting of a three-necked flask and a $H_2$ balloon ($p$=1.0 bar), 2.0 mM substrate gave only 3.6% conversion after 42 h. Therefore, the effect of hydrogen pressure on the biohydrogenation of 3-phenylpropionic acid was firstly investigated under different hydrogen pressure ($p$=0-25 bar). As shown in Figure S3, a hydrogen pressure of 5 bar was sufficient for the acid reduction.

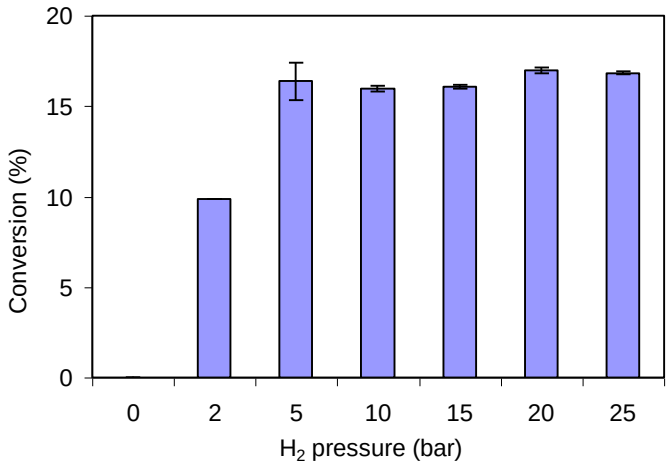



**Figure S3.** Effect of $H_2$ pressure on the hydrogenation of 3-phenylpropionic acid. Reaction conditions: *P. furiosus* frozen cells (100 g $L^{-1}$), 3-phenylpropionic acid (2.0 mM), sodium phosphate buffer (100 mM, pH 7.0), 50°C for 45 h.

*Effect of catalyst loading on the hydrogenation of 3-phenylpropionic acid*

*P. furiosus* cells at different concentrations were employed to catalyze the hydrogenation of 3-phenylpropionic acid under 5 bar $H_2$ pressure. As shown in Figure S4, in the absence of *P. furiosus* cells no conversion was observed and the conversion linearly depended on the concentration of cells applied. A cell concentration of 150 g $L^{-1}$ was used through the subsequent experiments.

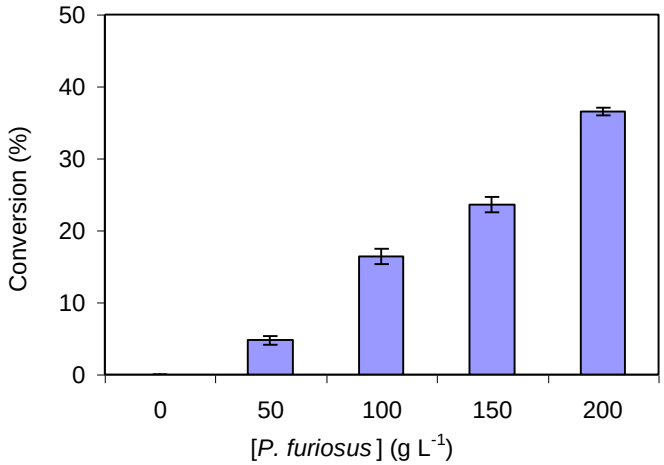



**Figure S4.** Effect of cell concentration on the hydrogenation of 3-phenylpropionic acid. Reaction conditions: 3-phenylpropionic acid (2.0 mM), sodium phosphate buffer (100 mM, pH 7.0), $H_2$ ($p$=5 bar), 50°C for 45 h.

*pH profile of the hydrogenation of 3-phenylpropionic acid*

The pH profile was determined using 100 mM sodium phosphate buffer of various pH values from 5.5 to 8.0. As shown in Figure S5, full conversion was observed in pH 6.5 sodium phosphate buffer. Lower or higher pH caused a drop of the conversion especially under basic environment.

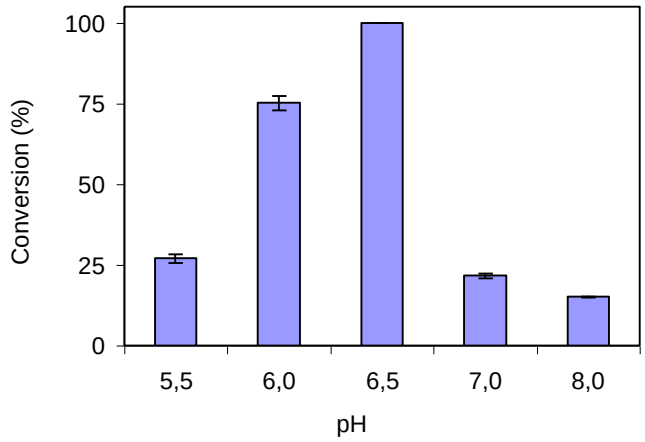



**Figure S5.** Hydrogenation of 3-phenylpropionic acid at different pH. Reaction conditions: 3-phenylpropionic acid (2.0 mM), *P. furiosus* frozen cells (150 g $L^{-1}$), sodium phosphate buffer (100 mM,), $H_2$ (*p*=5 bar), 50°C for 45 h.

*Temperature profile of hydrogenation of 3-phenylpropionic acid*

The optimum reaction temperature was determined under 5 bar $H_2$ pressure at different temperatures in the range of 30-90°C. Interestingly, the highest conversion was reached at the reaction temperature of 40°C, which was not expected for *P. furiosus*. We suspected the poorer $H_2$ solubility at higher temperature to account for this unexpected temperature profile. As a result, 40°C was chosen as the favorable reaction temperature.

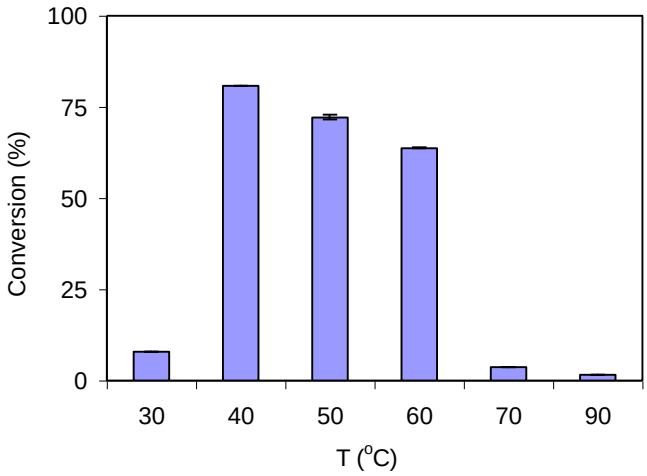



**Figure S6.** Hydrogenation of 3-phenylpropionic acid at different temperature. Reaction conditions: *P. furiosus* frozen cells (150 g $L^{-1}$), 3-phenylpropionic acid (20 mM), sodium phosphate buffer (100 mM, pH 6.5), $H_2$ (*p*=5 bar), for 45 h.

*Preparative synthesis of 5-hexen-1-ol*

A 200 ml of 100 mM sodium phosphate buffer (pH 6.5) containing 5-hexenoic acid (250 mg) and *P. furiosus* frozen cells (40 g) was flushed with $N_2$ followed with $H_2$ (*p*=5.0 bar). The mixture was stirred

for 48 h at 40$^{o}$C. GC analysis showed the selective transformation of 5-hexenoic acid to 5-hexen-1-ol in 99% conversion. Cells were removed by centrifugation and the supernatant was extracted with methyl tertiary-butyl ether. The organic layers were dried over anhydrous $Na_2SO_4$, evaporated under vacuum and passed over a silica column. The isolated product, 5-hexen-1-ol, was afforded as a colorless liquid. $^{1}$H NMR (400 MHz, $CDCl_3$): $\delta$ 1.44-1.49 (m, 2H), 1.56-1.61 (m, 2H), 1.92 (s, 1H), 2.09 (q, 2H, *J*=6.8Hz), 3.63 (t, 2H, *J*=6.6Hz), 4.95 (d, 1H, *J*=10.1Hz), 5.01 (d, 1H, *J*=17.2Hz), 5.75-5.85 (m, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 25.0, 33.0, 33.9, 62.6, 114.5, 138.9.

## References

1. G. Fiala and K. O. Stetter, *Arch. Microbiol.* 1986, **145**, 56-61.