Supplementary Information

Coupling of permeabilized microorganisms for efficient enantioselective reduction of ketone with cofactor recycling

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Analytic conditions. *Analysis of conversion of **1** to **2** by GC*. Column: Chrompack Optima-5 (25 m × 0.32 mm); temperature program: 50 °C for 10 min, increase to 280 °C at a rate of 10 °C/min, keep at 280 °C for 2 min. Retention time: 2.01 and 5.25 min for **1**, 4.16 min for **2**.

*Analysis of ee of compound **2** by GC*. Column: Lipodex-A (25 m × 0.25 mm); temperature program: 40 °C to 120 °C at 5 °C/min, increase to 170 °C at 45 °C /min; retention time: 11.89 min for (*S*)-**2** and 12.21 min for (*R*)-**2**.

*Analysis of conversion of **3** to **4** by HPLC*. Column: Nucleosil 120-5 C18 (125 mm × 8 mm). Eluent: acetonitrile/10mM K-phosphate Buffer (pH = 7.0), 1/9; flow rate: 0.5 mL/min; UV detection: 210 nm; Retention time: 3.1 min for **3**, 1.32 min for **4**.

*Analysis of ee of compound **4** by HPLC.* Column: Chiralcel OB-H (250 mm × 4.6 mm); eluent: *n*-hexane/2-propanol 75/25; flow rate: 0.5ml/min; UV detection: 210 nm; retention time: 15.6 min for (+)-**4** and 21.2 min for (-)-**4.**

Production of cells. *Bacillus pumilus* Phe-C3 was grown in 1/2 Evans medium[13] containing 50 mM glucose and 5 mM phenylalanine in a shaking flask at 25 °C and 300 rpm. The cells were harvested at the late exponential phase at 36 h with an OD_{450} of 8.6. *Pseudomonas* sp. Tyr-F10 was grown in 1/2 Evans medium containing 50 mM glucose and 5 mM tyrosine in a shaking flask at 25°C and 300 rpm. The cells were harvested at the late exponential phase with an OD_{450} of 9.5 at 15 h. *Bacillus subtilis* BGSC 1A1 were grown in 1/2 Evans medium containing 50 mM glucose and 0.05% yeast extract in a shaking flask at 37°C and 250 rpm. The cells were harvested at the late exponential phase

at 11 h with an OD_{450} of 6.2. The cell pallets of all three strains were separately kept at −80°C for further biotransformation.

Coupling of permeabilized cells of* B. pumilus *Phe-C3 and* B. subtilis *BGSC 1A1 for bioreduction of ethyl 3-keto-4,4,4-trifluorobutyrate 1 (20 mM) at an initial $NADP^+$ concentration of 0.20 mM. To a suspension of permeabilized cells of *B. pumilus* Phe-C3 (6.7 g cdw/L) and *B. subtilis* BGSC 1A1 (13-27 g cdw/L) in 10 mL 100 mM Tris-buffer (pH = 7.0) was added substrate **1** (18.4 mg, 0.10 mmol, 10 mM), glucose (100 mg, 0.55 mmol, 55 mM), and $NADP^+$ (1.6 mg, 2.0 μmol, 0.20 mM). The mixture was shaken at 300 rpm and 25°C. Additional substrate **1** (15.8 mg, 0.086 mmol, 10 mM) was added at 9 h. 200 μL aliquots were taken out at different time point for analysis. After centrifugation, 100 μL supernatant was diluted with 400 μL Tris-buffer (pH = 8.0) and then extracted with 500 μL chloroform containing 2 mM hexadecane as the internal standard. The organic phase was separated, dried over Na_2SO_4, and filtrated. The samples were used for the analysis of conversion and the ee of the product ethyl 3-hydroxy-4,4,4-trifluorobutyrate (*R*)-**2**. The results are summarized in Table 1 and Figure S-1.

Coupling of permeabilized cells of* Pseudomonas *sp. Tyr-F10 and* B. subtilis *BGSC 1A1 for bioreduction of methyl 3-keto-3-(3'-pyridyl)-propionate 3 (20 mM) at an initial $NADP^+$ concentration of 0.20 mM. To a suspension of permeabilized cells of *Pseudomonas* sp. Tyr-F10 (6.7 g cdw/L) and *B. subtilis* BGSC 1A1 (13-27 g cdw/L) in 10 mL 100 mM Tris-buffer (pH = 8.0) was added substrate **3** (17.9 mg, 0.10 mmol, 10 mM), glucose (100 mg, 0.55 mmol, 55 mM), and $NADP^+$ (1.6 mg, 2.0 μmol, 0.20 mM).

The mixture was shaken at 300 rpm and 25°C. Additional substrate **3** (15.4 mg, 0.086 mmol, 10 mM) was added at 9 h. At different time point, 200 μL aliquots were taken out for analysis. After centrifugation, 100 μL supernatant was mixed with 400 μL Tris-buffer (PH = 8.0) with 5 mM benzyl acetone as the internal standard; the samples were used for the analysis of the conversion of the product methyl 3-hydroxyl-3-(3'-pyridyl)-propionate **4**. For the ee detection, the aqueous sample was extracted with $CHCl_3$, the organic phase was dried over Na_2SO_4, filtrated and evaporated, and the residue was dissolved in *n*-hexane/2-propanol 75/2 for HPLC analysis. The results are summarized in Table 1 and Figure S-2.

Bioreduction of ethyl 4,4,4-trifluorobutyrate 1 (120 mM) with permeabilized cells of* B. pumilus *Phe-C3* and B. subtilis *BGSC 1A1 at different initial NADP$^+$ concentrations (0.04-0.20 mM). To a suspension of permeabilized cells of *B. pumilus* Phe-C3 (20 g cdw/L) and *B. subtilis* BGSC 1A1 (40 g cdw/L) in 10 mL Tris-buffer (pH = 7.0) was added $NADP^+$ (0.32-1.6 mg, 0.4-2.0 μmol, 0.04-0.20 mM), substrate **1** (110 mg, 0.60 mmol, 60 mM), and glucose (0.60 g, 3.3 mmol, 330 mM), and the mixtures were shaken at 25°C and 300 rpm. Additional substrate **1** was added at 5 h (91 mg, 0.49 mmol, 60 mM), and more glucose was supplied at 5 h (0.66 g, 3.7 mmol, 0.45 M), 10 h (0.44 g, 2.4 mmol, 0.32 M), 18 h (0.28 g, 1.6 mmol, 0.21 M), 22 h (0.27 g, 1.5 mmol, 0.21 M), and 26 h (0.25 g, 1.4 mmol, 0.21 M). Samples (300 μL) was taken at different time point, centrifuged, and 100 μL supernatant was diluted with 400 μL Tris-buffer (PH = 7.0), followed by extraction with 500 μL chloroform containing 2 mM hexadecane as the internal standard. The organic phase was separated, dried over Na_2SO_4, filtrated, and

analyzed by GC to quantify the concentration and ee of the product (*R*)-**2**. The reaction was stopped at 30 h, resulting in 6.4 mL reaction mixtures due to sample taking. The product concentration and TTN of the cofactor are shown in Figure 1 A-E, and the product formation is given in Figure S-3.

Bioreduction of ethyl 4,4,4-trifluorobutyrate 1 (120 mM) with permeabilized cells of* B. pumilus *Phe-C3* and B. subtilis *BGSC 1A1 at an initial NADP$^+$ concentration of 0.01 mM. To a suspension of permeabilized cells of *B. pumilus* Phe-C3 (20 g cdw/L) and *B. subtilis* BGSC 1A1 (40 g cdw/L) in 10 mL Tris-buffer (PH = 7.0) was added $NADP^+$ (0.08 mg, 0.10 μmol, 0.01 mM), substrate **1** (110 mg, 0.6 mmol, 60 mM), and glucose (0.80 g, 4.4 mmol, 440 mM), and the mixtures were shaken at 25°C and 300 rpm. Additional substrate **1** was added at 5 h (97 mg, 0.53 mmol, 60 mM), and more glucose was supplied at 5 h (0.70 g, 3.9 mmol, 440 mM), 14 h (0.68 g, 3.8 mmol, 440 mM), 19 h (0.16 g, 0.91 mmol, 110 mM), 24 h (0.16 g, 0.9 mmol, 110 mM), 29 h (0.63 g, 3.5 mmol, 440 mM) and 38 h (0.61 g, 3.4 mmol, 440 mM). Aliquots (300 μL) were taken at different time point, and samples were prepared and analyzed as described above. After 43 h reaction, 2.42 M glucose was added totally, and the volume of reaction mixtures reduced to 7.0 mL due to sample taking. 16.2 mM product (*R*)-**2** was formed in 92% ee, corresponding to a cofactor TTN of 1620. The product formation is given in Figure S-4.

Bioreduction of ethyl 4,4,4-trifluorobutyrate 1 (140 mM) with permeabilized cells of* B. pumilus *Phe-C3* and B. subtilis *BGSC 1A1 at an initial NADP$^+$ concentration of 0.12 mM for a longer reaction time. To a suspension of permeabilized cells of *B. pumilus*

Phe-C3 (20 g cdw/L) and *B. subtilis* BGSC 1A1 (40 g cdw/L) in 20 mL Tris-buffer (PH = 7.0) was added $NADP^+$ (1.9 mg, 2.4 μmol, 0.12 mM), substrate **1** (221 mg, 1.2 mmol, 60 mM), and glucose (1.60 g, 8.89 mmol, 0.44 M), and the mixtures were shaken at 25°C and 300 rpm. Additional substrate **1** was added at 5 h (208 mg, 1.16 mmol, 60 mM) and at 24 h (65 mg, 0.36 mmol, 20 mM), and more glucose was supplied at 5 h (1.50 g, 8.33 mmol, 0.44 M), 14 h (1.11 g, 6.17 mmol, 0.33 M), 20 h (1.07 g, 5.94 mmol, 0.33 M), 28 h (1.38 g, 7.67 mmol,0.44 M), 38 h (1.02 g, 5.67 mmol, 0.33 M), 44 h (0.98 g, 5.44 mmol, 0.33 M), 50 h (0.95 g, 5.28 mmol, 0.33 M), 53 h (0.93 g, 5.17 mmol, 0.33 M), and 62 h (0.91 g, 5.06 mmol, 0.33 M). Samples (300 μL) was taken at different time point, extracted with chloroform, and analyzed by GC to quantify the product concentration and product ee. After 68 h reaction, 14.6 mL reaction mixtures remained after sample taking, and 114.4 mM (81.7% yield, 21 g/L) of ethyl 3-hydroxy-4,4,4-trifluorobutyrate (*R*)-**2** was formed in 91% ee with a TTN of cofactor of 953. The product formation is given in Figure S-5.

Repeated use of the permeabilized cells for bioreduction of 1. Permeabilized cells of *B. pumilus* Phe-C3 (26.7 g cdw/L) and *B. subtilis* BGSC 1A1 (53.4 g/L) were suspended in 20 mL Tris-buffer (PH =7.0). $NADP^+$ (3.1 mg, 3.9 μmol, 0.20 mM) was added, and the mixtures were shaken at 25°C and 300 rpm. Substrate **1** was added at 0 min (221 mg, 1.2 mmol, 60 mM), 5 h (208 mg, 1.13 mol, 60 mM), 24 h (62 mg, 0.034 mol, 20 mM). Glucose was added at 0 min (1.60 g, 8.89 mmol,0.44 M), 5 h (1.50 g, 8.33 mmol,0.44 M), 14 h (1.09 g, 6.06 mmol, 0.33 M), 20 h (1.06 g, 5.89 mmol, 0.33 M), 24 h (1.00 g, 5.56 mmol, 0.33 M), 30 h (1.30 g, 7.22 mmol, 0.44 M), 49 h (0.91 g, 5.06 mmol, 0.33 M)

and the total glucose concentration is 2.64 M. 200μL aliquot were taken at different time points, extracted with chloroform, and analyzed by GC and chiral GC. After 67 h, 14.4 mL reaction solution was remained. 123.7 mM (88%, 331 mg, 1.78 mmol) of (*R*)-**2** (23 g/L) was formed in 91% e.e.

The permeabilized cells after the 67 h biotransformation described above was harvested and washed twice with Tris-buffer (PH = 7.0). The cells were suspended in 11 mL Tris-buffer (PH = 7.0) to a concentration of 78.3 g cdw/L which is nearly the same as in the first run reduction. The bioreduction of **1** was performed at the same condition as described above with externally added $NADP^+$ (1.8 mg, 2.3 μmol, 0.20 mM). Substrate **1** was added at 0 min (121 mg, 0.66 mmol, 60 mM) and 5 h (113 mg, 0.61 mmol, 60 mM), and glucose was added at different time point as described for the first run. 102 mM (85%, 152 mg, 0.82 mmol) product (*R*)-**2** was obtained in 8.0 ml remaining reaction solution after 67 h, corresponding to a product concentration of 19 g/L.

For the third time transformation, the cells after the 67 h from the second run were centrifuged, washed and suspended in 7.0 mL Tris-buffer (PH = 7.0) to a cell density of 78.3 g cdw/L). $NADP^+$(1.1 mg, 1.4 μmol, 0.20 mM) was added initially, and substrate was added at 0 min (77 mg, 0.42 mmol, 60 mM) and 5 h (50 mg, 0.27 mmol, 40 mM). Glucose was added at different time point as described for the first run. After 67 h reaction, 80.1 mM (80.1%, 53 mg, 0.29 mmol) of (*R*)-**2** in 3.6 ml remaining reaction solution was formed, corresponding to a product concentration of 14.9 g/L. All results are shown in Figure 2.

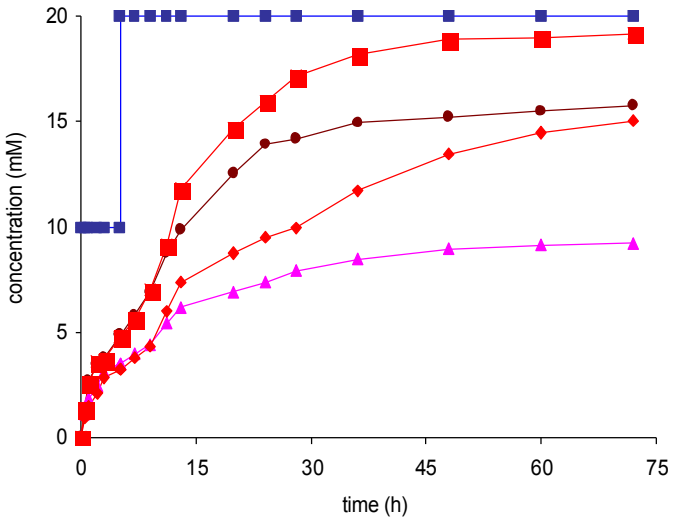

Figure S-1. Formation of (*R*)-**2** in the reduction of ethyl 3-keto-4,4,4-trifluoro-butyrate **1** (20 mM) with permeabilized cells of *B. pumilus* Phe-C3 (6.7 g cdw/L) and *B. subtilis* BGSC 1A1 at initial $NADP^+$ concentration of 0.20 mM, compared with the reduction with the whole cells of *B. pumilus* Phe-C3 (6.7 g cdw/L). (▲) whole cells of *B. pumilus* Phe-C3 with 55 mM glucose; (♦) permeabilized cells (1:2) with 55 mM glucose; (●) permeabilized cells (1:4) with 55 mM glucose; (■) permeabilized cells (1:4) with 110 mM glucose; (■) cumulative substrate **1**.

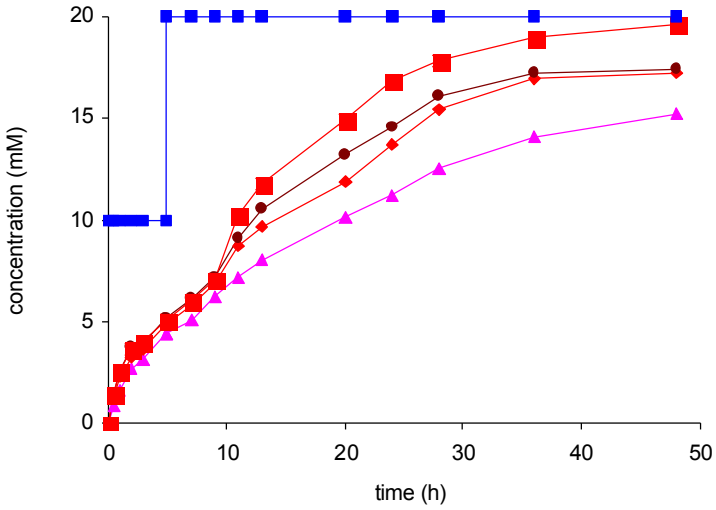

Figure S-2. Formation of (+)-**4** in the reduction of methyl 3-keto-pyridine propionate **3** (20 mM) with permeabilized cells of *Pseudomonas* sp. Tyr-F10 (6.7 g cdw/L) and *B. subtilis* BGSC 1A1 at initial $NADP^+$ concentration of 0.20 mM, compared with the reduction with the whole cells of *Pseudomonas* sp. Tyr-F10 (6.7 g cdw/L). (▲) whole cells of *Pseudomonas* sp. Tyr-F10 with 55 mM glucose; (♦) permeabilized cells (1:2) with 55 mM glucose; (●) permeabilized cells (1:4) with 55 mM glucose; (■) permeabilized cells (1:4) with 110 mM glucose. (■) cumulative substrate **3**.

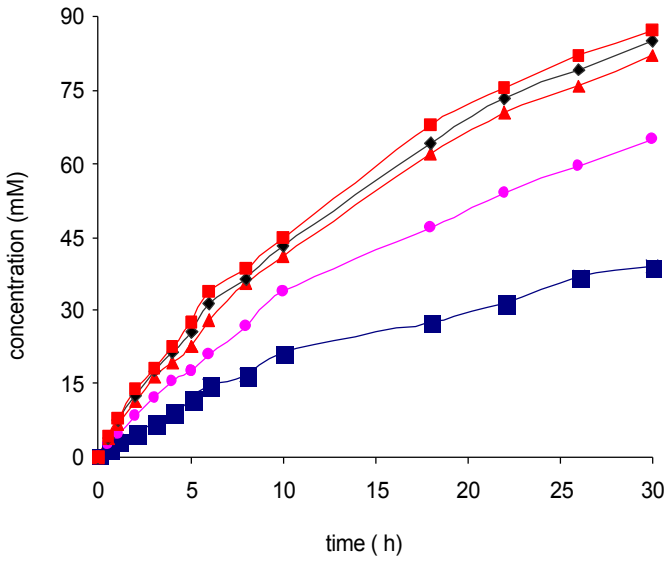

Figure S-3. Formation of (*R*)-**2** in the bioreduction of ethyl 4,4,4-trifluorobutyrate **1** (120 mM) with permeabilized cells of *B. pumilus* Phe-C3 (20 g cdw/L) and *B. subtilis* BGSC 1A1(40 g cdw/L) at different $NADP^+$ concentrations. (■) 0.04 mM $NADP^+$. (●) 0.08 mM $NADP^+$. (▲) 0.12 mM $NADP^+$. (♦) 0.16 mM $NADP^+$. (■) 0.20 mM $NADP^+$.

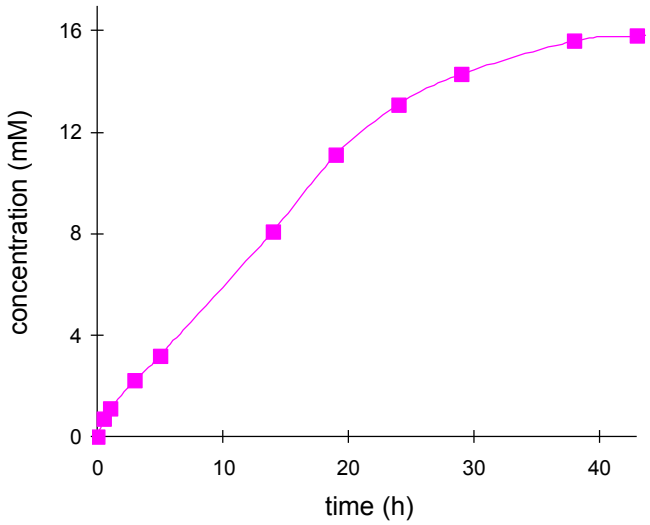

Figure S-4. Formation of (*R*)-**2** in the bioreduction of ethyl 4,4,4-trifluorobutyrate **1** (120 mM) with permeabilized cells of *B. pumilus* Phe-C3 (20 g cdw/L) and *B. subtilis* BGSC 1A1 (40 g cdw/L) at an initial $NADP^+$ concentration of 0.01 mM and a total glucose concentration of 2.42 M added at different time points.

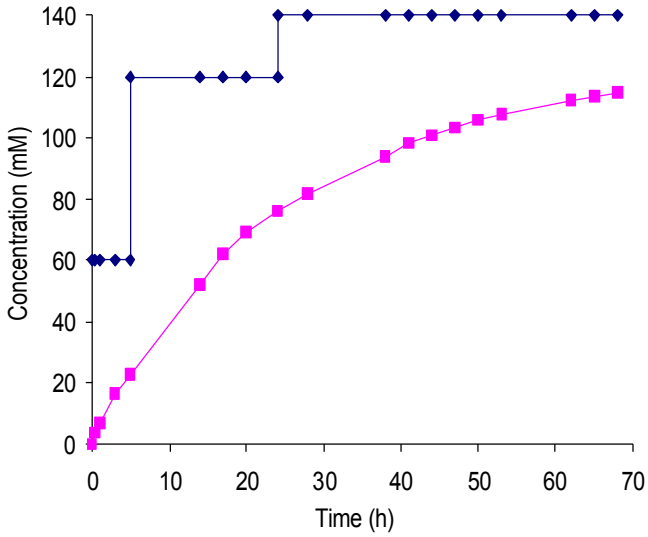

Figure S-5. Formation of (*R*)-**2** in the bioreduction of **1** (140 mM) with permeabilized cells of *Bacillus pumilus* Phe-C3 (20 g cdw/L) and *Bacillus subtilis* BGSC 1A1 (40 g cdw/L) at an initial $NADP^+$ concentration of 0.12 mM and a total glucose concentration of 3.63 M added at different time points. ♦ - cumulative substrate **1**; ■ - formed product (*R*)-**2**.