Electronic Supplementary Information

Whole-cell biocatalytic selective oxidation of 5-hydroxymethylfurfural to 5-hydroxymethyl-2-furancarboxylic acid

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Isolation and screening of *Comamonas testosteroni* SC1588

Soil samples were collected from the grounds of University Town (China, Guangzhou). Strain screening was conducted by an enrichment culture technique. Soil samples were mixed, and then 10 g of samples was added into 90 mL of sterile deionized water. Five milliliters of suspensions were inoculated into 50 mL of 1.8% nutrient broth (NB) medium containing 20 mM HMF, and then were cultivated aerobically at 150 r/min and 30 °C for 2 days. After dilution, the enriched cultures were spread on 1.8% NB agar media containing 20 mM HMF, and were incubated at 30 °C until colonies appeared. Then morphologically different colonies were isolated, and were subsequently streaked for five generations to obtain the purified single colony. To preliminarily evaluate their catalytic performances, the purified isolates (30 mg/mL) were incubated in 4 mL phosphate buffer (200 mM, pH 7.0) containing 20 mM HMF at 150 r/min and 30 °C. Aliquots were withdrawn from the reaction mixtures at specified time intervals for quantificational analysis of HMF and HMFCA using HPLC. *C. testosteroni* SC1588 proved to be the best biocatalyst for the synthesis of HMFCA, among the purified isolates.

16S rDNA gene sequence S871 S3 1392 bp

AAGTCGAACGGTAACAGGTCTTCGGATGCTGACGAGTGGCGAACGGGTGAGTAATACATCGGAACGT
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The GenBank accession number for the 16S rDNA gene sequence of the strain SC1588 is KX961682.

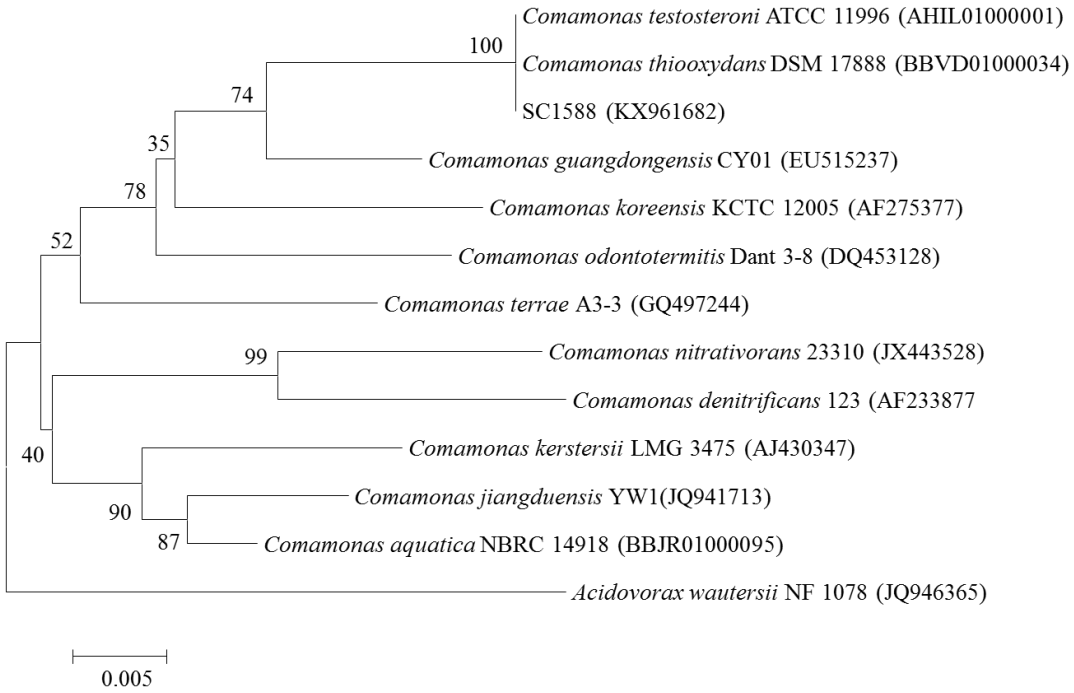

Figure S1. The phylogenetic tree of the train SC1588

Table S1. Physiological and biochemical characteristics of *Comamonas thiooxidans* DSM17888,[1] *C. testosteroni* KCTC2990,[1] and the strain SC1588

Characteristics	*C. thiooxidans* DSM17888	*C. testosteroni* KCTC2990	SC1588
Growth on			
Amino acids			
Histidine	−	+	+
Phenylalanine	−	+	+
Alanine	−	+	+
Glycine	−	+	+
Asparagine	−	+	+
Tryptophan	−	+	+
Methionine	−	−	−
Lysine	−	−	−
Aspartic acid	+	+	+
Serine	+	−	−
Sugar			
Glucose	+	+	−
Xylose	−	n.a.	−
Fructose	−	n.a.	−
Mannose	−	n.a.	−
Sucrose	−	n.a.	−

Others			
Testosterone	n.a.	n.a.	+
Mannitol	−	n.a.	−
Anaerobic growth	−	−	−
Fluorescent pigment	n.a.	n.a.	−
Growth at 42˚C	−	n.a.	−
Growth at pH 4.0	−	n.a.	−
Oxidase test	+	n.a.	+
Catalase test	+	n.a.	+
Hydrolysis of gelatin	−	n.a.	−
Hydrolysis of starch	+	+	−
Salt tolerance	n.a.	n.a.	2%
Methyl red test	−	n.a.	−
Voges-proskauer test	−	n.a.	−
Tween 80	n.a.	n.a.	−
Gram staining	−	n.a.	−
Morphology	Rods, motile	n.a.	Rods, motile

+ Positive; −, negative; n.a.: not available

Figure S1 shows the phylogenetic tree of the train SC1588, which was constructed using the neighbor-joining method,[2] based on the analysis of 16S rDNA of this strain. It was found that the 16S rDNA sequence of the strain SC1588 was almost the same as those of *C. thiooxidans* DSM17888 C and *C. testosteroni* KCTC2990. Therefore, physiological and biochemical properties of this strain was characterized according to bacterial identification methods,[3, 4] and were compared with other two strains[1] (Table S1). As shown in Table S1, the strain SC1588 show highly similar properties to *C. testosteroni* KCTC2990. More importantly, the strain SC1588 could grow on testosterone. Therefore, it was identified as one of *C. testosteroni* strains.

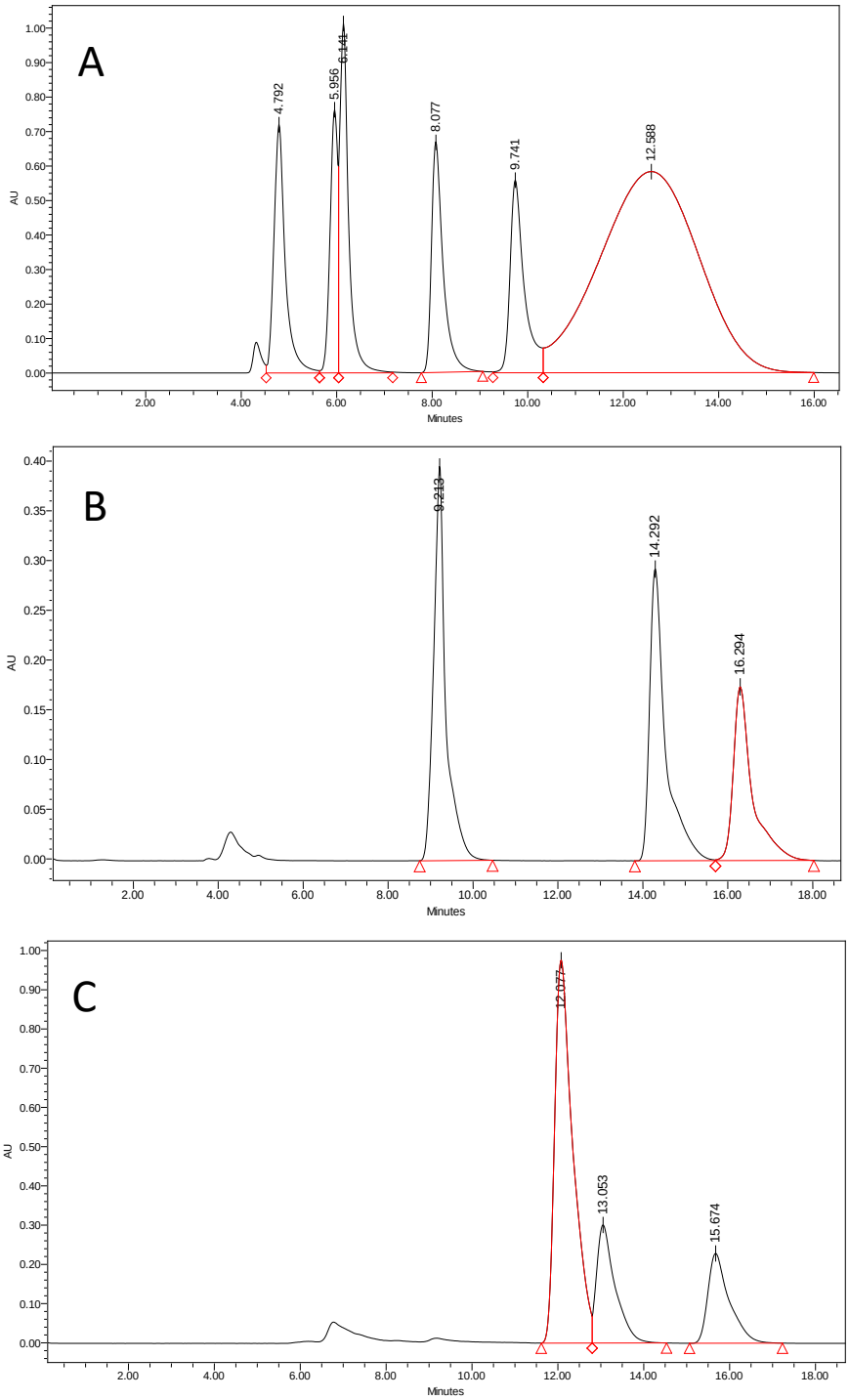

Figure S2. HPLC spectra: (A) HMF and its derivatives; (B): furfural and its derivatives; (C): 5-methylfurfural and its derivatives

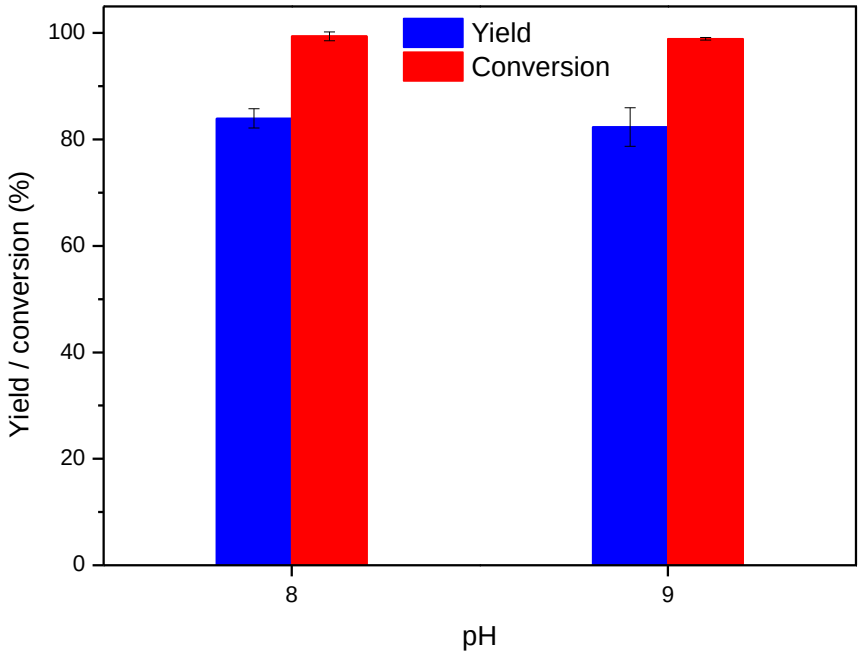

Figure S3. Whole-cell biocatalytic synthesis of HMFCA in 50 mM tris-HCl buffer

Conditions: 40 mM HMF, 30 mg/mL microbial cells, 4 mL tris-HCl buffer (50 mM, pH 8.0 or 9.0), 150 r/min, 30°C.

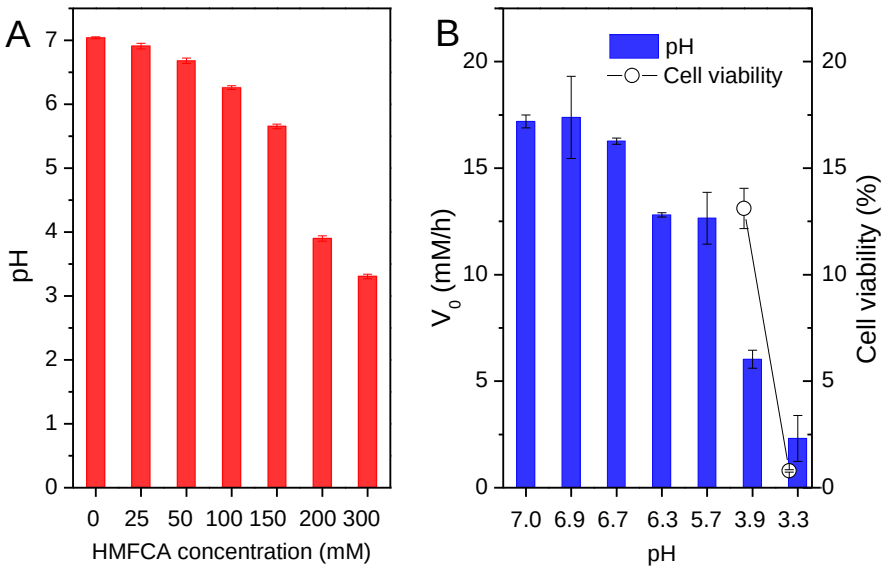

Figure S4. pH changes of the reaction mixtures with addition of HMFCA (A) and effect of pH on initial reaction rate and cell viability (B)

Conditions for Figure S4A: HMFCA of the designated concentration in 4 mL phosphate buffer (0.2 M, pH 7.0) at 25°C; conditions for Figure S4B: 50 mM HMF, 30 mg/mL microbial cells, 4 mL phosphate buffer (200 mM, pH 5.7-7.0) or citrate buffer (200 mM, pH 3.1- 3.9), 150 r/min, 30 °C

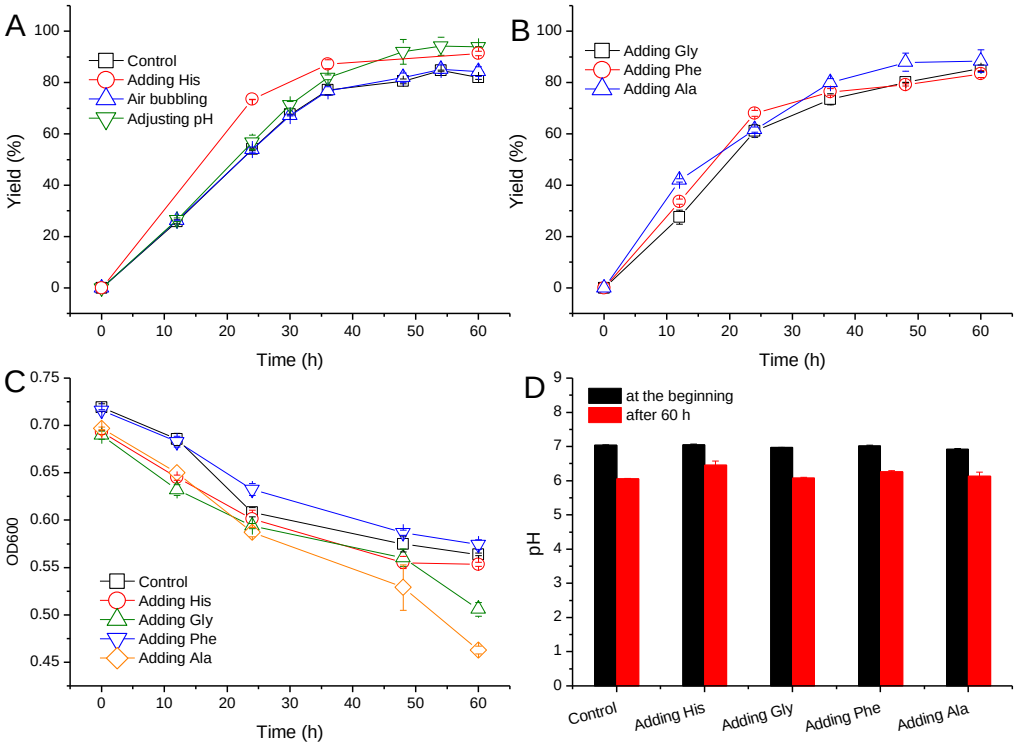

Figure S5. Improving HMFCA synthesis by using various strategies (A and B) and changes in OD600 values (C) and pH (D) during biocatalytic oxidation

General conditions: 130 mM HMF, 30 mg/mL microbial cells, 4 mL phosphate buffer (200 mM, pH 7.0), 150 r/min, 30 °C. Adding amino acid: 0.08 mmol amino acid (20 mM) was added at the beginning; Air bubbling: air was bubbled for 3 min every 12 h; pH-tuning: pH of the reaction mixture was adjusted to approximately 7.0 by using $NaHCO_3$ at 24 h. OD600 values corresponded to the absorbance of the reaction mixtures after dilution 21 times at 600 nm.

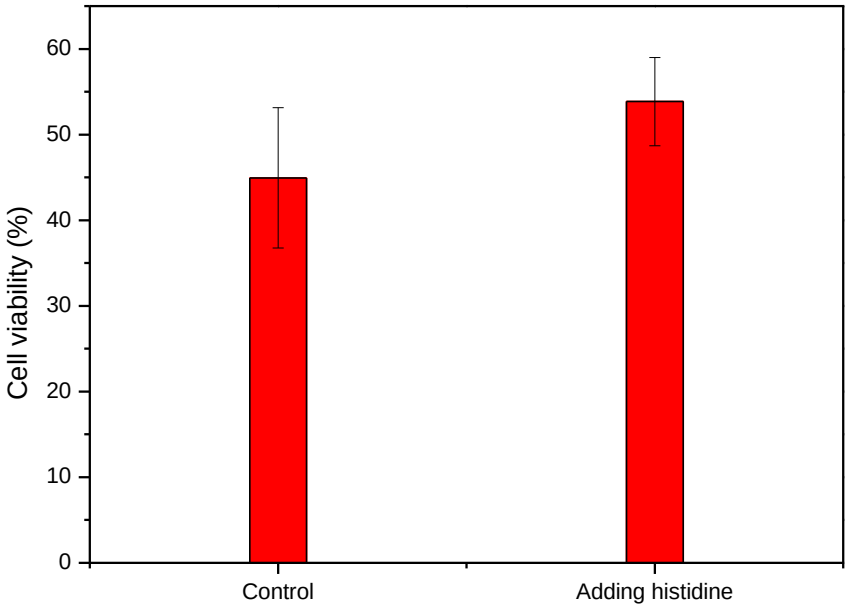

Figure S6. Effect of histidine addition on cell viability

Conditions: after 30 mg/mL microbial cells were incubated for 12 h in phosphate buffer (200 mM, pH 7.0) containing 150 mM HMF and 20 mM histidine at 150 r/min and 30 °C, cell viability was measured. Control: without histidine

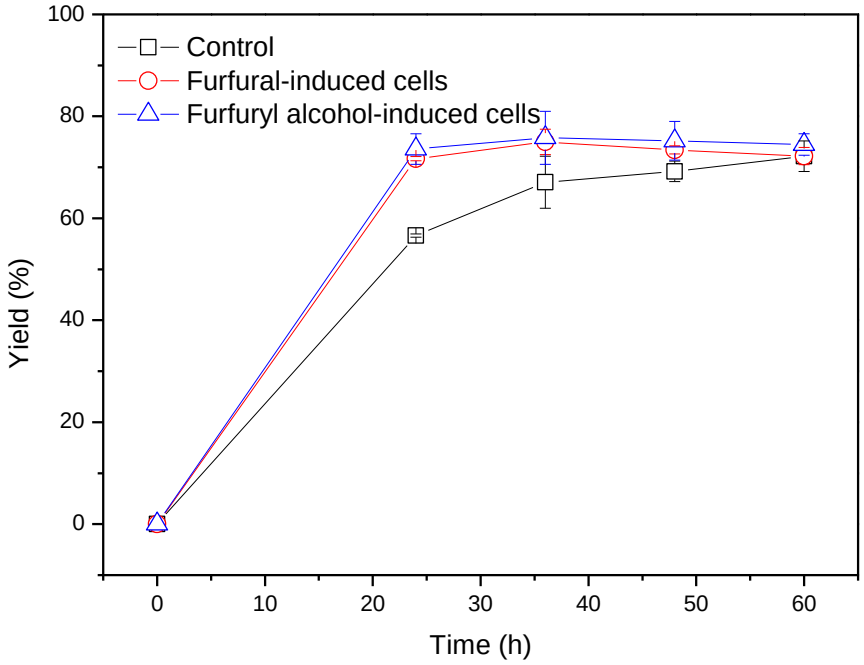

Figure S7. Time courses of HMFCA synthesis catalyzed by whole cells

Cultivation conditions: *C. testosteroni* SC1588 cells were pre-cultivated at 30 °C and 150 r/min

for 12 h in 1.8% nutrient broth. Then, the 1% seed culture was inoculated to the fresh nutrient broth (1.8%) containing **5 mM furfural or furfuryl alcohol**. After incubation at 30 °C and 150 r/min for 5 h, the cells were harvested by centrifugation (8000 g, 5 min, 4 °C) and washed twice with 0.85% NaCl solution, followed by being dispersed in phosphate buffer to give a cell concentration of 30 mg (cell wet weight)/mL. **Control**: neither furfural nor furfuryl alcohol were added during cultivation.

Reaction conditions: 150 mM HMF, 30 mg/mL microbial cells, 4 mL phosphate buffer (200 mM, pH 7.0), 30 °C, 150 r/min

References

1. K. D. Narayan, S. K. Pandey and S. K. Das, *Curr. Microbiol.*, 2010, **61**, 248-253.
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3. X. Z. Dong and M. Y. Cai, *Bacterial Identification Manual (in Chinese)*, China Science Publishing Co., Beijing, 2001.
4. *Bergey's Manual of Determinative Bacteriology*, Williams & Wilkins Co., Baltimore, Maryland, 1975.