Electronic Supplementary Material (ESI)

Engineering enzymatic cascades for efficient biotransformation of eugenol and taxifolin to silybin and isosilybin

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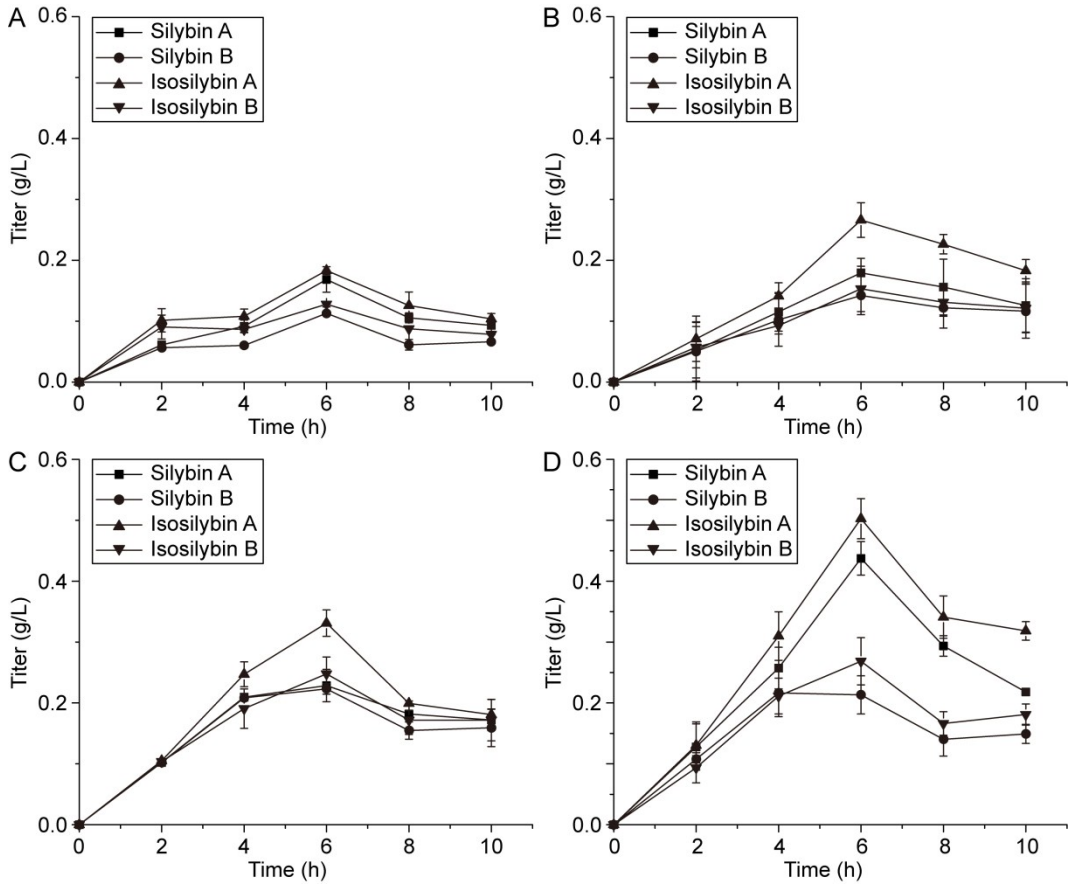

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22 **Figure S1** Silybin and isosilybin titers in bioconversions of different feeding rates.

23 The starting concentration was 5 mM eugenol and 1 mM taxifolin. The feeding rate

24 was **(A)** 6.25 μL/h, equal to 0.25 mM/h eugenol and 0.05 mM/h taxifolin, **(B)** 12.5

25 μL/h, equal to 0.5 mM/h eugenol and 0.1 mM/h taxifolin, **(C)** 25 μL/h, equal to 1

26 mM/h eugenol and 0.2 mM/h taxifolin, and **(D)** 62.5 μL/h, equal to 2.5 mM/h eugenol

27 and 0.5 mM/h taxifolin.

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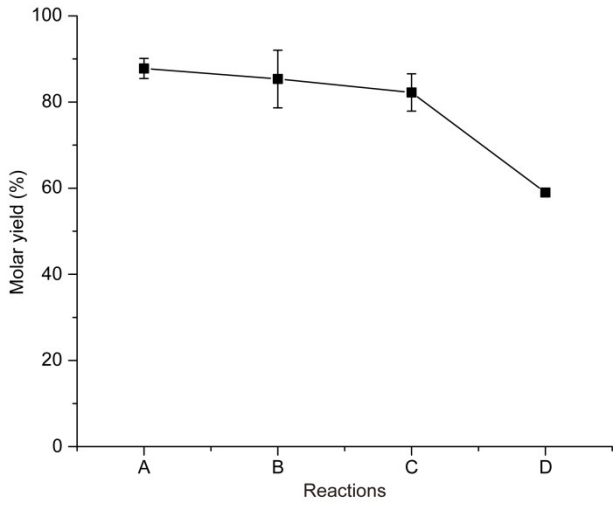

Figure S2 Molar yields of silybin and isosilybin in bioconversions of different feeding rates. The starting concentration was 5 mM eugenol and 1 mM taxifolin. The feeding rate was **(A)** 6.25 μL/h, equal to 0.25 mM/h eugenol and 0.05 mM/h taxifolin, **(B)** 12.5 μL/h, equal to 0.5 mM/h eugenol and 0.1 mM/h taxifolin, **(C)** 25 μL/h, equal to 1 mM/h eugenol and 0.2 mM/h taxifolin, and **(D)** 62.5 μL/h, equal to 2.5 mM/h eugenol and 0.5 mM/h taxifolin. The molar yield was calculated based on taxifolin.

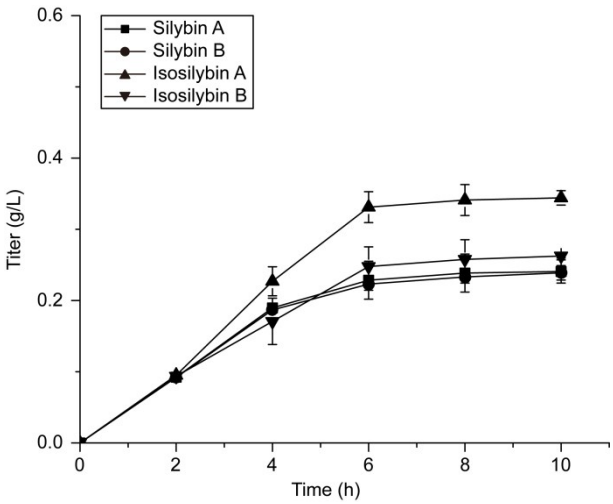

Figure S3 Bioconversion under pH6.0. The starting concentration was 5 mM eugenol and 1 mM taxifolin. The feeding rate was 25 μL/h. The bioconversion was carried out in the fermented broth, and the pH was adjusted to 6.0 using 1 M HCl.

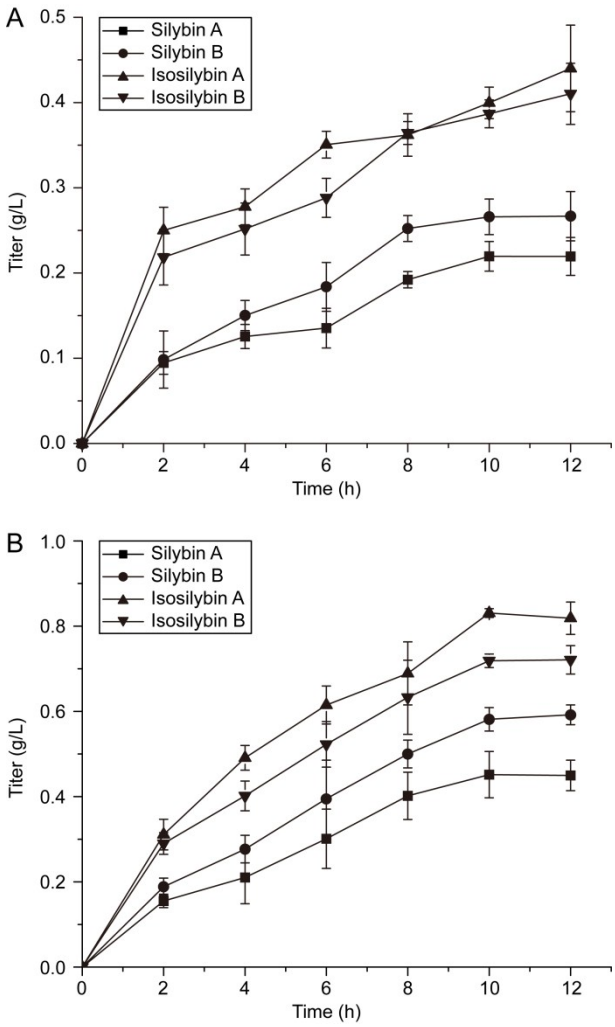

Figure S4 Scale-up of the bioconversion. The bioconversions were carried out in 1.5-L in a 3.0-L fermenter. The starting concentration was 5 mM eugenol and 1 mM taxifolin. The feeding rates were 1.5 mL/h **(A)** and 3.75 mL/h **(B)**, respectively.

Table S1 Ratio of isomers produced by different peroxidases

Peroxidase	Silybin A (%)	Silybin B (%)	Isosilybin A (%)	Isosilybin B (%)
EcoDyPrx02_536	16.39±3.02	15.55±2.66	43.30±3.63	24.75±7.07
EcoDyPrx01_K12	11.78±2.93	ND[1]	88.22±2.73	ND
SceCcP01	17.28±0.64	20.33±1.12	36.31±1.61	26.09±1.26
PpasCcP01	17.98±4.39	20.19±5.14	36.22±6.74	25.61±3.86
APX1	17.79±0.71	21.66±0.67	35.51±0.76	25.03±0.38

1. ND refers to not detected.

Table S2 Primers used in this study

Primers	Sequence (5’ to 3’)
SceCcP01 F	GGAATTCATGACTACTGCTGTTAGGCTTTTAC[1]
SceCcP01 R	CCGCTCGAGTAAACCTTGTTCCTCTAAAGTC
PpasCcP01 F	GGAATTCATGTCGTCCATTGCATTCCATACAT
PpasCcP01 R	CCAAGCTTACTCTGCTCGTCTAGTGTCCTG
APX1-PsVAO F1	GGAATTCATGCCGGTGGTTGATACCGA
APX1-PsVAO R1	CGAAATTCCTGGGTTTTGCTCATAGAACCACCAC
	CCATTTTCTTACGGGCTTCG
APX1-PsVAO F2	CTACGAAGCCCGTAAGAAAATGGGTGGTGGTTCT
	ATGAGCAAAACCCAGGAATTTC
APX1-PsVAO R2	CCAAGCTTACAGTTTCCAGGTGACGTGGC
PsVAO-APX1 F1	GGAATTCATGAGCAAAACCCAGGAATTTC
PsVAO-APX1 R1	ATTCGGTATCAACCACCGGCATAGAACCACCACC
	CAGTTTCCAGGTGACGTGGC
PsVAO-APX1 F2	GCCACGTCACCTGGAAACTGGGTGGTGGTTCTAT
	GCCGGTGGTTGATACCGAATATC
PsVAO-APX1 R2	CCAAGCTTACATTTTCTTACGGGCTTCG

1. Underlined letters refer to restriction enzymes or overlapping sequences.