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Supporting Information

Efficient testosterone production by engineered *Pichia pastoris* co-expressing human 17β-hydroxysteroid dehydrogenase type 3 and *Saccharomyces cerevisiae* glucose 6-phosphate dehydrogenase with NADPH regeneration

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Supplemental materials

Supplementary Table 1. Strains, plasmids and primers used in this study.

Name	Description	Sources
Strains		
Escherichia coli JM109	*recA1, endA1, gyrA96, thi-1, hsd R17(r_k^- m_k^+)supE44*	Invitrogen
Saccharomyces cerevisiae	Source of *ZWF1* gene	This lab
Pichia pastoris GS115	Host of *17β-hsd3* or *ZWF1*	Invitrogen
P. pastoris/CK	*P. pastoris* GS115 electro-transformed with pPIC3.5K and pPICZα as control	This study
P. pastoris/17β-HSD3$_{CK}$	*P. pastoris* GS115 over-expressing the uncodon-optimized *17β-hsd3* gene	This study
P. pastoris/17β-HSD3	*P. pastoris* GS115 over-expressing the codon-optimized *17β-hsd3* gene	This study
P. pastoris/17β-HSD3-G6PDH	*P. pastoris* GS115 over-expressing the codon-optimized *17β-hsd3* and *ZWF1* genes	This study
Plasmids		
pUC57-*17β-hsd3*$_{CK}$	The uncodon-optimized *17β-hsd3* gene delivered by pUC57	Shanghai Sangon Biological Engineering Technology & Services Co. Ltd
pUC57-*17β-hsd3*	The codon-optimized *17β-hsd3* gene delivered by pUC57	Shanghai Sangon Biological Engineering Technology & Services Co. Ltd
pPIC3.5K	9.0 kb, Amp^R	Invitrogen
pPICZα	3.6 kb, Zeocin^R	Invitrogen
pPIC3.5K-*17β-hsd3*$_{CK}$	9.9 kb, pPIC3.5K containing the uncodon-optimized *17β-hsd3* gene, Amp^R	This study
pPIC3.5K-*17β-hsd3*	9.9 kb, pPIC3.5K containing the codon-optimized *17β-hsd3* gene, Amp^R	This study

pPICZα-*ZWF1*	5.1 kb, pPICZα containing *ZWF1* gene, Zeocin^R	This study
Primers 5'-3'	
P1	CGGGATCCATGGGGGACGTCCTGGAACAG (*Bam*H I)
P2	CGGAATTCCTA**GTGGTGGTGGTGGTGGTG**CCTGACCTTGGTGTTG (*Eco*R I)
P3	CGGGATCCACCATGGGAGATGTACTAGAG (*Bam*H I)
P4	CGGAATTCTTA**GTGGTGGTGGTGGTGGTG**ACGAACTTTGGTATTC (*Eco*R I)
P5*	CTGTTCGAAACGATGAGTGAAGGCCCCGTCAAATTTGAAAAAAATACCG (*Bst*B I)
P6*	CACGCTCGAGCTAATTATCCTTCGTATCTTCTGGC (*Xho* I)

Notes: Amp^R ampicillin-resistant, Zeocin^R zeocin-resistant, the restriction enzyme sites were underlined, and the His-Taq coding region were bold typed.

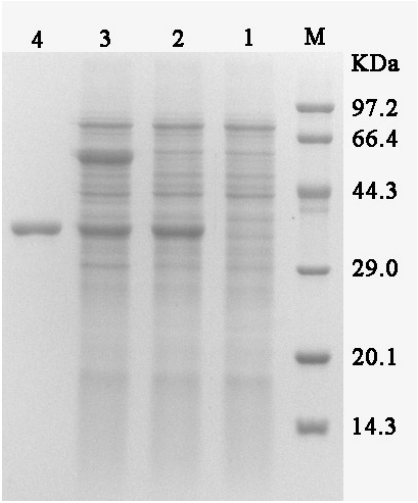
*P5, P6 the primers were in accordance with that reported by Geng et al. [1].

Supplementary Table 2. Summary of the purification procedure for the recombinant 17β-HSD3.

Step	Total activity* (U)	Total protein (mg)	Specific activity (U mg^{-1})	Purification (fold)	Yield (%)
Crude cell extract	201.32	39.32	5.12	1.00	100.00
HisTrap™ HP column	250.62	20.13	12.45	2.43	62.24

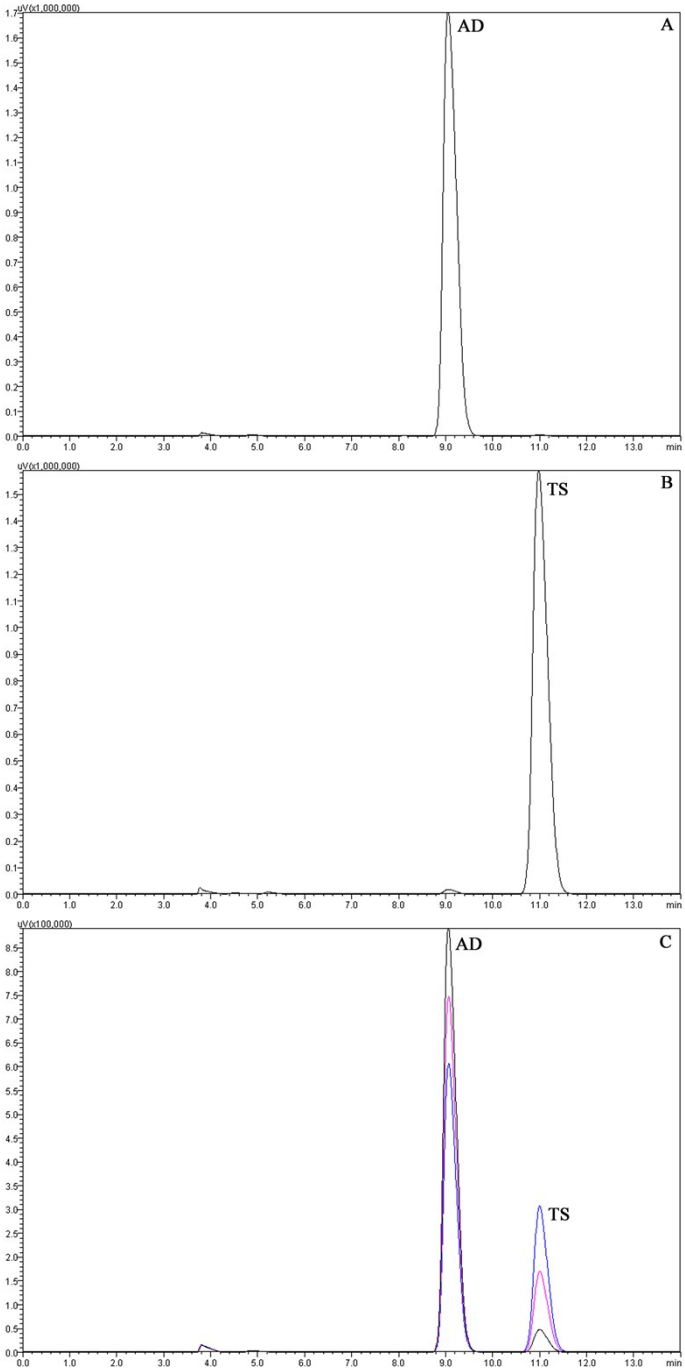
Notes: *One unit of enzyme activity defined as the amount of enzyme required to oxidize 1 μmol of AD to produce 1 μmol of TS at 37°C and pH 7.5 per min.

Supplementary Fig. 1



Supplementary Fig. 1. SDS-PAGE analysis of the expression of 17β-HSD3 and G6PDH in *P. pastoris* GS115. Lanes: (M) Protein marker; Lane 1, crude cell extracts of *P. pastoris*/CK; Lane 2, crude cell extracts of *P. pastoris*/17β-HSD3; Lane 3, crude cell extracts of *P. pastoris*/17β-HSD3-G6PDH; Lane 4, purified 17β-HSD3.

Supplementary Fig. 2



Supplementary Fig. 2. HPLC analysis of AD transformation. (A) The standard sample of AD; (B) The standard sample of TS; (C) The product by strain *P. pastoris*/17β-HSD3$_{CK}$ (black line), *P. pastoris*/17β-HSD3 (pink line) and *P. pastoris*/17β-HSD3-G6PDH (blue line).

References

1. Geng YW, Zhang RZ, Xu Y, Wang SS, Sha C, et al. (2011) Coexpression of a carbonyl reductase and glucose 6-phosphate dehydrogenase in *Pichia pastoris* improves the production of (S)-1-phenyl-1, 2-ethanediol. Biocatalysis and Biotransformation 29: 172-178.