

## SUPPLEMENTARY MATERIALS

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### **Development of high methanol tolerance *Pichia pastoris* based on iterative adaptive laboratory evolution**

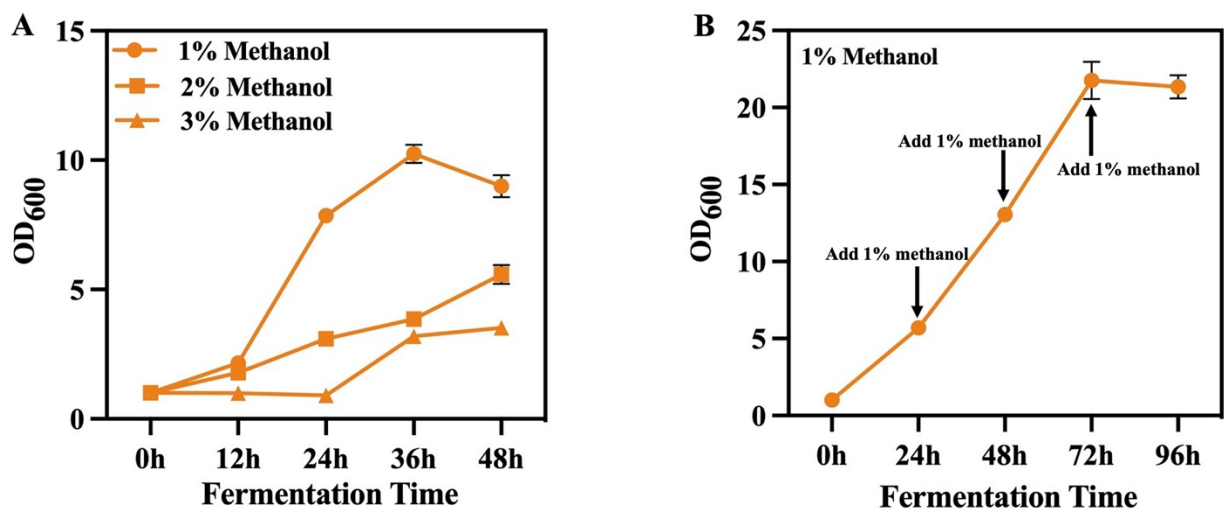
Shuai Wang<sup>a</sup>, Yuanyuan Wang<sup>a</sup>, Qingyan Yuan<sup>a</sup>, Liu Yang<sup>a</sup>, Fengguang Zhao<sup>b</sup>, Ying Lin<sup>a</sup>,  
Shuangyan Han<sup>a\*</sup>

*<sup>a</sup>School of Biology and Biological Engineering, South China University of Technology,  
Guangzhou 510006, Guangdong, China*

*<sup>b</sup>School of Light Industry and Engineering, South China University of Technology, Guangzhou,  
510006, Guangdong, China*

\* Corresponding author.

*E-mail address: [syhan@scut.edu.cn](mailto:syhan@scut.edu.cn) (S. Han)*



**Fig. S1** (A) Growth curves of *Pichia pastoris* GS115 with initial methanol concentrations of 1%, 2% and 3%. (B) Growth curve of *Pichia pastoris* GS115 with an initial methanol concentration of 1% and 1% methanol was added every 24h. Values are the average of at least three biological replicates with error bars indicating one standard deviation.

**Table S1** Primers used in this study.

Names	Characteristics	
Strains		
GS115	<i>his4<sup>-</sup></i>	Invitrogen
WS026-4/14 (MMC)	GS115 evolution with MMC	This study
WS026-4/14 (MMC-SFC)	WS026-4/14 (MMC) evolution with flask culture	This study
GS115-PET2	GS115 harboring plasmid pPICZA-PET2	This study
Plasmid		
pPICZA	Expression vector with P <sub>AOX1</sub> promoter, P <sub>AOX1</sub> terminator and Zeo <sup>R</sup> marker	Invitrogen
pPICZA-PET2	pPICZA carrying gene <i>PET2</i>	This study

Primers	Sequence (5'→3')	Objective
PET2-F	atggctgatcgacttgtttctaac	PCR primers for <i>PET2</i> from GS115
PET2-R	ctacaaactcttttcttctttgt	
P1	gagaagtccaagaacaacaacc	RT-qPCR primers for <i>CRD1</i>
P2	tgacaggttcagcatcttttagtgg	
P3	gaaaggcatcagagccatcgt	RT-qPCR primers for <i>GEP4</i>
P4	ctatgtccctcgtcgtcgttcg	
P5	tctccgatttactctggcggcact	RT-qPCR primers for <i>PET1</i>
P6	aagagttgtgacttcgcttttggtg	
P7	gacagagtgacctcgttccatt	RT-qPCR primers for <i>PET2</i>
P8	tctttgctgtgaatggctcctc	
P9	acggtccagggtagatgtagac	RT-qPCR primers for <i>PGS1</i>
P10	gtgctgtaacccaataaaccttc	
P11	tgacggtaaagtgccagattgc	RT-qPCR primers for <i>PAS</i>
P12	agaggcgggagcaaccccaaat	
P13	caagtgaccaatcgtgag	RT-qPCR primers for <i>PSD1</i>
P14	aatcgggtgtaatctcctgg	