Supplemental material

Metabolic Engineering and Adaptive Evolution for Efficient Production of L-lactic acid in *Saccharomyces cerevisiae*

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Table S1 Strains and plasmids used in this study.

Strains and plasmids	Relevant characteristics	References
Strains		
S. cerevisiae CEN.PK2-1C	*MATa ura3-52 leu3-5,112 trp1-289 his3Δ MAL2-8^c SUC2*	EUROSCARF
S.c-0	*S. cerevisiae* CEN.PK2-1C	EUROSCARF
S.c-PΔ-L	*S. cerevisiae* CEN.PK2-1C *pdc1*Δ::P_{PDC1}-*LcLDH*-T::*his*	This study
S.c-PΔ-B	*S. cerevisiae* CEN.PK2-1C *pdc1*Δ::P_{PDC1}-*BoLDH*-T::*his*	This study
S.c-PΔ-R	*S. cerevisiae* CEN.PK2-1C *pdc1*Δ::P_{PDC1}-*RoLDH*-T::*his*	This study
S.c-PΔAΔ-B	S.c-PΔ-B *adh1*Δ::*leu*	This study
S.c-PΔAΔ-BE	S.c-PΔ-B *adh1*Δ::P_{ADH1}-*eutE*-T::*leu*	This study
S.c-NO.1	S.c-PΔAΔ-BE (pY16)	This study
S.c-NO.2	S.c-PΔAΔ-BE (pY16-JEN1)	This study
S.c-NO.3	S.c-PΔAΔ-BE (pY16-ADY2)	This study
S.c-NO.4	S.c-PΔAΔ-BE (pY16-ESBP6)	This study
S.c-NO.5	S.c-PΔAΔ-BE (pY16-JEN1-ADY2)	This study
S.c-NO.6	S.c-PΔAΔ-BE (pY16-JEN1-ESBP6)	This study
S.c-NO.7	S.c-PΔAΔ-BE (pY16-ADY2-ESBP6)	This study
S.c-NO.8	S.c-PΔAΔ-BE (pY16-JEN1-ADY2-ESBP6)	This study
S.c-NO.2-100	the evolved S.c-NO.2	This study
Plasmids		
pY16	CEN6/ARSH4, Amp, URA3, P_{TEF}	Turbo
pY16-JEN1	CEN6/ARSH4, Amp, URA3, P_{TEF}-JEN1	This study
pY16-ADY2	CEN6/ARSH4, Amp, URA3, P_{TEF}-ADY2	This study
pY16-ESBP6	CEN6/ARSH4, Amp, URA3, P_{TEF}-ESBP6	This study
pY16-JEN1-ADY2	CEN6/ARSH4, Amp, URA3, P_{TEF}-JEN1, P_{TEF}-ADY2	This study
pY16-JEN1-ESBP6	CEN6/ARSH4, Amp, URA3, P_{TEF}-JEN1, P_{TEF}-ESBP6	This study
pY16-ADY2-ESBP6	CEN6/ARSH4, Amp, URA3, P_{TEF}-ADY2, P_{TEF}-ESBP6	This study
pY16-JEN1-ADY2-ESBP6	CEN6/ARSH4, Amp, URA3, P_{TEF}-JEN1, P_{TEF}-ADY2, P_{TEF}-ESBP6	This study
pLAZ10-LDH	pLAZ10 with *LDH* gene from *Bovine*	(1)

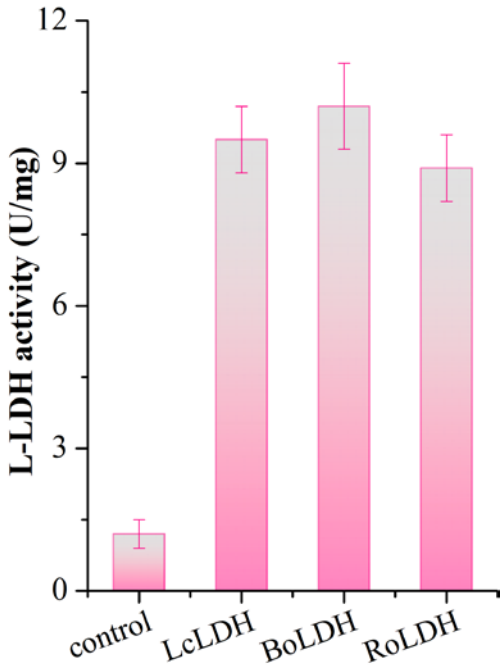

FIG S1 The specific activities of *Lc*LDH, *Bo*LDH, and *Ro*LDH in the engineered *S. cerevisiae*, S.c-PΔ-L, S.c-PΔ-B, and S.c-PΔ-R, respectively.

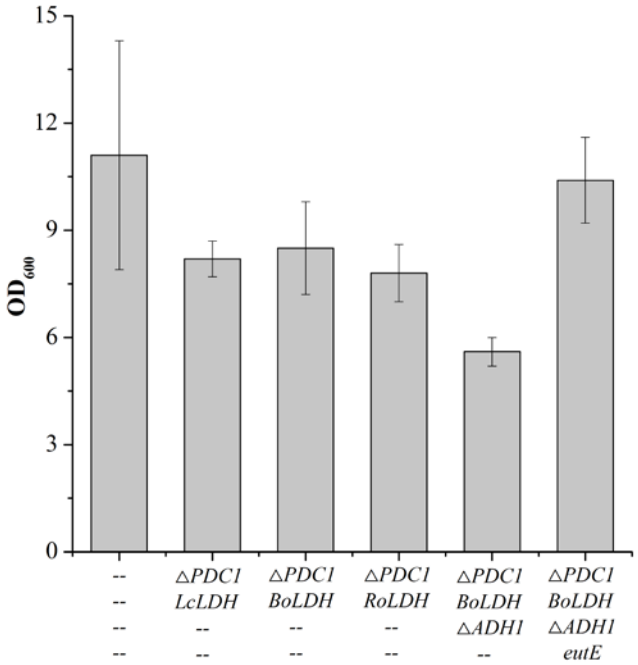

FIG S2 Effect of gene expression or deletion on cell growth.

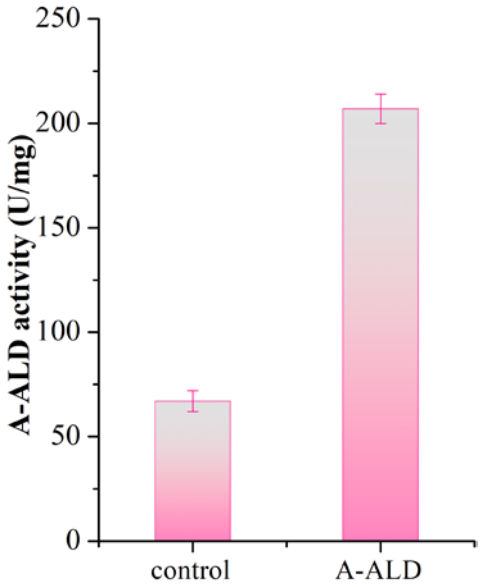

FIG S3 The specific activities of A-ALD in the engineered strain S.c-PΔAΔ-BE and the control strain S.c-PΔAΔ-B.

References

1. **Bianchi MM, Brambilla L, Protani F, Liu CL, Lievense J, Porro D.** 2001. Efficient homolactic fermentation by *Kluyveromyces lactis* strains defective in pyruvate utilization and transformed with the heterologous LDH gene. Appl Environ Microbiol **67:**5621-5625.