Supplementary Materials for

Metabolic engineering of *Escherichia coli* for production of D-Panthenol from 3-aminopropanol and glucose

Junping Zhou,[a,b] Zheng Zhang,[a,b] Xinyuan Xin,[a,b] Yinan Xue,[a,b] Yihong Wang,[a,b] Xueyun Feng,[a,b] Bo Zhang,[a,b] Man Zhao,[a,b] Zhiqiang Liu*[a,b] and Yuguo Zheng[a,b]

[a]*The National and Local Joint Engineering Research Center for Biomanufacturing of Chiral Chemicals, Zhejiang University of Technology, Hangzhou 310014, China.*

[b]*Key Laboratory of Bioorganic Synthesis of Zhejiang Province, College of Biotechnology and Bioengineering, Zhejiang University of Technology, Hangzhou 310014, China.*

***Corresponding author:**

Zhi-Qiang Liu, Key Laboratory of Bioorganic Synthesis of Zhejiang Province, College of Biotechnology and Bioengineering, Zhejiang University of Technology, Hangzhou 310014, China

E-mail: microliu@zjut.edu.cn (Z.-Q. Liu)

Phone number: +86-571-88320379

Fax number: +86-571-88320630

Table S1. Bacterial strains and plasmids used in this study

Strains	Characteristics	Sources
E. coli DH5α	F⁻ Φ80d/*lacZ*ΔM15, Δ(*lacZYA-argF*) U169, *recA*1, *endA*1, *hsdR*17 (*r*K⁻ , *m*K⁺), *phoA*, *supE*44, λ⁻ , *thi*-1, *gyrA*96, *relA*1	Tsingke, China
E. coli W3110	*Escherichia coli* subsp. W3110	Laboratory stored
B. subtilis	*Bacillus subtilis* subsp. 168	Laboratory stored
C. glutamicum	*Corynebacterium glutamicum* subsp. ATCC 13032	Laboratory stored
P. putida	*Pseudomonas putida* subsp. KT2400	Laboratory stored
P. aeruginosa	*Pseudomonas aeruginosa subsp*. PAO1	Laboratory stored
DPA11A	*E.coli* W3110 Trc-*lpd-panE-panB*-ilvC /ΔavtAΔilvAΔglk/ilvG[a]/ilvE[b]/coaA[c]/ilvA*	Laboratory stored
DPN1	DPA11A Δ*panC*	This study
DPN0-2	DPA11A Δ*poxB*	This study
DPN0-3	DPA11A Δ*ppsA*	This study
DPN0-4	DPA11A Δ*ldhA*	This study
DPN0-5	DPA11A Δ*pflB*	This study
DPN0-6	DPA11A Δ*yfbQ*	This study
DPN0-7	DPA11A Δ*aroG*	This study
DPN2	DPA11A Δ*panC* Δ*poxB*	This study
DPN3	DPA11AΔ*panC* Δ*poxB* Δ*yfbQ*	This study
DPN4	DPA11AΔ*panC* Δ*poxB* Δ*yfbQ* Δ*pflB*	This study
DPN5	DPA11AΔ*panC* Δ*poxB* Δ*yfbQ* Δ*pflB* Δ*ppsA*	This study
DPN6	DPA11AΔ*panC* Δ*poxB* Δ*yfbQ* Δ*pflB* Δ*ppsA* Δ*ldhA*	This study
DPN7	DPA11AΔ*panC* Δ*poxB* Δ*yfbQ* Δ*pflB* Δ*ppsA* Δ*ldhA* Δ*fumB*::*alsS*$^{B.subtilis}$-*ilvC-ilvD*	This study
DPN8	DPA11AΔ*panC* Δ*poxB* Δ*yfbQ* Δ*pflB* Δ*ppsA* Δ*ldhA* Δ*fumB*::*alsS*$^{B.subtilis}$-*ilvC-ilvD*Δ*yjhE*::*alsS*$^{B.subtilis}$	This study
DPN8-1	DPA11AΔ*panC* Δ*poxB* Δ*yfbQ* Δ*pflB* Δ*ppsA* Δ*ldhA* Δ*fumB*::*alsS*$^{B.subtilis}$-*ilvC-ilvD*Δ*yjhE*::*alsS*$^{B.subtilis}$ Δ*rpnD*::*alsS*$^{B.subtilis}$	This study
DPN8-2	DPA11AΔ*panC* Δ*poxB* Δ*yfbQ* Δ*pflB* Δ*ppsA* Δ*ldhA* Δ*fumB*::*alsS*$^{B.subtilis}$-*ilvC-ilvD* Δ*yjhE*::*alsS*$^{B.subtilis}$ Δ*tdcD-tdcE*::*serA*$^{B.subtilis}$-*glyA*$^{B.subtilis}$	This study
DPN9	DPA11AΔ*panC* Δ*poxB* Δ*yfbQ* Δ*pflB* Δ*ppsA* Δ*ldhA* Δ*fumB*::*alsS*$^{B.subtilis}$-*ilvC-ilvD*Δ*yjhE*::*alsS*$^{B.subtilis}$Δ*rpnD*:: *panB*$^{C. glutamicum}$	This study
DPN10	DPA11AΔ*panC* Δ*poxB* Δ*yfbQ* Δ*pflB* Δ*ppsA* Δ*ldhA* Δ*fumB*::*alsS*$^{B.subtilis}$-*ilvC-ilvD*Δ*yjhE*::*alsS*$^{B.subtilis}$Δ*rpnD*::	This study

	panB$^{C.\ glutamicum}$ Δ*ppsA*::*panB*$^{C.\ glutamicum}$	
DPN11	DPA11AΔ*panC* Δ*poxB* Δ*yfbQ* Δ*pflB* Δ*ppsA* Δ*ldhA* ΔfumB::alsS$^{B.subtilis}$-*ilvC-ilvD*Δ*yjhE*::*alsS*$^{B.subtilis}$Δ*rpnD*::*panB*$^{C.glutamicum}$Δ*ppsA*::*panB*$^{C.glutamicum}$Δ*ydeU*::*panB*$^{B.subtilis}$	This study
DPN12	DPA11AΔ*panC* Δ*poxB* Δ*yfbQ* Δ*pflB* Δ*ppsA* Δ*ldhA* ΔfumB::*alsS*$^{B.subtilis}$-*ilvC-ilvD*Δ*yjhE*::*alsS*$^{B.subtilis}$Δ*rpnD*::*panB*$^{C.glutamicum}$Δ*ppsA*::*panB*$^{C.glutamicum}$Δ*ydeU*::*panB*$^{B.subtilis}$ Δ*yjiT*::*panB*$^{B.subtilis}$	This study
DPN12-1	DPA11AΔ*panC* Δ*poxB* Δ*yfbQ* Δ*pflB* Δ*ppsA* Δ*ldhA* Δ*fumB*::*alsS*$^{B.subtilis}$-*ilvC-ilvD*Δ*yjhE*::*alsS*$^{B.subtilis}$Δ*rpnD*::*panB*$^{C.glutamicum}$Δ*ppsA*::*panB*$^{C.glutamicum}$Δ*ydeU*::*panB*$^{B.subtilis}$ Δ*yjiT*::*panB*$^{B.subtilis}$Δ*yeiG*::*zwf*	This study
DPN12-2	DPA11AΔ*panC* Δ*poxB* Δ*yfbQ* Δ*pflB* Δ*ppsA* Δ*ldhA* Δ*fumB*::*alsS*$^{B.subtilis}$-*ilvC-ilvD*Δ*yjhE*::*alsS*$^{B.subtilis}$Δ*rpnD*::*panB*$^{C.glutamicum}$Δ*ppsA*::*panB*$^{C.glutamicum}$Δ*ydeU*::*panB*$^{B.subtilis}$ Δ*yjiT*::*panB*$^{B.subtilis}$ Δ*yeiG*::*gnd*	This study
DPN12-3	DPA11AΔ*panC* Δ*poxB* Δ*yfbQ* Δ*pflB* Δ*ppsA* Δ*ldhA* Δ*fumB*::*alsS*$^{B.subtilis}$-*ilvC-ilvD*Δ*yjhE*::*alsS*$^{B.subtilis}$ Δ*rpnD*::*panB*$^{C.glutamicum}$Δ*ppsA*::*panB*$^{C.glutamicum}$Δ*ydeU*::*panB*$^{B.subtilis}$ Δ*yjiT*::*panB*$^{B.subtilis}$ Δ*yeiG*::*pntAB*	This study
DPN12-4	DPA11AΔ*panC* Δ*poxB* Δ*yfbQ* Δ*pflB* Δ*ppsA* Δ*ldhA* Δ*fumB*::*alsS*$^{B.subtilis}$-*ilvC-ilvD*Δ*yjhE*::*alsS*$^{B.subtilis}$ Δ*rpnD*::*panB*$^{C.glutamicum}$ Δ*ppsA*::*panB*$^{C.glutamicum}$Δ*ydeU*::*panB*$^{B.subtilis}$ Δ*yjiT*::*panB*$^{B.subtilis}$Δ*yahk*	This study
DPN12-5	DPA11AΔ*panC* Δ*poxB* Δ*yfbQ* Δ*pflB* Δ*ppsA* Δ*ldhA* Δ*fumB*::*alsS*$^{B.subtilis}$-*ilvC-ilvD*Δ*yjhE*::*alsS*$^{B.subtilis}$Δ*rpnD*::*panB*$^{C.glutamicum}$Δ*ppsA*::*panB*$^{C.glutamicum}$Δ*ydeU*::*panB*$^{B.subtilis}$ Δ*yjiT*::*panB*$^{B.subtilis}$ Δ*stha*	This study
DPN13	DPA11AΔ*panC* Δ*poxB* Δ*yfbQ* Δ*pflB* Δ*ppsA* Δ*ldhA* ΔfumB::*alsS*$^{B.subtilis}$-*ilvC-ilvD*Δ*yjhE*::*alsS*$^{B.subtilis}$ Δ*rpnD*::*panB*$^{C.glutamicum}$ Δ*ppsA*::*panB*$^{C.glutamicum}$Δ*ydeU*::*panB*$^{B.subtilis}$ Δ*yjiT*::*panB*$^{B.subtilis}$Δ*pgi*	This study
DPN13-1	DPA11AΔ*panC* Δ*poxB* Δ*yfbQ* Δ*pflB* Δ*ppsA* Δ*ldhA* ΔfumB::*alsS*$^{B.subtilis}$-*ilvC-ilvD*Δ*yjhE*::*alsS*$^{B.subtilis}$Δ*rpnD*::*panB*$^{C.glutamicum}$ Δ*ppsA*::*panB*$^{C.glutamicum}$Δ*ydeU*::*panB*$^{B.subtilis}$ Δ*yjiT*::*panB*$^{B.subtilis}$Δ*pgi* Δ*yeiG*::*zwf*	This study
DPN13-2	DPA11AΔ*panC* Δ*poxB* Δ*yfbQ* Δ*pflB* Δ*ppsA* Δ*ldhA* ΔfumB::*alsS*$^{B.subtilis}$-*ilvC-ilvD*Δ*yjhE*::*alsS*$^{B.subtilis}$Δ*rpnD*::*panB*$^{C.glutamicum}$Δ*ppsA*::*panB*$^{C.glutamicum}$Δ*ydeU*::*panB*$^{B.subtilis}$ Δ*yjiT*::*panB*$^{B.subtilis}$Δ*pgi* Δ*yeiG*::*gnd*	This study
DPN13-3	DPA11AΔ*panC* Δ*poxB* Δ*yfbQ* Δ*pflB* Δ*ppsA* Δ*ldhA* ΔfumB::*alsS*$^{B.subtilis}$-*ilvC-ilvD*Δ*yjhE*::*alsS*$^{B.subtilis}$Δ*rpnD*::*panB*$^{C.glutamicum}$Δ*ppsA*::*panB*$^{C.glutamicum}$Δ*ydeU*::*panB*$^{B.subtilis}$ Δ*yjiT*::*panB*$^{B.subtilis}$Δ*pgi* Δ*yeiG*::*pntAB*	This study

Plasmids	Characteristics	Sources
pCas	epA101 (Ts), KanR, Pcas-as9ParaB-Red *lacI*q Ptrc-sgRNA-pMB1	Laboratory stored
pTarget	pMB1 aadA PpJ23119-sgRNA-(target gene)	Laboratory stored
pTrc99a (K)	pTrc99A (Replace AmpR with KanR)	Laboratory stored
pTrc99a-*panC*$^{E.coli}$	pTrc99a (K), KanR, containing the *panC* gene from *E. coli* W3110	This study
pTrc99a-*panC*$^{B.subtilis}$	pTrc99a(K), KanR, containing the *panC* gene from *B. subtilis* 168	This study
pTrc99a-*panC*$^{P.putida}$	pTrc99a(K), KanR, containing the *panC* gene from *P. putida* KT2400	This study
pTrc99a-*panC*$^{P. aeruginosa}$	pTrc99a(K), KanR, containing the *panC* gene from *P. aeruginosa* PAO1	This study
pTrc99a-*panC*$^{C.glutamicum}$	pTrc99a(K), KanR, containing the *panC* gene from *C. glutamicum* ATCC 13032	This study
pTrc99a-*panC*(P28A)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(P28A) gene	This study
pTrc99a-*panC*(M30A)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(M30A) gene	This study
pTrc99a-*panC*(L40A)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(L40A) gene	This study
pTrc99a-*panC*(N58A)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(N58A) gene	This study
pTrc99a-*panC*(N61A)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(N61A) gene	This study
pTrc99a-*panC*(F62A)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(F62A) gene	This study
pTrc99a-*panC*(R123A)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123A) gene	This study
pTrc99a-*panC*(H126A)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(H126A) gene	This study
pTrc99a-*panC*(I133A)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(I133A) gene	This study
pTrc99a-*panC*(V134A)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(V134A) gene	This study
pTrc99a-*panC*(L137A)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(L137A) gene	This study
pTrc99a-*panC*(K151A)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(K151A) gene	This study
pTrc99a-*panC*(Q155A)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(Q155A) gene	This study

pTrc99a-*panC*(M178A)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(M178A) gene	This study
pTrc99a-*panC*(L186A)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(L186A) gene	This study
pTrc99a-*panC*(R189A)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R189A) gene	This study
pTrc99a-*panC*(F62G)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(F62G) gene	This study
pTrc99a-*panC*(F62V)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(F62V) gene	This study
pTrc99a-*panC*(F62L)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(F62L) gene	This study
pTrc99a-*panC*(F62I)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(F62I) gene	This study
pTrc99a-*panC*(F62P)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(F62P) gene	This study
pTrc99a-*panC*(F62S)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(F62S) gene	This study
pTrc99a-*panC*(F62T)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(F62T) gene	This study
pTrc99a-*panC*(F62H)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(F62H) gene	This study
pTrc99a-*panC*(F62W)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(F62W) gene	This study
pTrc99a-*panC*(F62C)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(F62C) gene	This study
pTrc99a-*panC*(F62D)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(F62D) gene	This study
pTrc99a-*panC*(F62E)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(F62E) gene	This study
pTrc99a-*panC*(F62K)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(F62K) gene	This study
pTrc99a-*panC*(F62Y)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(F62Y) gene	This study
pTrc99a-*panC*(F62M)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(F62M) gene	This study
pTrc99a-*panC*(F62N)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(F62N) gene	This study
pTrc99a-*panC*(F62Q)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(F62Q) gene	This study
pTrc99a-*panC*(F62R)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(F62Q) gene	This study
pTrc99a-*panC*(R123G)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123G) gene	This study

pTrc99a-*panC*(R123V)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123V) gene	This study
pTrc99a-*panC*(R123L)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123L) gene	This study
pTrc99a-*panC*(R123I)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123I) gene	This study
pTrc99a-*panC*(R123P)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123P) gene	This study
pTrc99a-*panC*(R123S)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123S) gene	This study
pTrc99a-*panC*(R123T)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123T) gene	This study
pTrc99a-*panC*(R123H)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123H) gene	This study
pTrc99a-*panC*(R123W)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123W) gene	This study
pTrc99a-*panC*(R123C)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123C) gene	This study
pTrc99a-*panC*(R123D)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123D) gene	This study
pTrc99a-*panC*(R123E)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123E) gene	This study
pTrc99a-*panC*(R123K)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123K) gene	This study
pTrc99a-*panC*(R123Y)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123Y) gene	This study
pTrc99a-*panC*(R123M)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123M) gene	This study
pTrc99a-*panC*(R123N)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123N) gene	This study
pTrc99a-*panC*(R123Q)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123Q) gene	This study
pTrc99a-*panC*(R123G)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123G) gene	This study
pTrc99a-*panC*(R123G)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123G) gene	This study
pTrc99a-*panC*(R123F)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123F) gene	This study
pTrc99a-*panC*(R189G)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R189G) gene	This study
pTrc99a-*panC*(R189V)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R189V) gene	This study
pTrc99a-*panC*(R189L)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R189L) gene	This study

pTrc99a-*panC*(R189I)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R189I) gene	This study
pTrc99a-*panC*(R189P)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R189P) gene	This study
pTrc99a-*panC*(R189S)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R189S) gene	This study
pTrc99a-*panC*(R189T)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R189T) gene	This study
pTrc99a-*panC*(R189H)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R189H) gene	This study
pTrc99a-*panC*(R189W)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R189W) gene	This study
pTrc99a-*panC*(R189C)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R189C) gene	This study
pTrc99a-*panC*(R189D)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R189D) gene	This study
pTrc99a-*panC*(R189E)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R189E) gene	This study
pTrc99a-*panC*(R189K)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R189K) gene	This study
pTrc99a-*panC*(R189Y)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R189Y) gene	This study
pTrc99a-*panC*(R189M)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R189M) gene	This study
pTrc99a-*panC*(R189N)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R189N) gene	This study
pTrc99a-*panC*(R189Q)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R189Q) gene	This study
pTrc99a-*panC*(R189F)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R189Q) gene	This study
pTrc99a-*panC*(R123Q/R189A)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123Q/R189A) gene	This study
pTrc99a-*panC*(R123Q/R189I)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123Q/R189I) gene	This study
pTrc99a-*panC*(R123A/R189A)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123A/R189A) gene	This study
pTrc99a-*panC*(R123A/R189I)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123A/R189I) gene	This study
pTrc99a-*panC*(R123N/R189I)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123N/R189I) gene	This study
pTrc99a-*panC*(R123Q/R189I)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123Q/R189I) gene	This study
pTrc99a-*panC*(R123Q/R189I/F	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123Q/R189I/F62A) gene	This study

62A)		
pTrc99a-M3	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123Q/R189I/F62I) gene	This study
pTrc99a-*panC*(R123Q/R189I/F62M)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123Q/R189I/F62I) gene	This study
pTrc99a-*panC*(R123A/R189I/F62I)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123Q/R189I/F62I) gene	This study
pTrc99a-*panC*(R123A/R189I/F62M)	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123Q/R189I/F62I) gene	This study
pTrc99a-M3-*ilvBN*	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123Q/R189I/F62I), *ilvBN* genes	This study
pTrc99a-M3-*alsS*	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123Q/R189I/F62I), *alsS* genes	This study
pTrc99a-M3-*ilvCD*	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123Q/R189I/F62I), *ilvCD* genes	This study
pTrc99a-M3-*panB*$^{E.coli}$	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123Q/R189I/F62I), *panB*$^{E.coli}$ genes	This study
pTrc99a-M3-*panB*$^{C.glutamicum}$	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123Q/R189I/F62I), *panB*$^{C.glutamicum}$ genes	This study
pTrc99a-M3-*panB*$^{P.putida}$	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123Q/R189I/F62I), *panB*$^{P.putida}$ genes	This study
pTrc99a-M3-*panB*$^{B.subtilis}$	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123Q/R189I/F62I), *panB*$^{B.subtilis}$genes	This study
pTrc99a-M3-*panE*	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123Q/R189I/F62I), *panE* genes	This study
pTrc99a-CBS	pTrc99a(K), KanR, containing the *panC*$^{E.coli}$(R123Q/R189I/F62I), *panB*$^{B.subtilis}$ and *ilvBN* genes	This study
pET28a-*panC*(WT)	pET28a(+), KanR, *panC*$^{E.coli}$ expression, T7 promoter	This study
pET28a-*panC*(R123Q/R189I)	pET28a(+), KanR, *panC*$^{E.coli}$(R123A/R189I) expression, T7promoter	This study
pET28a -*panC*(R123Q/R189I/F62I)	pET28a(+), KanR, *panC*$^{E.coli}$(R123A/R189I/F62I) expression, T7 promoter	This study

Table S2. Primers used in this study

Primer	Sequence (5'—3')
zwf-f	TTTGCCTCGCCAAGCCCTTTG
zwf-R	CACTGCTGCATTCAGCCGTCTC
pgl-F	GGAAGATGGCAGCGTGTTGAGT
pgl-R	CCCGGCGGCAATCAGATACTTG
gnd-F	TGCTTGCGGACCAGAGAGAACT
gnd-R	CGCTGTCGCTGATTACCGAGTC
rpe-F	GCTGGTCGAAGATTGCCGAACC
rpe-R	GGTGGCGTGAAGGTGAACAACA
tkta-F	TGAGGTGCAGCAGGCTGTAGAT
tkta-R	ACAACCCGCAGAATCCGTCCT
tktb-F	AACGGACCGACGGCACTGAT
tktb-R	GCACATAACCGCCACGAGCAA
16S-F	CCTTACGACCAGGGCTACAC
16S-R	CAATCCGGACTACGACGC

For RT-qPCR

Table S3. By-product and pyruvate production of the engineered strains in shake flask cultures with the LB medium

Strains	Pyruvate (g L^{-1})	Formate (g L^{-1})	Acetate (g L^{-1})	L-alanine (g L^{-1})	L-lactate(g L^{-1})
DPA11A	1.64± 0.01	0.50 ± 0.05	0.62± 0.03	0.70 ± 0.03	0.95 ± 0.04
DPN1	1.60 ± 0.02	0.51 ± 0.06	0.63 ± 0.11	0.73 ± 0.09	0.93 ± 0.05
DPN2	1.67 ± 0.05	0.54 ± 0.03	0.23 ± 0.02	0.72 ± 0.12	0.87 ± 0.05
DPN3	1.70 ± 0.10	0.43 ± 0.02	0.25 ± 0.02	0.52 ± 0.02	0.92 ± 0.06
DPN4	1.69 ± 0.09	0.12 ± 0.01	0.21 ± 0.08	0.43 ± 0.03	0.85 ± 0.04
DPN5	1.92 ± 0.08	0.23 ± 0.06	0.31 ± 0.07	0.36 ± 0.04	0.95 ± 0.03
DPN6	2.01 ± 0.04	0.22 ± 0.06	0.32 ± 0.07	0.33 ± 0.04	0.55 ± 0.03
DPN8	1.32 ± 0.14	0.14 ± 0.05	0.34 ± 0.09	0.23 ± 0.06	0.45 ± 0.09
DPN12	0.75 ± 0.12	0.13 ± 0.05	0.42 ± 0.07	0.3 3± 0.07	0.51 ± 0.10

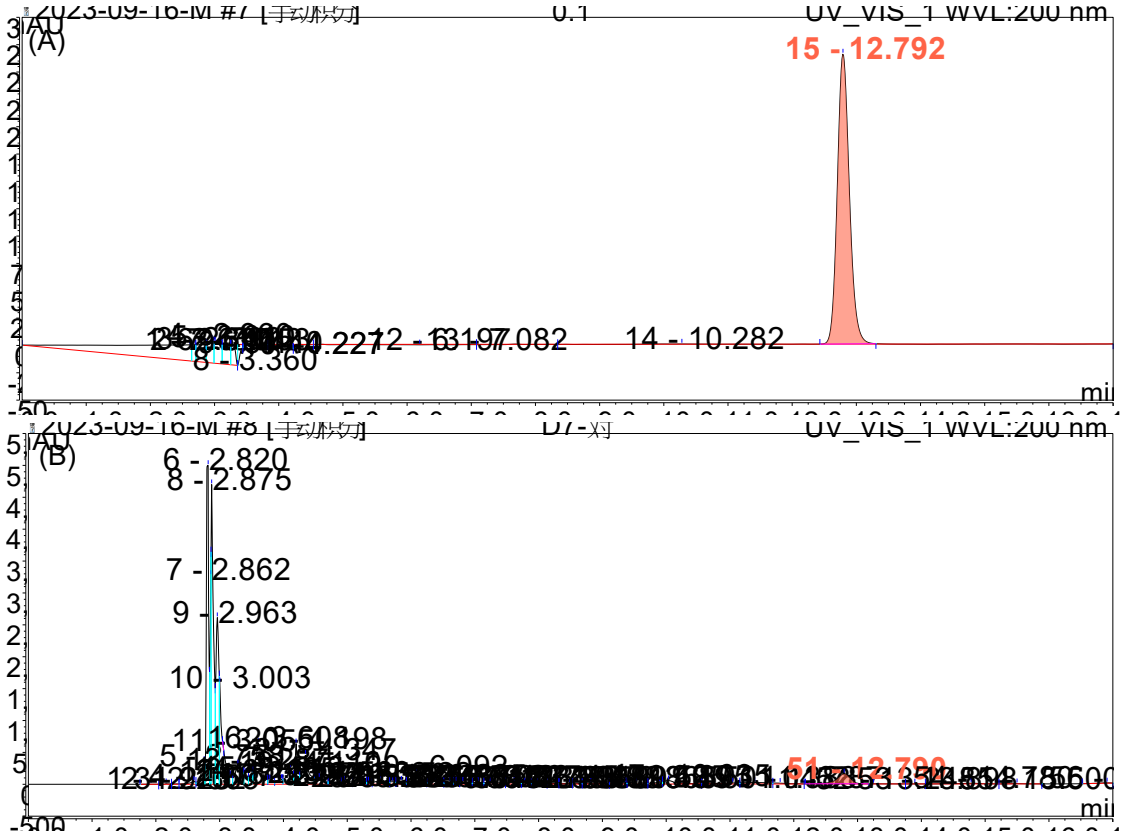

Fig. S1 HPLC analysis of D-panthenol standard (A) and fermentation broth sample (B).

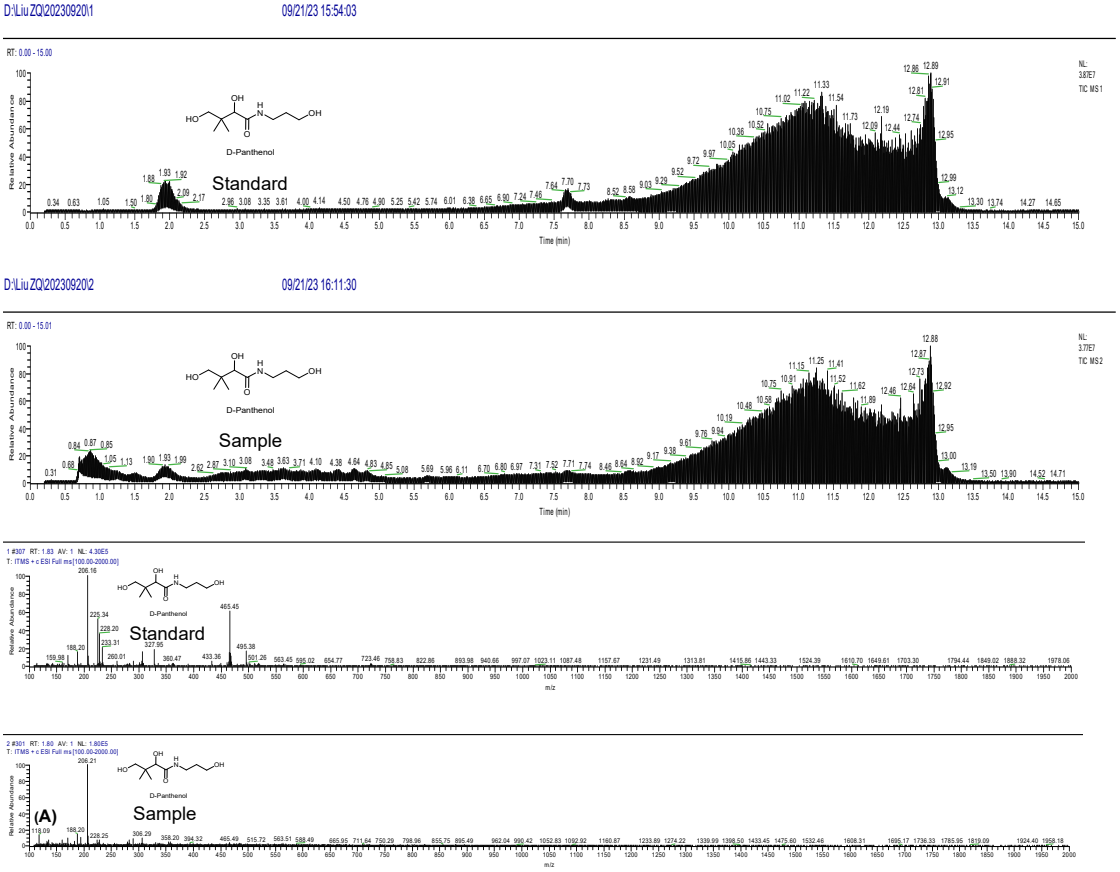

Fig. S2 Verification of D-panthenol from DPA1-ptrc99a-panC fermentation broth using LC-MS analysis. (A-B) HPLC analysis of standard and sample. (C-D) LC-MS analysis of standard substance and sample. The LC-MS and mass spectra were obtained using Thermo LTQ XLTM, equipped with a BEH C18 column (100 mm × 2.1 mm, 1.7 μm) and an ESI source in negative ion mode used for MS analysis. The flow rate was set at 0.4 mL/min, and the mo-bile phases were (A) 0.1% of formic acid in water and (B) 0.1% of formic acid in acetonitrile. While gradient elution was performed from 2% B to 98% B in 15 min, and all chromatographic separations were carried out using same gradient elution procedure.

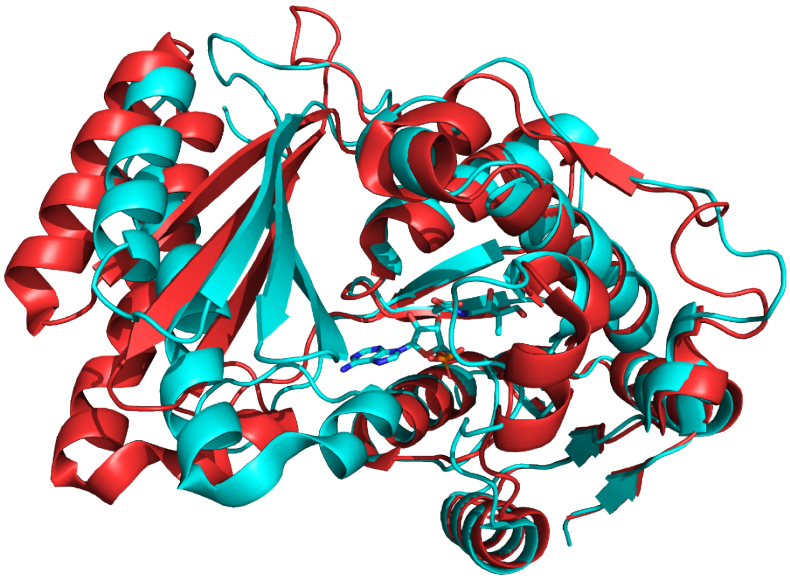

Fig. S3 Aligned X-ray structures of AMP and vitamin B5 *Campylobacter jejuni* PS (blue color, PDB: 3UY4) and *E. coli* PS (red color).

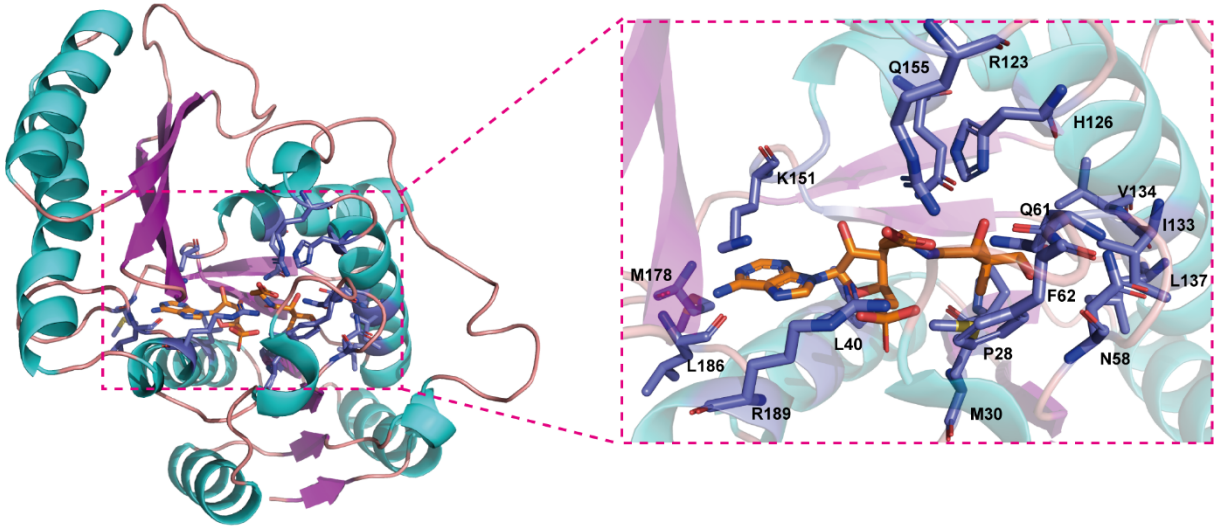

Fig. S4 Screening for mutation sites using alanine scanning strategy. Select sites 28, 30, 40, 58, 61, 62, 123, 126, 126, 133, 134, 137, 137, 115, 155, 178, 186, 189 as mutation sites.

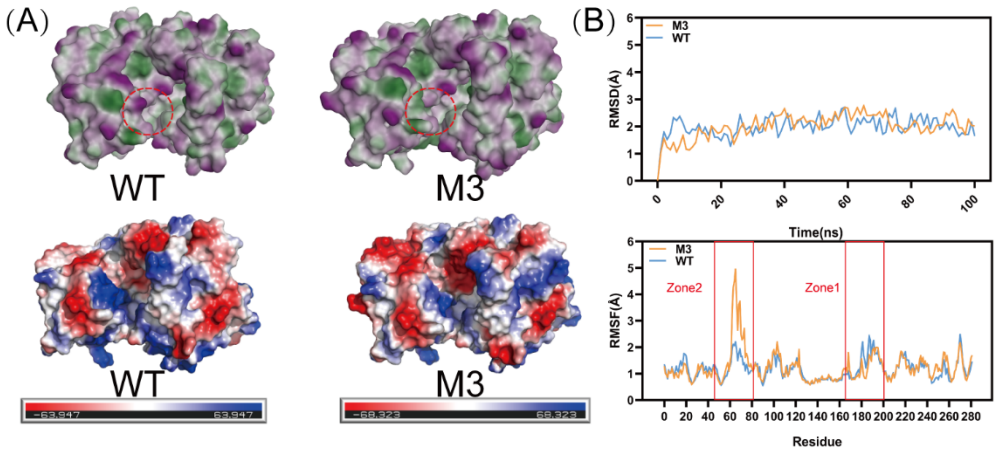

Fig. S5 (A)PS electrostatic potential energy diagrams and hydrophobic interaction diagrams. The darker the purple color, the more hydrophilic, and the darker the green color, the more hydrophobic. (B) 100 ns molecular dynamic (MD) analysis of wild type PS and variant M3.

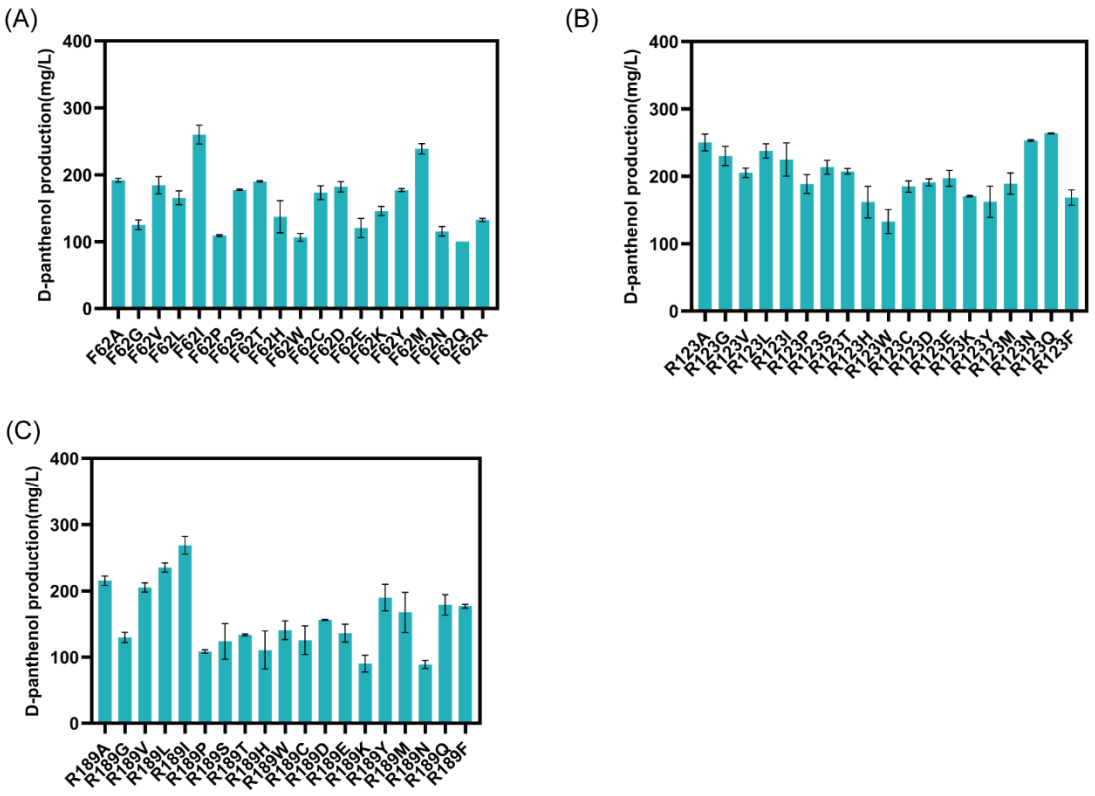

Fig. S6 The effect of saturation mutation on D-panthenol production.

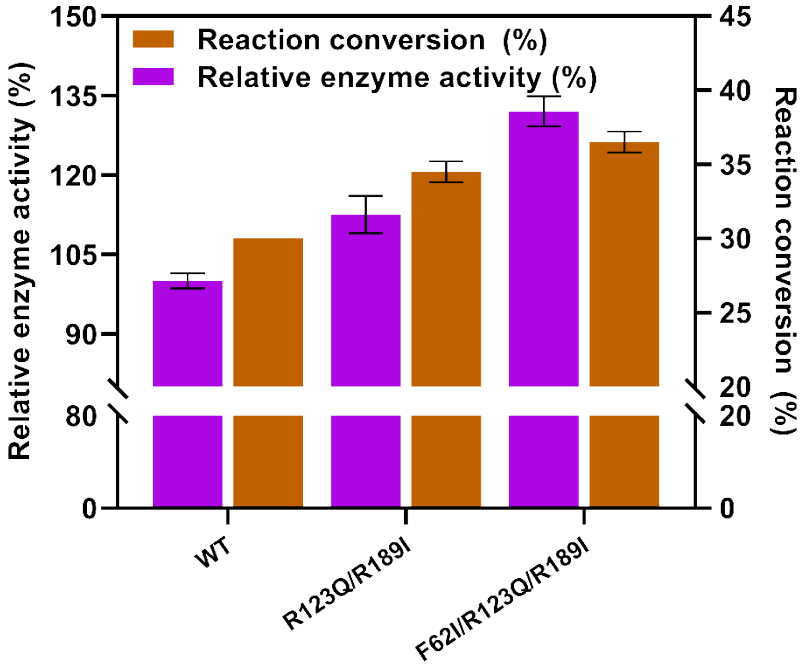

Fig. S7 Relative activity of the cell-free extracts of the wild-type (WT) PS and its variants evolved in protein engineering. The plasmids pET28a-*panC* (WT), pET28a- *panC* (R1231/R189I) and pET28a- *panC* (F62I/R1231/R189I) were transformed into *E. coli* BL21 (DE3) for expression. They were incubated in 100 mL LB medium containing 100 ug/ml for 2 to 3 hours in a shaker at 37°C. Protein expression was induced by adding 100 uL of 0.1 M IPTG to the shake flask for 12 h. The temperature of the shaker was adjusted to 28°C. Cells were collected by centrifugation at 4°C, washed twice with 50 mM Tris-HCl (pH 7.0) and resuspended in buffer containing 50 mM Tris-HCl (pH 7.0). Recombinant proteins were affinity purified by a Ni-NTA column. The purity of the purified enzyme was tested by SDS-PAGE, and the protein concentration was measured by BCA protein assay kit (Vazyme, Nanjing, China). The reaction mixture (1.1 mL) consisted of $PS_{E.\ coli}$ enzyme [7 Mm], (R)-pantoate [25 mM], 3-aminopropanol [25 mM], ATP [5 mM], $MgCl_2$ [10 mM] in Tris-HCl buffer [50 mM, pH 7]. The reaction mixture was incubated at 30 °C for 24 h. The enzyme was inactivated by heating at 70 °C for 5 min and the precipitated enzyme was removed by filtration.

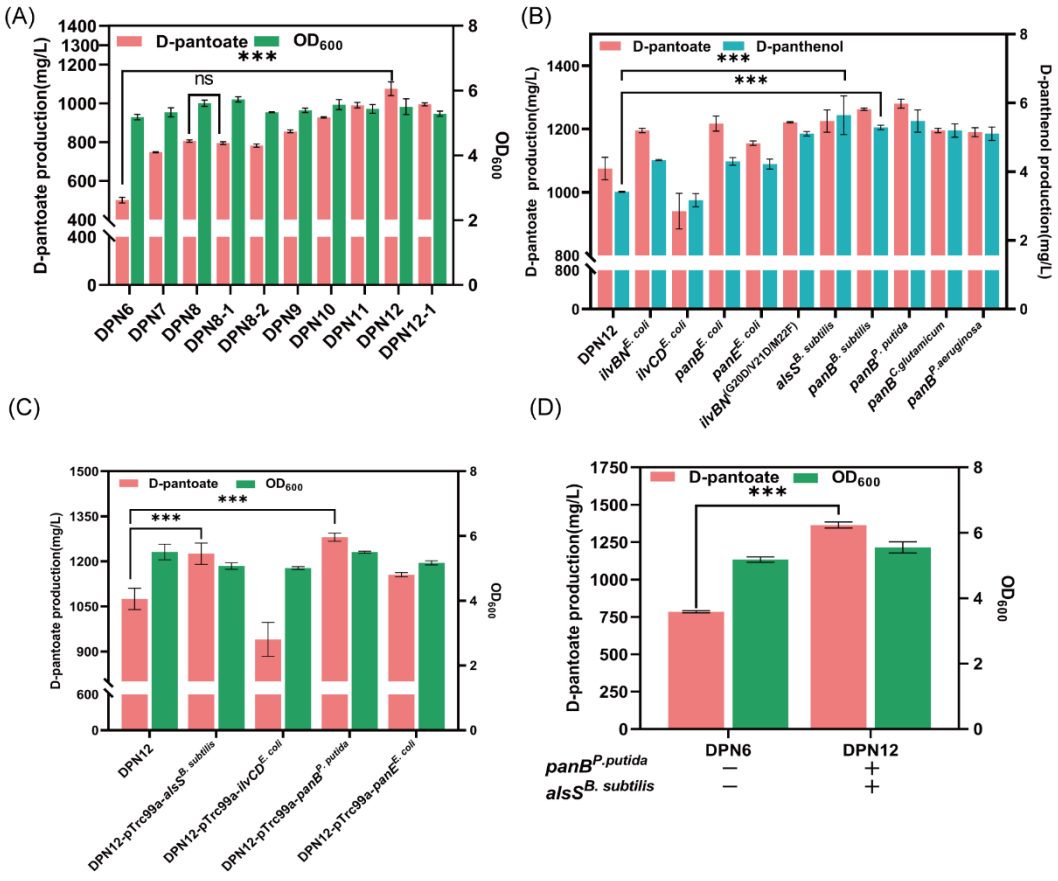

Fig. S8 Enhancing and screening of D-pantoate pathway genes. (A) Effects of increasing D-Pantoate pathway genes in the genome on D-Pantoate production. (B) Screening for plasmid overexpression of D-pantoate pathway genes. (C) Effect of overexpression of optimal genes on pantothenic acid production and biomass. (D) Effect of main pathway enhancement on the production capacity of strain D-pantothenic acid

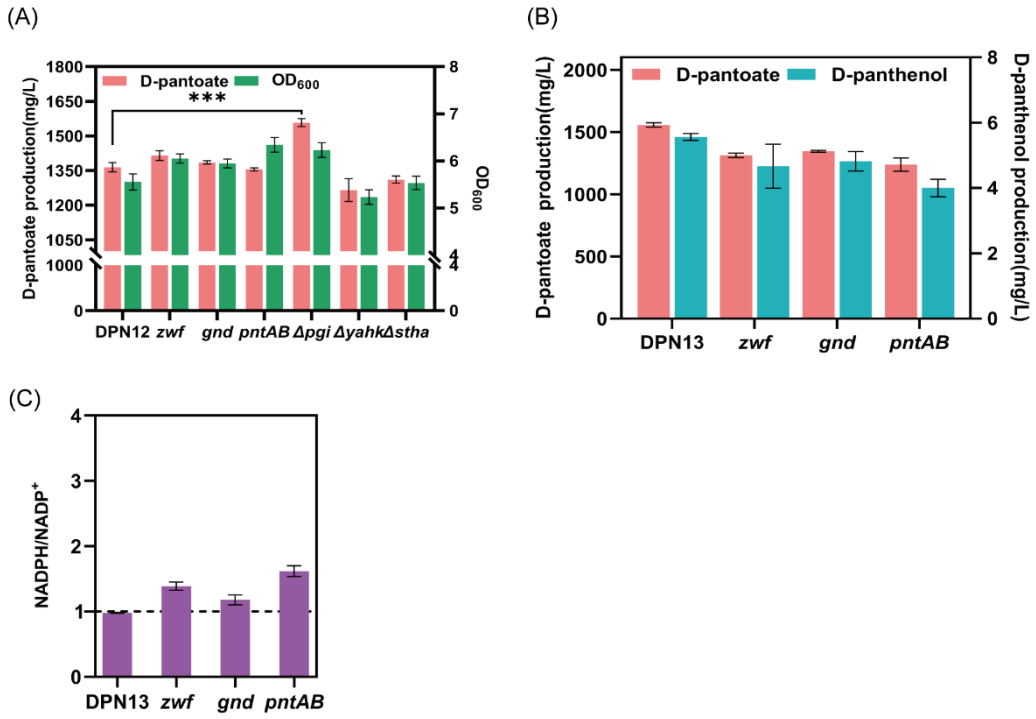

Fig. S9 A fine ratio of NADPH and NADP⁺ in cells promotes cell growth and D-pantoate synthesis. (A) Effects of overexpression of NADPH-generating genes and knockout-depleting genes on D-pantoate production. (B) Effect of continued overexpression of NADPH-producing genes on D-pantothenic acid and D-panthenol production (C) Differently engineered strains intracellular NADPH/NADP+ ratio.

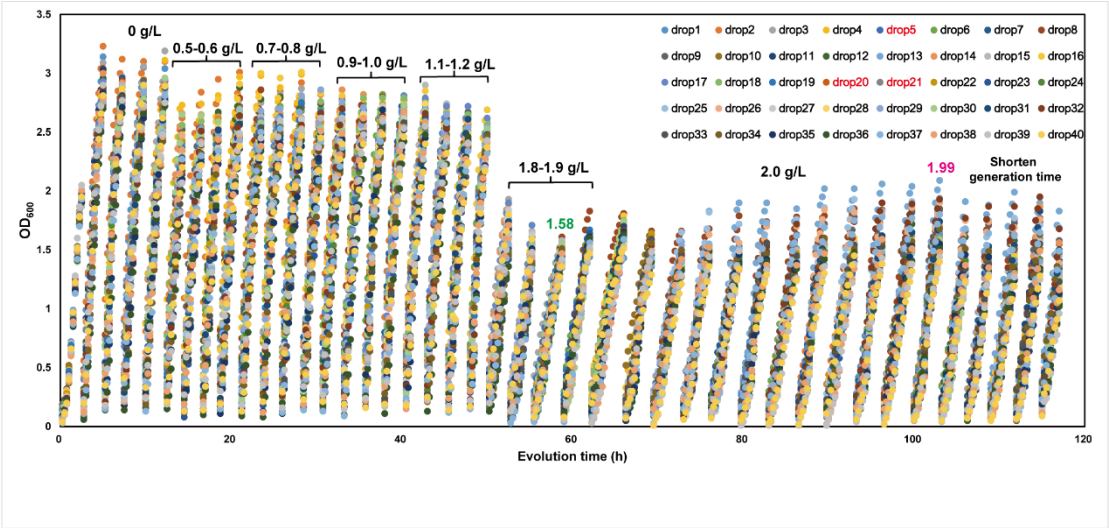

Fig. S10 The strain was domesticated for good tolerance of 3-aminopropanol by microbial microdroplet culture (MMC). The passaging time was 4 h in the first 37 bands and 3.5 h in the next 5 bands. Each droplet can be regarded as a shake flask in the traditional shake flask adaptation evolution. (Drop losses in drop5, drop20 and drop21 during passaging are shown in red).

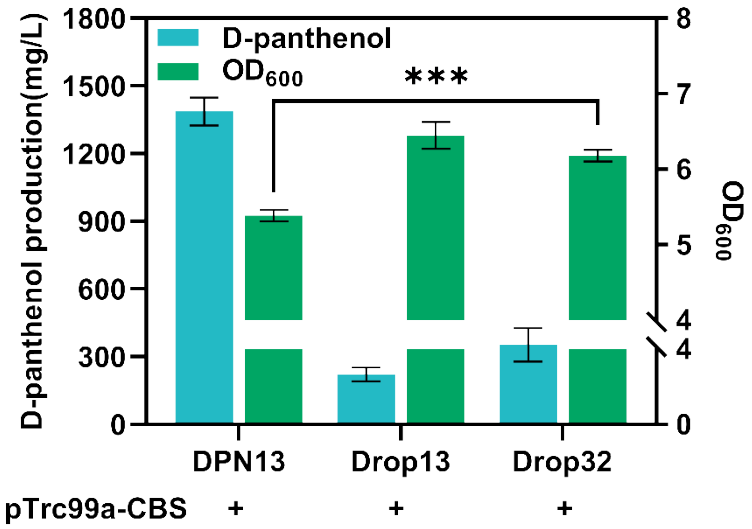

Fig. S11 D-panthenol and biomass production by shake flask fermentations of the adapted evolved strain versus the control strain DPN13.