Article

Identification and Combinatorial Overexpression of Key Genes for Enhancing ε-poly-L-lysine Biosynthesis in *Streptomyces albulus*

Hongjian Zhang [†], Hao Yang [†], Chongyang Zhang, Daojun Zhu, Liang Wang, Jianhua Zhang and Xusheng Chen *

Key Laboratory of Industrial Biotechnology, Ministry of Education, School of Biotechnology, Jiangnan University, Wuxi 214122, China; hjzhang@jiangnan.edu.cn (H.Z.); 18752112690@163.com (H.Y.); zhangchong-yang@cathaybiotech.com (C.Z.); zhu_daojun@163.com (D.Z.); wangl@jiangnan.edu.cn (L.W.); jhzh882@163.com (J.Z.)

* Correspondence: chenxs@jiangnan.edu.cn

† These authors contributed equally to this work.

Table S1. Primers used in this study.

Primers	Sequence (5'-3')
Primers for gene overexpression	
ppc-F	ggttggtaggatccacatatgCCCCGCCAAGACGTGCTC
ppc -R	ctatgacatgattacgaattcTCAGCCGGTGTTGCGCAG
zwf-F	ggttggtaggatccacatatgGCCGTCGGGCCTGGTCAT
zwf-R	ctatgacatgattacgaattcTCATGGCCGACGCCAGCT
dapA-F	ggttggtaggatccacatatgCTTGACCGGGCACCCGCG
dapA-R	ctatgacatgattacgaattcTCAGCCGGTGGGGCGCAG
lysA-F	ggttggtaggatccacatatgTCCCGGAACAACCAGCTACG
lysA-R	ctatgacatgattacgaattcTCAGAGGCCGATGTCGCG
pyc-F	ggttggtaggatccacatatgATGGTCTCGTCACCCGGC
pyc-R	cgcggccgcggatcctctagaTCAGGCGAGTTGGACGAGAA
Primers for validation of gene overexpression	
p1B139-F	TTGCGCCCGATGCTAGTCG
p1B139-R	GCACGACAGGTTTCCCGACTG
O-*pyc*-F	GACTCCGTGGTCGGCTTCCT
O-*pyc*-R	ACCGCTACATACTCGCTCTGCTA
O-*pls*-F	CGCGATGCTGTTGTGGG
O-*pls*-R	AACCGTTCGGCGTCCTT
O-*ppc*-F	CAACAGCGAAGAGCCCTACCG
O-*ppc*-R	GCGACCGCCTTGACGAACA
Primers for qRT-PCR	
Q-ppc-F	GCCATCCCGTGGGTGTT
Q-ppc -R	AGGAAGTTGCGGAAGAAGTG
Q-zwf-F	AGTGGGAGGACGAGGACTT
Q-zwf-R	TTGCCCTGGACGAACC
Q-*dapA*-F	TGGCACGCTGATGATTGGT

Q-*dapA*-R	CTCCCTCACCCGACTGGTCT
Q-*lysA*-F	CCACTTGGGCTCCCAGATC
Q-*lysA*-R	AGGGTGATGCCGTGTTGC
Q-*pyc*-F	TCGAGGCGATGAAGATGGA
Q-*pyc*-R	GGCGAGTTGGACGAGAAGAT
Q-*pls*-F	TCACCTACCGCGACCTGTG
Q-*pls*-R	AGGACGGCGAGGATGGA

Table S2. Chemical reaction rate equations in *S. albulus* WG-608.

Reaction number	Reaction
Glycolytic pathway	
r1	1Glucose→1Glucose-6-P
r2	1Glucose-6-P→1Fructose-6-P
r3	1Fructose-6-P→1Fructose-1,6-BP
r4	1Fructose-1,6-BP→2Glyceraldehyde-3-P
r4	1Glyceraldehyde-3-P→1Glycerate-3-P
r6	1Glycerate-3-P→1Phosphoenolpyruvate
r7	1Phosphoenolpyruvate→1Pyruvate
Pentose phosphate pathway	
r14	1Glucose-6-P→1Ribulose-5-P +2NADPH
r15	1Ribulose-5-P→1Xylulose-5-P
r16	1Ribulose-5-P→1Ribose-5-P
r17	1Xylulose-5-P+1R5P→1Glyceraldehyde-3-P+1Sedoheptulose-7-P
r18	1Glyceraldehyde-3-P+1Sedoheptulose-7-P→1Erythrose-4-P+1Fructose-6-P
r19	1Fructose-6-P +1Glyceraldehyde-3-P→1Xylulose-5-P+1Erythrose-4-P
TCA Cycle	
r8	1Pyruvate→1Acetyl Coenzyme A
r9	1Acetyl Coenzyme A+1Oxaloacetate→1Isocitrate
r10	1Isocitrate→1α-ketoglutarate
r11	1α-ketoglutarate→1Fumarate
r12	1Fumarate→1Oxaloacetate
r13	1Phosphoenolpyruvate→1Oxaloacetate
Amino acid synthesis pathway	
r20	1Ribose-5-P+1Gln +1Asp→1His+1α-ketoglutarate+1Fumarate
r21	1Erythrose-4-P+2Phosphoenolpyruvate+1Glu +1 NADPH→1α-ketoglutarate+1Phe
r22	1Erythrose-4-P+2Phosphoenolpyruvate+1Glu+1 NADPH→1α-ketoglutarate+1Tyr
r23	1Pyruvate+1Glu→1α-ketoglutarate+ 1Ala
r24	2Pyruvate+1Glu+1NADPH→1Val+1α-ketoglutarate
r25	2Pyruvate+1Glu+1NADPH +1Acetyl Coenzyme A→1Leu+1α-ketoglutarate
r26	1α-ketoglutarate→1Glu
r27	1Glu→1Glu$_{ex}$

r28	$2 \text{ Glu} + 1\text{Asp} + 1 \text{ NADPH} \rightarrow 1\text{Arg} + 1\text{Fumarate} + 1\alpha\text{-ketoglutarate}$
r29	$1\text{Glu} + 2 \text{ NADPH} \rightarrow 1\text{Pro}$
r30	$1\text{Glycerate-3-P} + 1\text{Glu} \rightarrow 1\text{Ser} + 1\alpha\text{-ketoglutarate}$
r31	$1\text{Ser} \rightarrow 1\text{Ser}_{ex}$
r32	$1\text{Ser} + 4 \text{ NADPH} \rightarrow 1\text{Cys}$
r33	$1\text{Ser} \rightarrow 1\text{Gly}$
r34	$1\text{Oxaloacetate} + 1\text{Glu} \rightarrow 1\alpha\text{-ketoglutarate} + 1\text{Asp}$
r35	$1\text{Asp} \rightarrow 1\text{Asp}_{ex}$
r36	$1\text{Asp} + 1\text{Glu} + 1\text{Pyruvate} + 2\text{NADPH} \rightarrow 1\text{Lys} + 1\alpha\text{-ketoglutarate}$
r37	$1\text{Asp} + 2\text{NADPH} + 1\text{Cys} \rightarrow 1\text{Met} + 1\text{Pyruvate}$
r38	$1\text{Asp} + 2\text{NADPH} \rightarrow 1\text{Thr}$
r39	$1\text{Thr} \rightarrow 1\text{Thr}_{ex}$
r40	$1\text{Thr} + 1\text{Pyruvate} + 1\text{NADPH} + 1\text{Glu} \rightarrow 1\text{Ile} + 1\alpha\text{-ketoglutarate}$
r41	$1\text{Lys} \rightarrow 1\text{Lys}_{ex}$
	ε-PL synthesis pathway
r42	$30\text{Lys} \rightarrow 1\varepsilon\text{-PL}$
	Cellular substance composition
r43	$0.154 \text{ Glucose-6-P} + 0.19 \text{ Fructose-6-P} + 0.194 \text{ Glyceraldehyde-3-P} + 0.816 \text{ Ribose-5-P} + 0.308 \text{ Erythrose-4-P} + 1.353 \text{ Glycerate-3P} + 0.711 \text{ Phosphoenolpyruvate} + 3.06 \text{ Pyruvate} + 2.132 \text{ Acetyl-Coenzyme A} + 1.071\alpha\text{-ketoglutarate} + 1.923 \text{ Oxaloacetate} + 16.06 \text{ NADPH} \rightarrow \text{Biomass}$

Table S3. Reaction rate equation of metabolic node.

Metabolites	Reaction rate equations
Glucose-6-P	$r1 - r2 - r14 - 0.154r43 = 0$
Fructose-6-P	$r2 - r3 - r19 + r18 - 0.19r43 = 0$
Fructose-1,6-BP	$r3 - r4 = 0$
Glyceraldehyde-3-P	$r4 - r5 + r17 - r18 - r19 - 0.194r43 = 0$
Glycerate-3-P	$r5 - r6 - r30 - 1.353r43 = 0$
Phosphoenolpyruvate	$r6 - r7 - r13 - 2r21 - 2r22 - 0.711r43 = 0$
pyruvate	$r7 - r8 - r23 - 2r24 - 2r25 - r36 + r37 - r40 - 3.06r43 = 0$
Acetyl-Coenzyme A	$r8 - r9 - r25 - 2.132r43 = 0$
Isocitrate	$r9 - r10 = 0$
α-ketoglutarate	$r10 - r11 + r20 + r21 + r22 + r23 + r24 + r25 - r26 + r28 + r30 + r34 + r40 + r36 - 1.071r43 = 0$
Fumarate	$r11 - r12 + r20 + r28 = 0$
Oxaloacetate	$r12 + r13 - r9 - r34 - 1.923r43 = 0$
Ribulose-5-P	$r14 - r15 - r16 = 0$
Xylulose-5-P	$r15 - r17 + r19 = 0$
Ribose-5-P	$r16 - r20 - r17 - 0.816r43 = 0$
Sedoheptulose-7-P	$r17 - r18 = 0$
Erythrose-4-P	$r18 + r19 - r21 - r22 - 0.308r43 = 0$
Glu	$r34 - r21 - r22 - r23 - r24 - r25 - r27 - 2r28 - r29 - r30 - r34 - r36 - r40 = 0$
Ser	$r30 - r31 - r32 - r33 = 0$
Asp	$r34 - r20 - r28 - r35 - r37 - r38 - r36 = 0$
Thr	$r38 - r39 - r40 = 0$
Lys	$r36 - r41 - r42 = 0$
NADPH	$2r14 - r21 - r22 - r24 - r25 - r28 - 2r29 - 4r32 - 2r37 - 2r38 - r40 - 2r36 - 16.06r43 = 0$

Table S4. Change of metabolite concentration and metabolic flux.

Metabolites	Reaction number	*S. albulus* M-Z18		*S. albulus* WG-608	
		Metabolite concentration changes（g/L）	Metabolic flux	Metabolite concentration changes（g/L）	Metabolic flux
Glucose	r1	60.000	100.00	51.240	100.00
His	r20	0.003	0.01	0.003	0.01
Phe	r21	0.001	0.00	0.001	0.00
Tyr	r22	0.004	0.01	0.008	0.02
Ala	r23	0.011	0.04	0.018	0.07
Val	r24	0.006	0.01	0.007	0.02
Leu	r25	0.008	0.02	0.016	0.04
Glu	r27	0.006	0.01	0.001	0.00
Arg	r28	0.002	0.00	0.002	0.00
Pro	r29	-0.001	0.00	0.015	0.05
Ser	r31	0.002	0.01	0.000	0.00
Cys-s	r32	0.003	0.01	0.006	0.02
Gly	r33	0.002	0.01	0.001	0.00
Asp	r35	0.010	0.02	0.004	0.01
Met	r37	0.005	0.01	0.001	0.00
Thr	r39	0.003	0.01	-0.002	-0.01
Ile	r40	0.001	0.00	0.002	0.01
Lys	r41	0.018	0.04	-0.001	0.00
ε-PL	r42	4.285	0.36	4.711	0.46
Biomass	r43	13.925	4.18	13.875	4.87
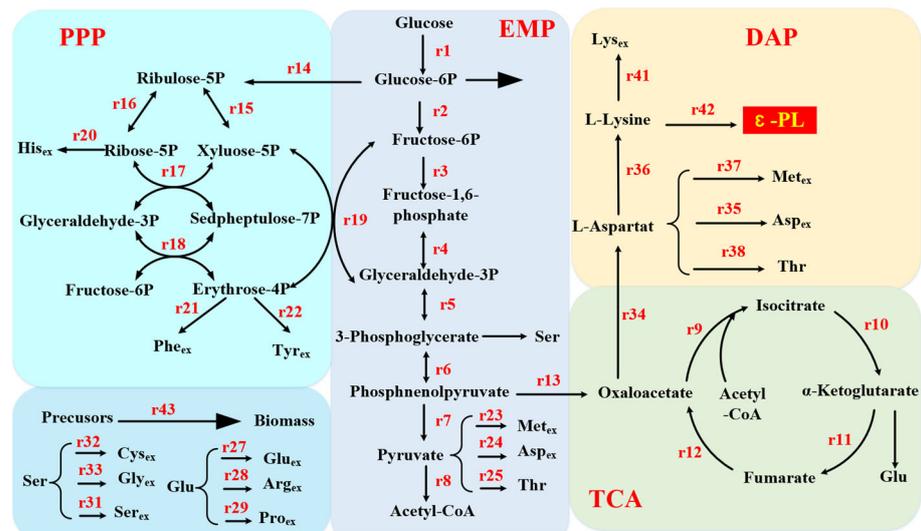
Figure S1. Metabolic network of ε -polylysine synthesis.

| *S. albulus* WG-608		*OE-pyc-ppc-pls*
35.50	ε-PL production (g/L)	39.52
64.70	DCW (g/L)	56.98
1372.5	Glucose consumption (g)	1545.00
0.55	Specific ε-PL production(g/g)	0.69
7.15	Rate of glucose consumption （g/h）	7.23
9.46	Yield （%）	9.57
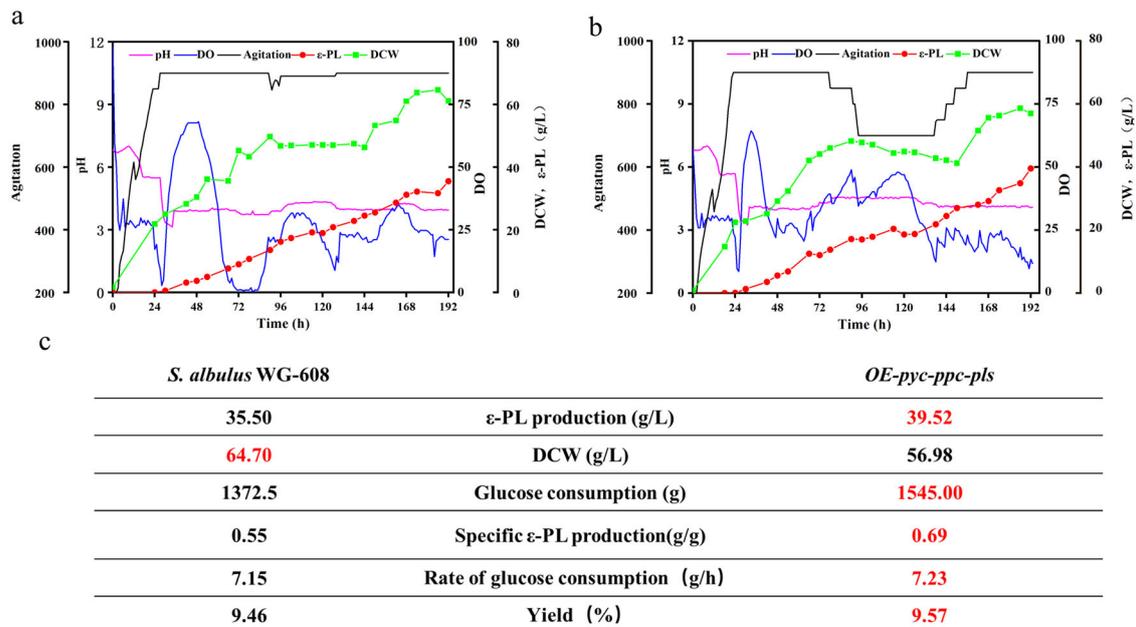
Figure S2. Fermentation process of *S. albulus* WG-608 and OE-*ppc-pyc-pls*. **a**: *S. albulus* WG-608. **b**: OE-*ppc-pyc-pls*. **c**: Comparison of fermentation parameters between *S. albulus* WG-608 and OE-*ppc-pyc-pls.* Red color indicates data with an advantage.