Supplementary Information

Efficient biosynthesis of resveratrol via combining phenylalanine and tyrosine pathways in *Saccharomyces cerevisiae*

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Supplementary Tables

Table S1. Resveratrol production in various hosts [a]

Strains (Parent strains)	Related gene cassettes	Notes	Carbon source	Resveratrol (mg/L)	Scale	References
E. coli						
S4 [c] [*E. coli* BL21 (DE3)]	*pCDFDuet-Pc4CL2-VvSTS; pIBR-181-ScmatBC*	Modular pathway engineering and the introduction of malonate assimilation pathway genes	Glucose	151	Flask (Batch)	[1]
N.A.[b c] [*E. coli* BL21 (DE3)]	*LacZ::EcaroGfbr, EctyrAfbr,* pCDFDuet-*TcTAL(Variants)-Pc4CL,* pETDuet-*VvSTS,* pACYC-*RtmatC-RtmatB-dCas9,* pCOLA-*fabF(high)/fabB(medium)/fabI(low)/fabH(low)*	Introduction of malonate assimilation pathway, Down regualtion of fatty acid biosynthesis pathway genes, Optimization the expression of *TcTAL* based on mRNA structure	Glucose	304.5	Flask (Batch)	[2]
N.A. [c] [*E. coli* BL21 (DE3)]	pET28a-*Vvsts,* pCDFDuet-1-*Setal,* pACYCDuet-1-*At4cl*	Construction of a new probabilistic computational model to simulate the microbial production of resveratrol	Glucose	172.8	Flask (Batch)	[3]
N.A. [*E. coli* BW25113 (DE3)]	*ΔtyrR ::VvSTS, ΔtrpED::RgTAL-Pc4CL*	First utilized the site-specific integration strategy to produce resveratrol in *Escherichia coli*	Glucose	4.6	Flask (Batch)	[4]

Unconventional yeast						
T4V-ARO47m [c] (*P. pastoris*)	CBS7435 *Δdnl4 Δhis4* pPGP-*HaTAL-At4CL-VvVST*, pPGPH-*ARO4*^{K229L}-*ARO7*^{G141S} [G418^{r}, Hyg^{r}]	Development of an L-Tyr chassis in *Pichia pastoristo* produce various aromatic secondary metabolites	Glycerol	451 1825	Flask (Batch) Bioreactor (Batch)	[5]
Ss-T4V-aro7m (*S. stipites*)	*ΔURA5, ΔADE2* *ADE2: PIR1p-HaTAL1-TEF1t, ENO1p-At4CL2-UAGt, TEF1p-VvVST1-GLN1t* *URA5: ENO1p-SsARO7*^{G139S}*-UAGt*	Production of resveratrol from a wide range of sugars as carbon sources by a using the yeast *Scheffersomyces stipitis*	Glucose Cellobiose Sucrose	238 530 669	Flask (Batch)	[6]
Y. lipolytica						
T2P2 (*Y. lipolytica* Pold)	*pTEF-FjTAL x2*, *pTEF-PAL x2*, *pTEF-C4H x2*, *pTEF-4CL1 x2*, *pTEF-VvVST x2*, Ura^{+}, Leu^{+}	Resveratrol production from glycerol, Multi-copy integration of pathway genes	Glycerol	430	Bioreactor (Batch)	[7]
N.A. (*Y. lipolytica* Po1fk)	Δ*ylTYR1*, Δ*ylTRP2*, Δ*ylTRP3*, Δ*ylARO8*, Δ*ylARO9*, Δ*ylPYK*, Δ*ylPHA2* *ylARO1, ylARO2, ylARO3, ylARO4, ylARO5, scARO4*^{K229L}, *aroG*^{S180F}, *ylTKT, bbxfpK, acxpk, rgTAL ylTYR1, vvSTS1, pc4CL2::Leu*	Mainly focus on the engineering the chassis for *de novo* synthesis of five aromatic-derived natural products and chemicals	Glucose	12.67	Flask (Batch)	[8]
ST9671 (*Y. lipolytica* W29)	*ku70Δ::SpCas9-EcDsdAMX4* *IntC2::PrTEFintron-FjTAL-TLip2* *IntC3::TPex20-At4CL1-PrGPD-PrTEFintron-VvVST1-TLip2* *IntE1::TPex20-YlARO7*^{G139S}*-PrGPD-PrTEFintron-YlARO4*^{K221L}*-TLip2* *IntE4,D1,E3,F3,A1::TPex20-FjTAL-PrTEFintron-TPex20-At4CL1-PrGPD-PrTEFintron-VvVST1-TLip2*	Integration of six copies of *FjTAL, At4CL1, VvVST1*; Significantly increase of resveratrol titer	Glucose	409 12400	Flask (Batch) Bioreactor (Fed-batch)	[9]

ST890 (*Y. lipolytica* Po1fk)	D17::P_{TEF}-*FjTAL*-T_{XPR2} F1-3::P_{TEF}-*ARO4*^{K221L}-T_{XPR2}-P_{TEF}-*ARO7*^{G139S}-T_{XPR2}-P_{TEF}-*ARO1*-T_{XPR2} C3::P_{TEF}-*AtPAL*-T_{XPR2}-P_{TEF}-*AtC4H*-T_{XPR2}-P_{TEF}-*AtATR2*-T_{XPR2} E1::P_{TEF}- *YlCYB5*-T_{XPR2}-P_{TEF}-*Aro3*^{K225L}-T_{XPR2} AXP*::*P_{TEF}-*CaFPK*-T_{XPR2}-P_{TEF}-*BsPTA*-T_{XPR2} 26S rDNA::$P_{8xUASTEFin}$-*Pc4CL1-EAAAK-VvSTS*-T_{XPR2}-P_{LEU}-*URA3*-T_{XPR2} ZETA::$P_{8xUASTEFin}$-*Pc4CL1-EAAAK-VvSTS*-T_{XPR2}-P_{LEU}-*LEU*-T_{XPR2} △DGA1	Multi-copy integration of *Pc4CL-EAAAK-VvSTS* and screening, Morphology control in Fed-batch fermentation	Glucose	819 22500	Flask (Batch) Bioreactor (Fed-batch)Flask	[10]
S. cerevisiae						
ST4152 (*S. cerevisiae* CEN.PK102-5B)	Ty-(P_{TEF1}-*HaTAL*, P_{PGK1}-*At4CL1*, P_{TEF1}-*VvVST1*), P_{TEF1}-*ScARO7*^{G141S}, P_{PGK1}-*ScARO4*^{K229L}, P_{TEF1}-*ScACC1*^{S659A,S1157A}	Multi-copy integration of *HaTAL-At4CL1-VvSTS1*	Glucose Ethanol	415.6 531.4	 Bioreactor (Fed-batch)	[11]
ST4992 (*S. cerevisiae* CEN.PK102-5B)	P_{TEF1}-*AtATR2*, P_{PGK1}-*CYB5*, P_{TEF1}-*VvVST1*) Ty-(P_{TDH3}-*AtPAL2*, P_{FBA1}-*AtC4H*, P_{PGK1}-*At4CL2*, P_{TEF1}-*VvVST1*), P_{TEF1}-*Sc*ARO7^{G141S}, P_{PGK1}-*ScARO4*^{K229L}, P_{TEF1}-*ScACC1*^{S659A,S1157A} Δ*ARO10*, P_{TDH3}-*EcaroL*, P_{TDH3}-*SeACS*^{L641P}	Multi-copy integration of *AtPAL-AtC4H- At4CL1-VvSTS1*	Glucose Glucose Ethanol	272.64 755 812	Flask (Batch) Bioreactor (Fed-batch)	[12]
Ethanol Red RBP (Ethanol Red)	*X4: P_{TDH3}-AtPAL2, P_{FBA1}-AtC4H, P_{PGK1}-At4CL2, P_{TEF1}-VvVST1*	The first report of lignocellulosic resveratrol production	Glucose + Ethanol Cellulose	187.07 151.65	Flask (Batch)	[13]

L501 (Ethanol Red)	*X4:* P_{TDH3}*-AtPAL2,* P_{FBA1}*-AtC4H,* P_{PGK1}*-At4CL2,* P_{TEF1}*-VvVST1* P_{PGK1}*-KIALC12,* P_{TEF1}*- KIALC4*	The first report on resveratrol production from lactose, relevant in dairy wastes	Lactose	210	Flask (Batch)	[14]
L543 (Ethanol Red)	*XII-4: ScCyb5←PGK1p- TEF1p→AtATR2* *X-4: TDH3p→AtPAL2, FBA1p→AtC4H,* *PGK1p→At4CL2, TEF1p→VvVST1* *X-3: TEF1p→RKI1, TDH3p→RPE1* *XI-3: TEF1p→TKL1, TDH3p→PsTAL1* *XII-2: TEF1p→CpXylA, TDH3p→PsSUT1* *XII-5: TEF1p→CpXylA, TDH3p→PsXYL3* *ΔGRE3::TEF1p→CpXylA*	The first report on the use of renewable carbon sources for resveratrol production from xylose and the use of winery by-products as a substrate to produce this stilbenoid	Glucose + Xylose All sugars in wine wastes	388 161.1 ~ 282.7	Flask (Batch)	[15]
BRT10 (*S. cerevisiae* BY4742)	*yorwΔ17*::HIS3/ T_{ADH1}*-VvSTS-*P_{PGK1}/ P_{TEF1}*-Pc4CL-*T_{CYC1} *ura3*::T_{ADH1}*-RtTAL-*P_{PGK1}/P_{TDH3}*-VvSTS-*T_{CYC1}/ URA3 *cit2*Δ::T_{ADH1}*-RtTAL-*P_{PGK1}/P_{TDH3}*-VvSTS-*T_{CYC1} *leu2*::T_{ADH1}*- AtCPR1-*P_{PGK1}/P_{TDH3}*-AtC4H-* T_{CYC1}/LEU2 *lpp1*Δ::T_{ADH1}*-RtTAL-*P_{PGK1}/P_{TDH3}*-VvSTS-* T_{CYC1} *1309a*::T_{ADH1}*-ARO4* ^{K229L}*-*P_{PGK1}/P_{TEF1}*-ARO7* ^{G141S}*-*T_{CYC1} *511b*::T_{ADH1}*-EcAROL-*P_{PGK1}/P_{TEF1}*-ARO2-*T_{CYC1} *pdc6*Δ::P_{TEF1}*-ScACC1* $^{S659A/S1157A}$*-*T_{CYC1} *dpp1*Δ::T_{ADH1}*-VvSTS-*P_{PGK1}/P_{TEF1}*-Pc4CL-*T_{CYC1} *lys2*::LYS2	Taking the advantage of bi-functional *RtTAL*, enabling the simultaneously use of phenylalanine and tyrosine by only one enzyme; Recover the lysine pathway; A new record for the resveratrol titer in *S. cerevisiae*	Glucose	1155 4100	Flask (Batch) Bioreactor (Fed-batch)	This study

a. Other examples especially start with precursors can refer to some previous reviews [16-18] or reports [9]; *b*. N.A., not available; *c*. strains bearing plasmids for genes expression

Table S2 Plasmids used in this study

Plasmids	**Description**	**Reference**
pCas	2μ/pUC, G418/Kan, *S. pyogenese* Cas9	[19]
CIT2-pCas	pCas with *gRNA* specific for site c*it2* gttatggtcatgctgtgcta	This study
LPP1-pCas	pCas with *gRNA* specific for site *lpp1* gccatgacagagatcatcct	This study
PHA2-pCas	pCas with *gRNA* specific for site *pha2* tcagcgacaaaagtaaacag	This study
1309a-pCas	pCas with *gRNA* specific for site *1309a* cctgtggtgactacgtatcc	This study
511b-pCas	pCas with *gRNA* specific for site *511b* cagtgtatgccagtcagcca	This study
PDC6-pCas	pCas with *gRNA* specific for site *pdc6* gatgcgtgcgtaaccatcgg	This study
911b-pCas	pCas with *gRNA* specific for site *911b* gtaatattgtcttgtttccc	This study
DPP1-pCas	pCas with *gRNA* specific for site *dpp1* gatcgttgccaacctgttga	This study
G418	2μ/pUC, G418/Kan, T_{ADH1}- P_{PGK1}/P_{TEF1}-T_{CYC1}	This study
G418-PV	T_{ADH1}-*Pc4CL*-P_{PGK1}/P_{TEF1}-*VvSTS*- T_{CYC1}	This study
G418-TS	T_{ADH1}-*RtTAL*- P_{PGK1}/P_{TDH3}-*VvSTS*- T_{CYC1}	This study
G418-C4H	T_{ADH1}-*AtCPR1*- P_{PGK1}/P_{TDH3}-*AtC4H*- T_{CYC1}	This study
G418-Aro47	T_{ADH1}-*ARO4*^{K229L} - P_{PGK1}/P_{TEF1}-*ARO7*^{G141S} - T_{CYC1}	This study
G418-AroL2	T_{ADH1}-*EcAROL* - P_{PGK1}/P_{TEF1}-*ARO2* - T_{CYC1}	This study
G418-ACC1	T_{ADH1}- P_{PGK1}/P_{TEF1}-*ScACC1*$^{S659A/S1157A}$ - T_{CYC1}	This study

Table S3 Primers used in this study

1.1 Primers used to construct plasmids with expression cassettes

Primers	**Sequence (5'-3')**	**Notes**
T_{CYC1}-F	tcatgtaattagttatgtcacgcttaca	For G418 backbone
T_{ADH1}-R	agttataaaaaaaataagtgtatacaaattttaaag	
G418-PV-F1	cacttatttttttataacttacttttggcaaatcaccagagg	DNA fragments for G418-PV
G418-PV-R1	atgggtgattgtgttgctcca	
G418-PV-F2	ggagcaacacaatcacccattgttttatatttgttgtaaaaagtagataattact	
G418-PV-R2	cttcaacggaagccatttgtaattaaaacttagattagattgctatg	
G418-PV-F3	caaaatggcttccgttgaagaattca	
G418-PV-R3	tgacataactaattacatgatcaattggtaacggttggaaca	
G418-TS-F0	atggcttccgttgaagaattca	Backbone
G418-TS-F1	cacttatttttttataacttaagccaacattttcaataaaacatt	DNA fragments for G418-TS
G418-TS-R1	acaatggctcctagaccaactagtcaaa	
G418-TS-F2	gttggtctaggagccattgttttatatttgttgtaaaaagtagataattact	
G418-TS-R2	caacgctagtatacgcacagatattataacatctgcac	
G418-TS-F3	ctgtgcgtatactagcgttgaatgttagcgtca	
G418-TS-R3	aattcttcaacggaagccatttgtttgtttatgtgtgtttattcga	
G418-C4H-F1	cacttatttttttataacttcaccagacatctctgaggtatcttc	DNA fragments for G418-C4H
G418-C4H-R1	atgacttctgctttgtatgcttccg	
G418-C4H-F2	gcatacaaagcagaagtcattgttttatatttgttgtaaaaagtagataattact	
G418-C4H-R2	ccaacaacaacaagtccatttgtttgtttatgtgtgtttattcga	
G418-C4H-F3	aatggacttgttgttgttggaaaag	
G418-C4H-R3	tgacataactaattacatgatcaacagtttcttggcttcataacg	
G418-Aro47-F1	cacttatttttttataactctatttcttgttaacttctcttctttgtctg	DNA fragments for G418-Aro47
G418-Aro47-R1	gggtgttactctacatggtgttgctgctatcacc	
G418-Aro47-F2	caccatgtagagtaacacccatgaaatggtgaga	
G418-Aro47-R2	caatgagtgaatctccaatgttcgc	
G418-Aro47-F3	cattggagattcactcattgttttatatttgttgtaaaaagtagataattact	
G418-Aro47-R3	ctggttttgtgaaatccattttgtaattaaaacttagattagattgctatg	
G418-Aro47-F4	aatggatttcacaaaaccagaaactg	
G418-Aro47-R4	ctctagtggcaacagaactgaagttattcttatcatcaccatctcttt	
G418-Aro47-F5	cagttctgttgccactagagatatagaatg	
G418-Aro47-R5	tgacataactaattacatgattactcttccaaccttcttagcaagt	
G418-AroL2-F1	cacttatttttttataacttcaacaattgatcgtctgtgcc	DNA fragments for G418-AroL2
G418-AroL2-R1	taaaacaatgacacaacctctttttctgatcg	
G418-AroL2-F2	gaggttgtgtcattgttttatatttgttgtaaaaagtagataattact	
G418-AroL2-R2	cccaaacgttgacatttgtaattaaaacttagattagattgctatg	
G418-AroL2-F3	acaaaatgtcaacgtttgggaaactgtt	
G418-AroL2-R3	tgacataactaattacatgattaatgaaccacggatctggaga	
G418-ACC1-R0	tttgtaattaaaacttagattagattgctatg	Backbone

1.1 Primers used to construct plasmids with expression cassettes (continued)

Primers	Sequence (5'-3')	Notes
G418-ACC1-F1	atctaagttttaattacaaaatgagcgaagaaagcttattcga	
G418-ACC1-R1	accatcagctagttgacgcagtatgatatcacattta	DNA
G418-ACC1-F2	tgcgtcaactagctgatggtggtcttttgattgc	fragments
G418-ACC1-R2	tgaaacagcaacagccctgttcatacccatt	for
G418-ACC1-F3	acagggctgttgctgtttcagatttgtcatatgttgc	G418-ACC1
G418-ACC1-R3	tgacataactaattacatgattatttcaaagtcttcaacaatttttctt	

2.1 Primers used to construct plasmids pCas of specific editing sites

Primers	Sequence (5'-3')	Notes
CIT2-pCas-F	gttatggtcatgctgtgctagttttagagctagaaatagc	
CIT2-pCas-R	tagcacagcatgaccataacaaagtcccattcgccaccc	
LPP1-pCas-F	gccatgacagagatcatcctgttttagagctagaaatagc	
LPP1-pCas-R	aggatgatctctgtcatggcaaagtcccattcgccaccc	
PHA2-pCas-F	tcagcgacaaaagtaaacaggttttagagctagaaatagc	
PHA2-pCas-R	ctgtttacttttgtcgctgaaaagtcccattcgccaccc	
1309a-pCas-F	cctgtggtgactacgtatccgttttagagctagaaatagc	
1309a-pCas-R	ggatacgtagtcaccacaggaaagtcccattcgccaccc	
511b-pCas-F	cagtgtatgccagtcagccagttttagagctagaaatagc	
511b-pCas-R	tggctgactggcatacactgaaagtcccattcgccaccc	
PDC6-pCas-F	gatgcgtgcgtaaccatcgggttttagagctagaaatagc	
PDC6-pCas-R	ccgatggttacgcacgcatcaaagtcccattcgccaccc	
911b-pCas-F	gtaatattgtcttgtttcccgttttagagctagaaatagc	
911b-pCas-R	gggaaacaagacaatattacaaagtcccattcgccaccc	
DPP1-pCas-F	gatcgttgccaacctgttgagttttagagctagaaatagc	
DPP1-pCas-R	tcaacaggttggcaacgatcaaagtcccattcgccaccc	

3.1 Primers used to amplify fragments to genomic editing in BY4742

Primers	Sequence (5'-3')	Notes
YORW-Up-F	tgtgcacaaaggccataatattatg	
YORW-Up-R	attaccgaggcataaaaaaatatagagtgtactaggatggcatgagttatggttg	
Y-HIS3-F	ttggtaactgtgcaaccataactcatgccatcctagtacactctatatttttttatg	
Y-HIS3-R	ctcttattgaccacacctctaccggcatgccgactacataagaacacctttggtg	Fragments
Y-TADH1-F	acgatgttccctccaccaaaggtgttcttatgtagtcggcatgccggtagaggtg	to form BR1
Y-TCYC1-R	gaattttgagagcccacttttgttggggacgattgcaaattaaagccttcgagc	
YORW-Down-F	ggttttgggacgctcgaaggctttaatttgcaatcgtccccaacaaaagtg	
YORW-Down-R	aaagctggctcccctagac	
△PHA2-UP-F	acatactaccttgcagttcc	
△PHA2-UP-R	accccacaggcaccactgtttacttttgtcgctgataattgaaggatgaatgcgg	Fragments to delete *pha2*
△PHA2-Down-F	aacttcattatatttccgcattcatccttcaattaatgcctgggatttcttgacg	
△PHA2-Down-R	aatcgtgtcgtgtgttcgac	

3.1 Primers used to amplify fragments to genomic editing in BY4742 (Continued)

Primers	**Sequence (5'-3')**	**Notes**
Ura3-Up-F	gtgaatttgcagtggtaacg	Fragments to form BRT2
Ura3-Up-R	tcttattgaccacacctctaccggcatgccgacactatctcttagcatctttaatc	
Ura3- TADH1-F	gtagaaaaggattaaagatgctaagagatagtgtcggcatgccggtagaggtg	
Ura3-TCYC1-R	aaaaatgatgaattgaaattaagggttctcgaggcaaattaaagccttcgagcgtc	
CYC-UraM-F	gaaggttttgggacgctcgaaggctttaatttgcctcgagaacccttaatttcaattc	
Leu-UraM-R	ccgagattcccggggcagtggattcaagtgctctgaagctctaatttgtgagtttag	
Ura3-Down-F	cacaaattagagcttcagagcacttgaatccactgccccgggaatctcggtcgtaa	
Ura3-Down-R	tctttatatttacatgctaaaaatgggc	
Leu2-Up-F	acatcgagaccaagaagaacattgc	
Leu2-Up-R	atgccgatacaatccttgcccgtgatgataatatatagtagtaacctgaaaaatag	
Leu- TADH1-F	actactatatattatcatcacgggcaaggattgtatcggcatgccggtagaggtg	Fragments to form BRT3
Leu-TCYC1-R	catcttacgatacctgagtattcccacagttgcaaattaaagccttcgagcgtc	
CYC-LEUM-F	gaaggttttgggacgctcgaaggctttaatttgcaactgtgggaatactcaggtatc	
Leu-LEUM-R	atgttaaagtgcaattctttttccttatcacgtctaccctatgaacatattccattttg	
Leu2-Down-F	aaattacaaaatggaatatgttcatagggtagacgtgataaggaaaaagaattgc	
Leu-Down-R	cataccacgttgaacggatc	
CIT2-Up-F	agttgttgccacaacataag	Fragments to integrate at site *cit2*
CIT2-Up-R	gctcttattgaccacacctctaccggcatgccgaggaactgtcatttttcttgttac	
CIT2-tADH-F	ttaataatactagtaacaagaaaaatgacagttcctcggcatgccggtagaggtg	
CIT2-tCYC1-R	cagaggggtgtaaaagtaggatgtaatccgcaaattaaagccttcgagcgtc	
CIT2-Down-F	gaaggttttgggacgctcgaaggctttaatttgcggattacatcctacttttac	
CIT2-Down-R	gaacgtaaaacaagtaaaaatgtag	
LPP1-Up-F	cttatacgtctcccaatcatg	Fragments to integrate at site *lpp1*
LPP1-Up-R	gctcttattgaccacacctctaccggcatgccgatatgacgagtttccttaggc	
LPP1-tADH-F	tcctacatcaacgcctaaggaaactcgtcatatcggcatgccggtagaggtg	
LPP1-tCYC1-R	gcttttattctttcctgataggactctgtaagtgcaaattaaagccttcgagcgtc	
LPP1-Down-F	gaaggttttgggacgctcgaaggctttaatttgcacttacagagtcctatcagg	
LPP1-Down-R	gaagcaagattctcgaaaatac	
1309a-UP-F	cgttgttgatcttgtagtctg	Fragments to integrate at site *1309a*
1309a-UP-R	cgctcttattgaccacacctctaccggcatgccgagatcctaaactgcgtcatag	
1309a-ADH1-F	atcaaagaaacttactatgacgcagtttaggatctcggcatgccggtagaggtg	
1309a-CYC1-R	ataaacaaggggctttacgatggagtagtagagcaaattaaagccttcgagcgtc	
1309a-Down-F	gaaggttttgggacgctcgaaggctttaatttgctctactactccatcgtaaagcc	
1309a-Down-R	cggcaaaattaaacgaaaacc	
511b-Up-F	gcaacggtttttgaatgctatg	Fragments to integrate at site *511b*
511b-UP-R	ctcttattgaccacacctctaccggcatgccgagaaatctgtaccaaccgtatagg	
511b-ADH1-F	agggtctctttcacctatacggttggtacagatttctcggcatgccggtagaggtg	
511b-CYC1-R	aagaaaatagaagcaaacgacgtaatgccggcaaattaaagccttcgagcgtc	
511b-Down-F	gaaggttttgggacgctcgaaggctttaatttgccggcattacgtcgtttgcttc	
511b-Down-R	caccagcaaagtcaacctcagag	

3.1 Primers used to amplify fragments to genomic editing in BY4742 (Continued)

Primers	**Sequence (5'-3')**	**Notes**
911b-UP-F	atggtcgagaagatgagatatg	Fragments
911b-UP-R	gctcttattgaccacacctctaccggcatgccgacatttatatttatgcccattcaac	to integrate
TADH1-F	tcggcatgccggtagaggtg	at site *911b*
PTDH3-R	atactagcgttgaatgttagcg	
911b-Down-F	gttgttgacgctaacattcaacgctagtatcctggagaagtaaatgaaaaatg	
911b-Down-R	ctcaattttccctttgtcatc	
PDC6-Up-F	aatgttatagagttcacacc	Fragments
PDC6-Up-R	acattttgaagctatggtgtgtgggggatcactttgttggcaatatgtttttgc	to integrate
PDC6-TEF1-F	cacgtaatatagcaaaaacatattgccaacaaaagtgatcccccacacaccatag	at site *pdc6*
PDC6-CYC1-R	caacaataattcgtttgagtacactactaatggcgcaaattaaagccttcgagcgtc	
PDC6-Down-F	agaaggttttgggacgctcgaaggctttaatttgcgccattagtagtgtactcaaac	
PDC6-Down-R	gagcacttgttatttataaaaag	
DPP1-Up-F	atagttttccagtagtgcac	
DPP1-Up-R	gctcttattgaccacacctctaccggcatgccgaggtcgtttgctatgatttaattc	Fragments
DPP1-tADH-F	gcaaagaatcagaattaaatcatagcaaacgacctcggcatgccggtagaggtg	to integrate
DPP1-tCYC1-R	aatacgtatatttcgtatgtcatgtggagtatatagcaaattaaagccttcgagcgtc	at site *dpp1*
DPP1-Down-F	gaaggttttgggacgctcgaaggctttaatttgctatatactccacatgacatacg	
DPP1-Down-R	acatagtatgtgttaagggg	
Lys2-F	acaaaggaaagcagttgctttc	Complement
Lys2-R	gtggagattgaaaagagctg	of Lys2

Table S4 Cell densities and resveratrol titers of strains BRT9 and BRT10 cultured in YPD medium

Strains	YPD-20G		YPD-40G	
	OD_{600}	**Resveratrol (mg/L)**	**OD_{600}**	**Resveratrol (mg/L)**
BRT9	15.75 ±0.38*	486.68 ±7.25	24.48 ±0.03	1139.94 ±63.34
BRT10	16.43 ±0.17	505.75 ±12.31	25.71 ±0.50	1078.81 ±53.97

Note: YPD-20G, YPD medium with 20 g/L glucose; YPD-40G, YPD medium with 40 g/L glucose. Statistical analysis was performed by using Student's t-test (two tailed; two-sample assuming equal variance; *$p<0.05$, and signs was not shown when $p>0.05$), the result of strain BRT9 was compared with strain BRT8, and the result of bRT10 was compared with strain BRT9. The displayed average values and standard deviations were calculated from three independent biological experiments.

Supplementary Figures

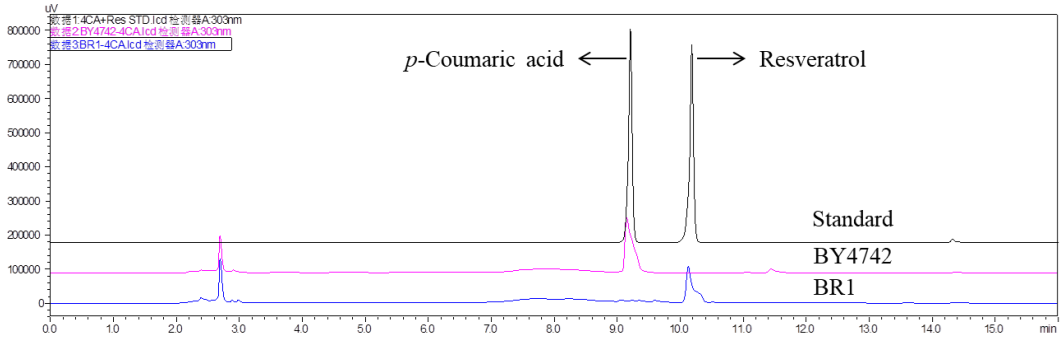

Fig. S1 Production of resveratrol in engineered strain BR1. Standards of *p*-coumaric acid and resveratrol, and samples of strain BR4742 and BR1 were analyzed by HPLC. Standards were dissolved in methanol, and fermentative broths of strain BY4742 and BR1 were mixed with ethanol. Strains were cultured in YPD medium with 0.5 mmol/L *p*-coumaric acid for 72 h.

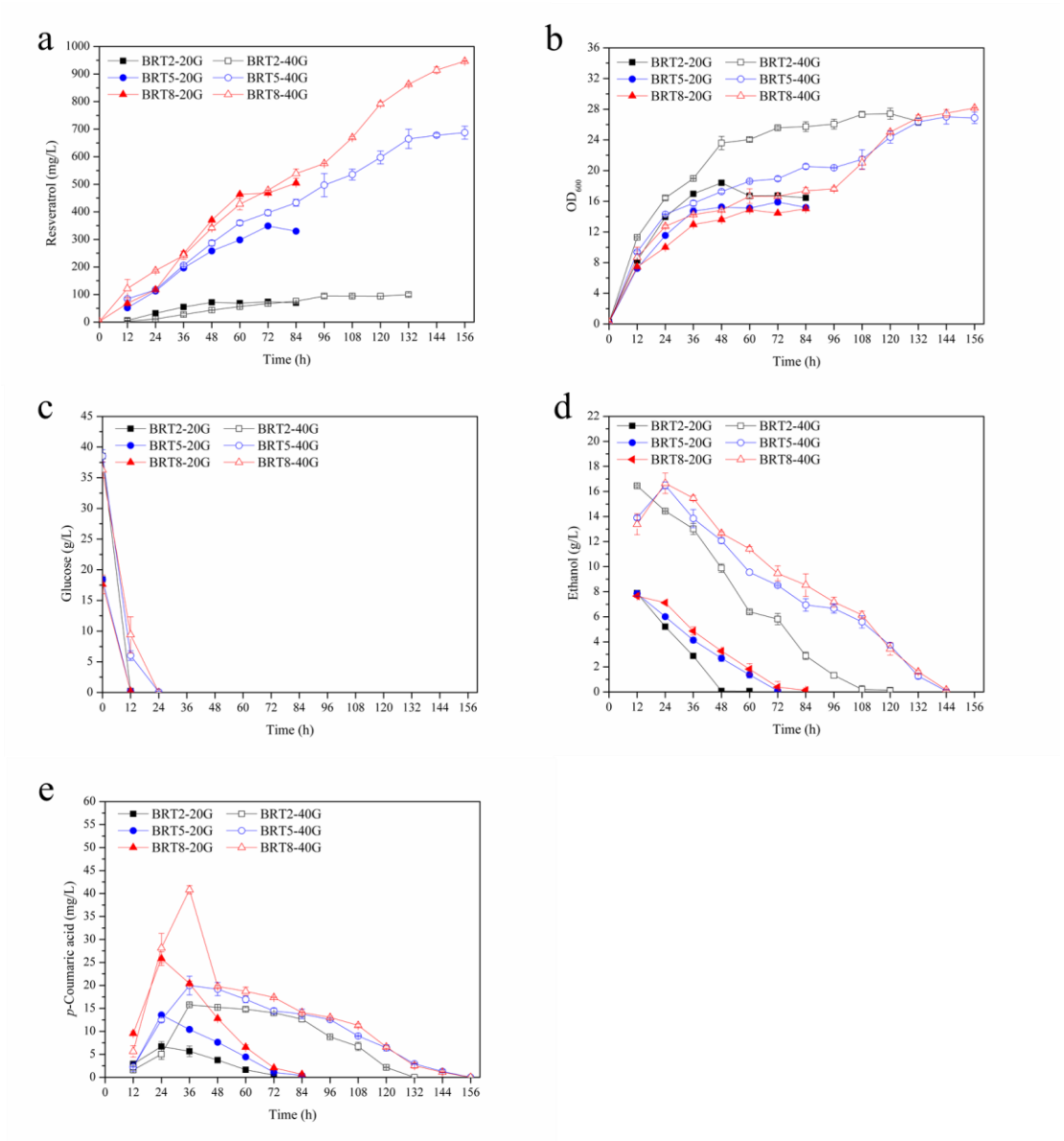

Fig. S2 Time courses of (a) resveratrol production, (b) cell growth, (c) glucose consumption, (d) ethanol concentration and (e) *p*-coumaric acid concentration of strains BRT2, BRT5 and BRT8 cultured in YPD medium with 20 g/L and 40 g/L glucose respectively. Error bars represent standard deviations from three independent biological experiments.

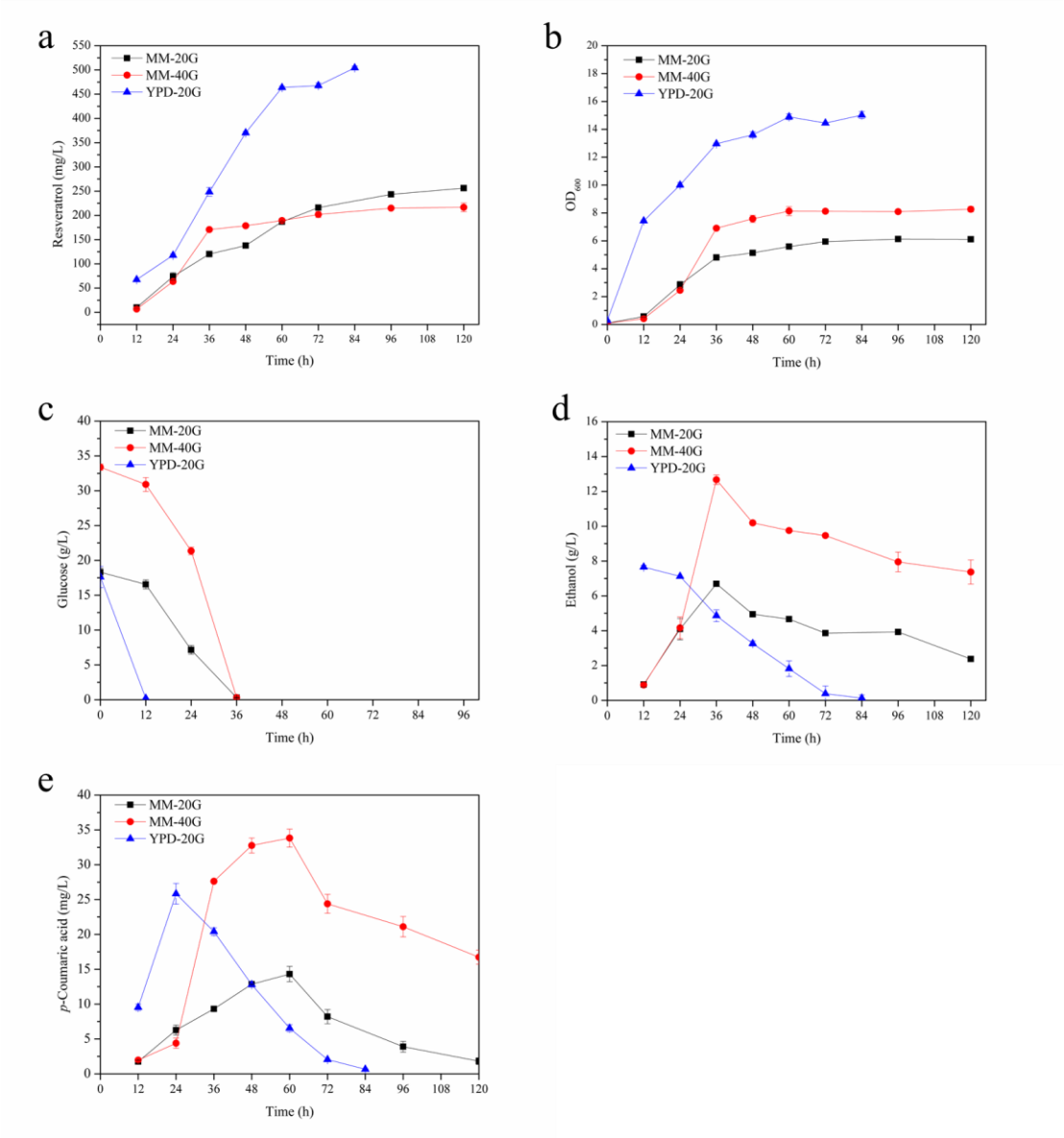

Fig. S3 Time courses of (a) resveratrol production, (b) cell growth, (c) glucose consumption, (d) ethanol concentration and (e) *p*-coumaric acid concentration of strain BRT8 cultured in different media. Error bars represent standard deviations from three independent biological experiments.

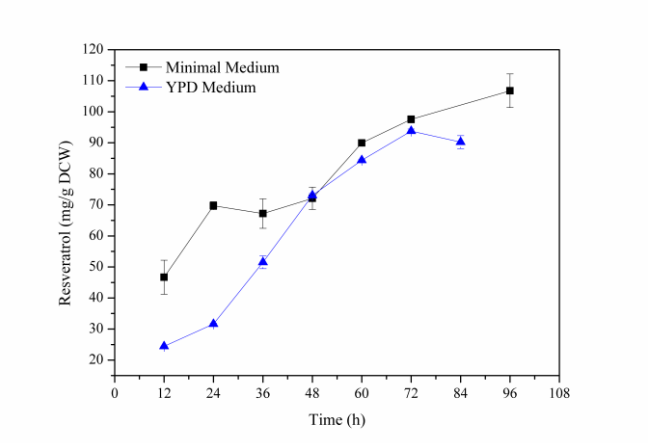

Fig. S4 Time course of resveratrol yield on dry cell weight when strain BRT8 was cultured in YPD or minimal medium. DCW: dry cell weight. Error bars represent standard deviations from three independent biological experiments.

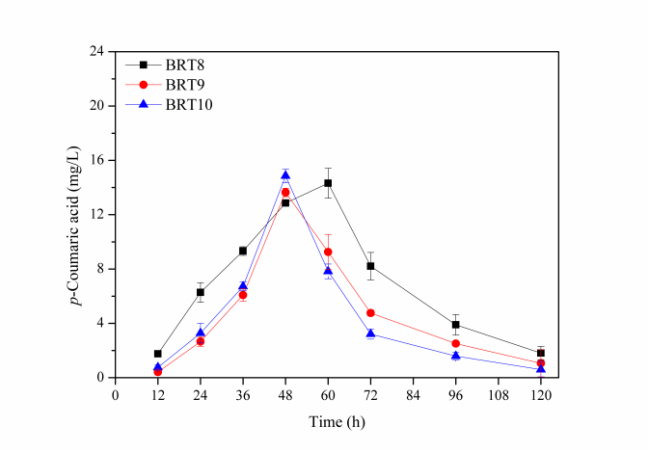

Fig. S5 Time course of *p*-coumaric acid concentration of strains BRT8, BRT9 and BRT10 cultured in minimal medium. 0.5 g/L lysine was added in the minimal medium for the growth of strain BRT8 and BRT9. Error bars represent standard deviations from three independent biological experiments.

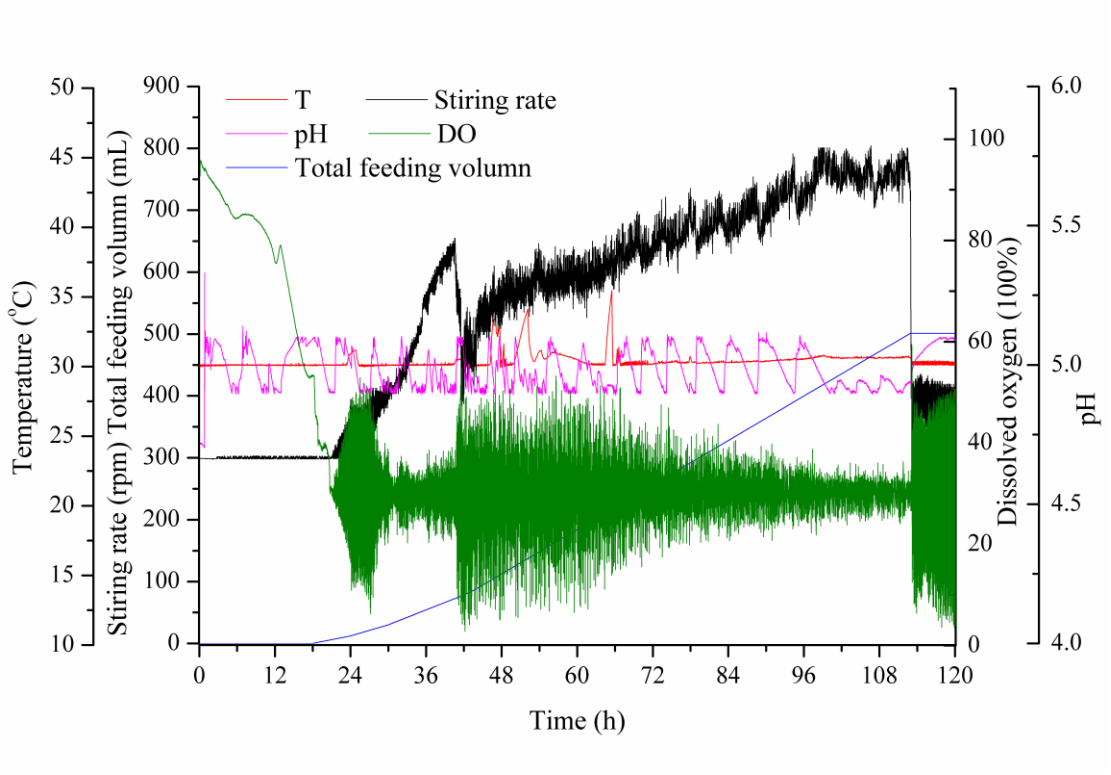

Fig. S6 Parameters during the fed-batch fermentation in the bioreactor using the non-auxotrophic strain BRT10 in minimal medium.

References

1. Shrestha A, Pandey RP, Pokhrel AR, Dhakal D, Chu LL, Sohng JK. Modular pathway engineering for resveratrol and piceatannol production in engineered *Escherichia coli*. Appl Microbiol Biotechnol. 2018;102(22):9691-706.

2. Wu J, Zhou P, Zhang X, Dong M. Efficient de novo synthesis of resveratrol by metabolically engineered *Escherichia coli*. J Ind Microbiol Biotechnol. 2017;44(7):1083-95.

3. Cotner M, Zhan J, Zhang Z. A computational metabolic model for engineered production of resveratrol in *Escherichia coli*. ACS Synth Biol. 2021;10(8):1992-2001.

4. Liu X, Lin J, Hu H, Zhou B, Zhu B. *De novo* biosynthesis of resveratrol by site-specific integration of heterologous genes in *Escherichia coli*. FEMS Microbiol Lett. 2016;363(8).

5. Kumokita R, Bamba T, Inokuma K, Yoshida T, Ito Y, Kondo A, et al. Construction of an L-tyrosine chassis in *Pichia pastoris* enhances aromatic secondary metabolite production from glycerol. ACS Synth Biol. 2022;11(6):2098-107.

6. Kobayashi Y, Inokuma K, Matsuda M, Kondo A, Hasunuma T. Resveratrol production from several types of saccharide sources by a recombinant *Scheffersomyces stipitis* strain. Metab Eng Commun. 2021;13:e00188.

7. He Q, Szczepanska P, Yuzbashev T, Lazar Z, Ledesma-Amaro R. De novo production of resveratrol from glycerol by engineering different metabolic pathways in *Yarrowia lipolytica*. Metab Eng Commun. 2020;11:e00146.

8. Gu Y, Ma J, Zhu Y, Ding X, Xu P. Engineering *Yarrowia lipolytica* as a chassis

for *de novo* synthesis of five aromatic-derived natural products and chemicals. ACS Synth Biol. 2020;9(8):2096-106.

9. Saez-Saez J, Wang G, Marella ER, Sudarsan S, Cernuda Pastor M, Borodina I. Engineering the oleaginous yeast *Yarrowia lipolytica* for high-level resveratrol production. Metab Eng. 2020;62:51-61.

10. Liu M, Wang C, Ren X, Gao S, Yu S, Zhou J. Remodelling metabolism for high-level resveratrol production in *Yarrowia lipolytica*. Bioresour Technol. 2022;365:128178.

11. Li M, Kildegaard KR, Chen Y, Rodriguez A, Borodina I, Nielsen J. De novo production of resveratrol from glucose or ethanol by engineered *Saccharomyces cerevisiae*. Metab Eng. 2015;32:1-11.

12. Li M, Schneider K, Kristensen M, Borodina I, Nielsen J. Engineering yeast for high-level production of stilbenoid antioxidants. Sci Rep. 2016;6:36827.

13. Costa CE, Moller-Hansen I, Romani A, Teixeira JA, Borodina I, Domingues L. Resveratrol production from hydrothermally pretreated eucalyptus wood using recombinant industrial *Saccharomyces cerevisiae* strains. ACS Synth Biol. 2021;10(8):1895-903.

14. Costa CE, Romani A, Teixeira JA, Domingues L. Resveratrol production for the valorisation of lactose-rich wastes by engineered industrial *Saccharomyces cerevisiae*. Bioresour Technol. 2022;359:127463.

15. Costa CE, Romaní A, Møller-Hansen I, Teixeira JA, Borodina I, Domingues L. Valorisation of wine wastes by *de novo* biosynthesis of resveratrol using a

recombinant xylose-consuming industrial *Saccharomyces cerevisiae* strain. Green Chem. 2022.

16. Feng C, Chen J, Ye W, Liao K, Wang Z, Song X, et al. Synthetic biology-driven microbial production of resveratrol: Advances and perspectives. Front Bioeng Biotechnol. 2022;10:833920.

17. Ibrahim GG, Yan J, Xu L, Yang M, Yan Y. Resveratrol production in yeast hosts: Current status and perspectives. Biomolecules. 2021;11(6).

18. Shrestha A, Pandey RP, Sohng JK. Biosynthesis of resveratrol and piceatannol in engineered microbial strains: achievements and perspectives. Appl Microbiol Biotechnol. 2019;103(7):2959-72.

19. Reider Apel A, d'Espaux L, Wehrs M, Sachs D, Li RA, Tong GJ, et al. A Cas9-based toolkit to program gene expression in *Saccharomyces cerevisiae*. Nucleic Acids Res. 2017;45(1):496-508.