**Tables and Figures**

**De Novo Biosynthesis of****Sakuranetin from Glucose by Engineered *Saccharomyces cerevisiae***

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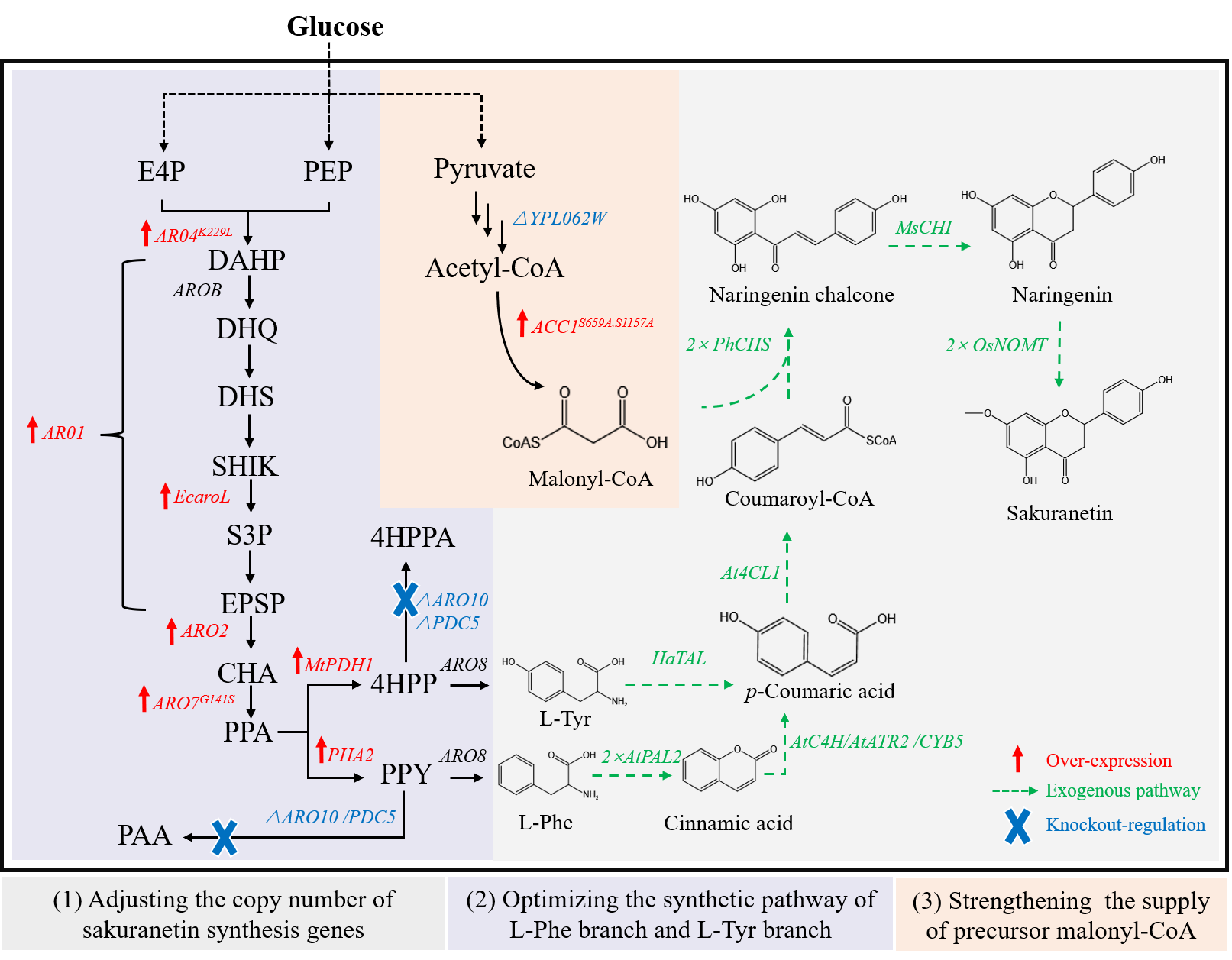
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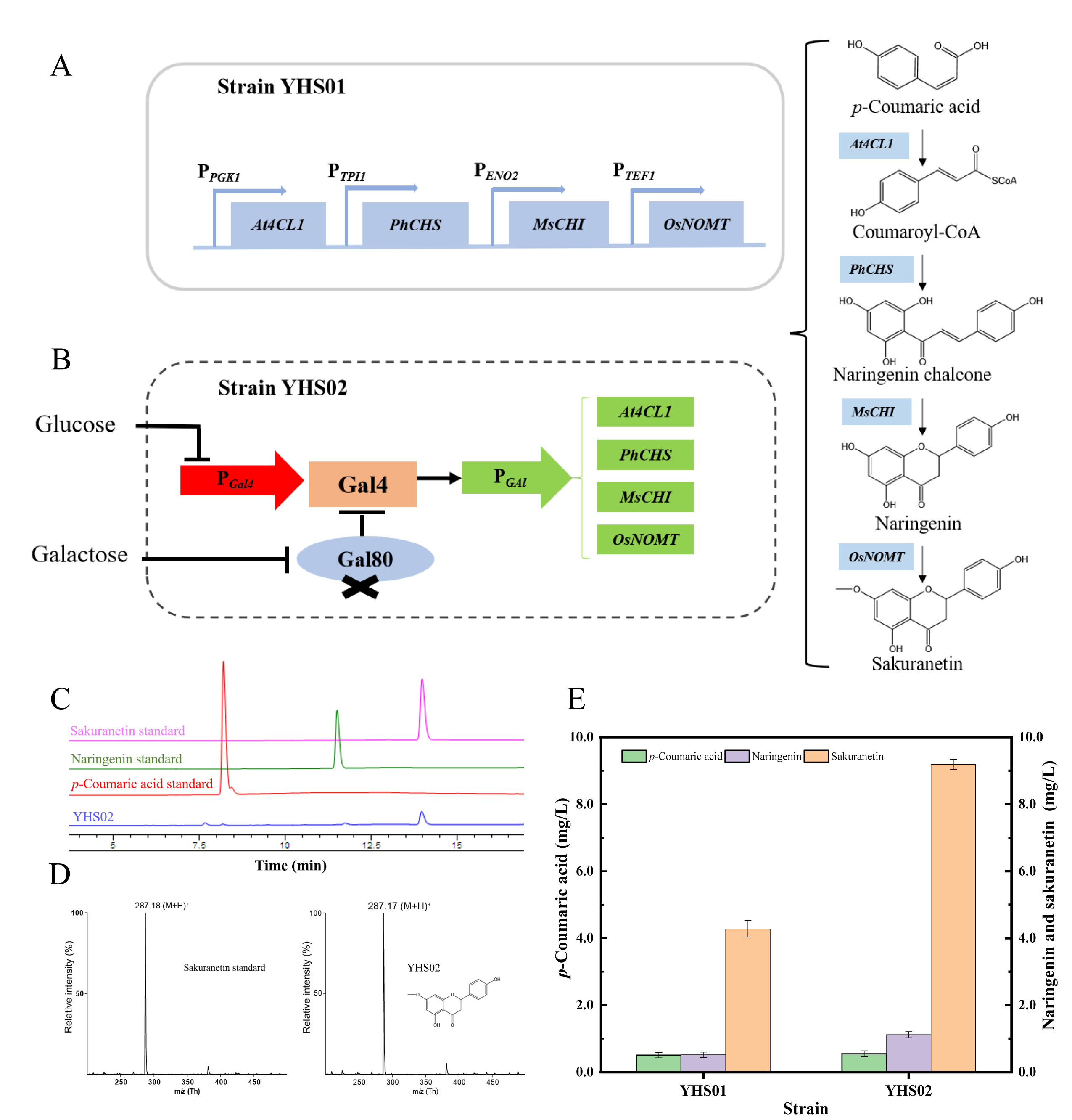
Weihong Zhong，E-mail: whzhong@zjut.edu.cn, Fax/Tel: (86)-571-88813378.

**Table 1** Strains used or constructed in this study.

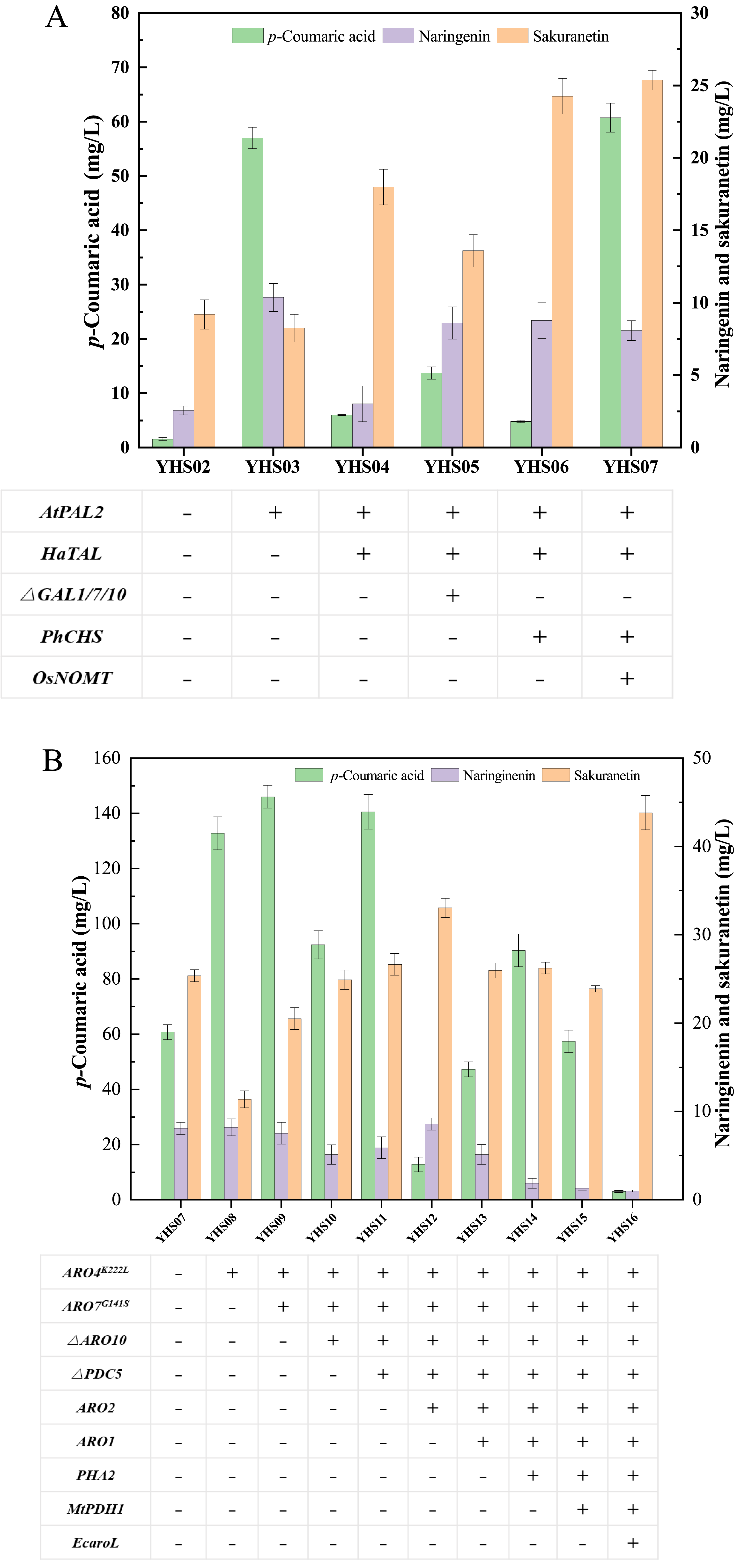
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| --- | --- | --- |
| Strain Name | Genotype/description | Source |
| CEN.PK2-1C | *MATa*; *ura3-52*; *trp1-289*; *leu2-3112*; *his3Δ1*; *MAL2-8C*; *SUC2* | Our lab |
| YT00 | CEN.PK2-1C, *IX1*:: *TEFp-SpCas9-ADH2t* | Our lab |
| YT01 | C00, *XII2*:: (*GPM1p-AtPAL2-ADH1t*)*-*(*GPDp-AtC4H-CYC1t*) | Our lab |
| YT02 | C01, *XI3*:: (*ADH1t-CYB5-PGK1p*)*-*(*HXT7p-AtATR2-CYC1t*) | Our lab |
| YHS01 | C02, *X3*:: (*ENO2p-MsCHI-PGK1t*)*-* (*TPI1p-PhCHS-TPI1t*)*-*(*TEF1p-OsNOMT-TEF1t*)*-*(*PGK1p-At4CL1-HXT7t*) | This study |
| YHS02 | C02, △*GAL80*:: *(ADH1t-At4CL1-GAL10p)-(GAL1p-OsNOMT-CYC1t)-(PGK1t-MsCHI-GAL2p)-(GAL7p-PhCHS-TPS1t)* | This study |
| YHS03 | YHS02, *HO-1*:: *GPM1p-AtPAL2-ADH1t* | This study |
| YHS04 | YHS03, *XVI1*:: *GAL7p-HaTAL-TPS1t* | This study |
| YHS05 | YHS04, △*GAL1/7/10* | This study |
| YHS06 | YHS04, *X5*:: *TPI1p-PhCHS-TPI1t* | This study |
| YHS07 | YHS06, *X5*:: (*TPI1p-PhCHS-TPI1t*)*-*(*TEF1p-OsNOMT-TEF1t*) | This study |
| YHS08 | YHS07, *TRP1*::*TEF1p-ARO4K229L-CYC1t* | This study |
| YHS09 | YHS07, *TRP1*::(*TEF1p-ARO4K229L-TEF1t*)*-*(*PGK1p-ARO7G141S-HXT7t*) | This study |
| YHS10 | YHS09, △*ARO10* | This study |
| YHS11 | YHS10, △*PDC5* | This study |
| YHS12 | YHS11, *XII5*:: *GPDp-ARO2-CYC1t* | This study |
| YHS13 | YHS11, *ARO1p*::(*GPDp-ARO2-CYC1t*)-*ENO2p* | This study |
| YHS14 | YHS13, *III1*:: (*GPM1p-PHA2-ADH1t*)*-URA3* | This study |
| YHS15 | YHS14, *X2*:: *FBA1p-MtPDH1-CYC1t* | This study |
| YHS16 | YHS15, *HO-2*:: *LEU2*-(*PDC1p-EcaroL-ADH3t*) | This study |
| YHS17 | YHS16, △*YPL062W* | This study |
| YHS18 | YHS16, △*YPL062W*:: *PGK1t-ACC1S659A,S1157A-GAL2p* | This study |

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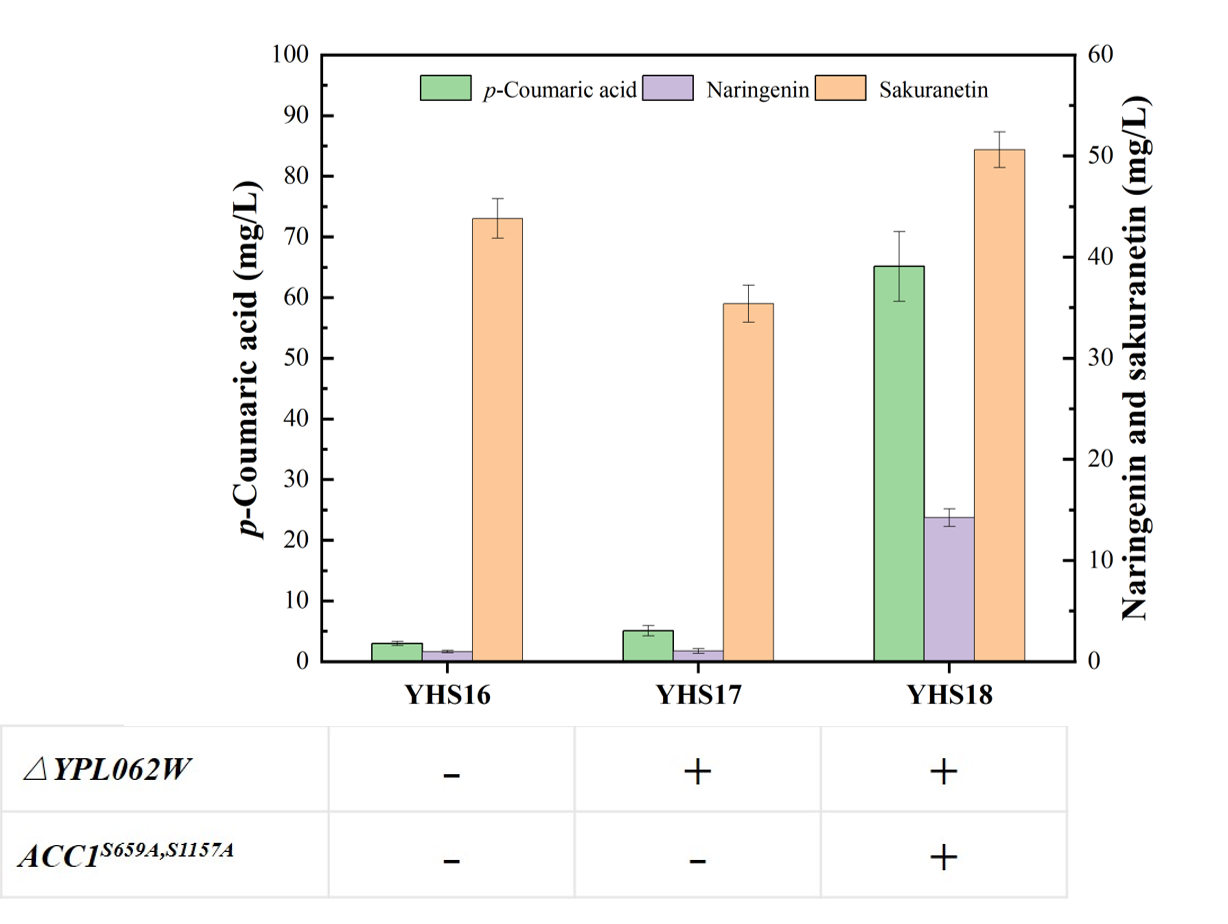
**Fig. 1.** Schematic representation of engineering strategies for sakuranetin production in *S. cerevisiae*.The biosynthetic pathway of sakuranetin starting with glucose. An optimized Sakuranetin biosynthesis pathway was reconstructed, by employing *AtPAL2, HaTAL, AtC4H, AtATR2,*  *CYB5, At4CL1, PsCHS, MsCHI,* and *OsNOMT*. PEP, phosphoenolpyruvate; E4P, erythose-4-phosphate; DAHP, 3-deoxy-D-arabino heptulosonate-7-phosphate; DHQ, 3-dehydroquinate; DHS, 3-dehydro-shikimate; SA, shikimate; S3P, shikimate-3-phosphate; EPSP, 5-enolpyruvyl-shikimate-3-phosphate; CHA, chorismate; PPA, prephenate; PAA, phenylacetaldehyde; PPY, phenylpyruvate; 4-HPP, 4-hydroxy phenylpyruvate; 4-HPAA, 4-hydroxy-phenylacetaldehyde; L-Phe, phenylalanine; L-Tyr, tyrosine; CIA, cinnamic acid; *p*-HCA, *p*-coumaric acid. Gene abbreviations: AtPAL2, phenylalanine ammonia lyase 2; AtC4H, cinnamate-4-hydroxylase; At4CL1, 4-coumarate:CoA ligase 1; AtATR2, cytochrome P450 reductase 2; CYB5, Cytochrome b5; HaTAL, tyrosine ammonia lyase; ARO4K229L, L-tyrosine feedback-insensitive DAHP synthase; ARO7G141S, L-tyrosine feedback-insensitive chorismate mutase; MtPDH1, tyrosine prephenate dehydrogenase; *EcaroL*, shikimate kinase 2; PHA2, prephenate dehydratase; ARO8, aromatic aminotransferase. PhCHS*,* chalcone synthase; MsCHI*,* chalcone isomerase; OsNOMT*,* naringenin-7-O-methyltransferase; ACC1S659A,S1157A, acetyl-CoA carboxylase; *YPL062W*, a distant genetic locus.

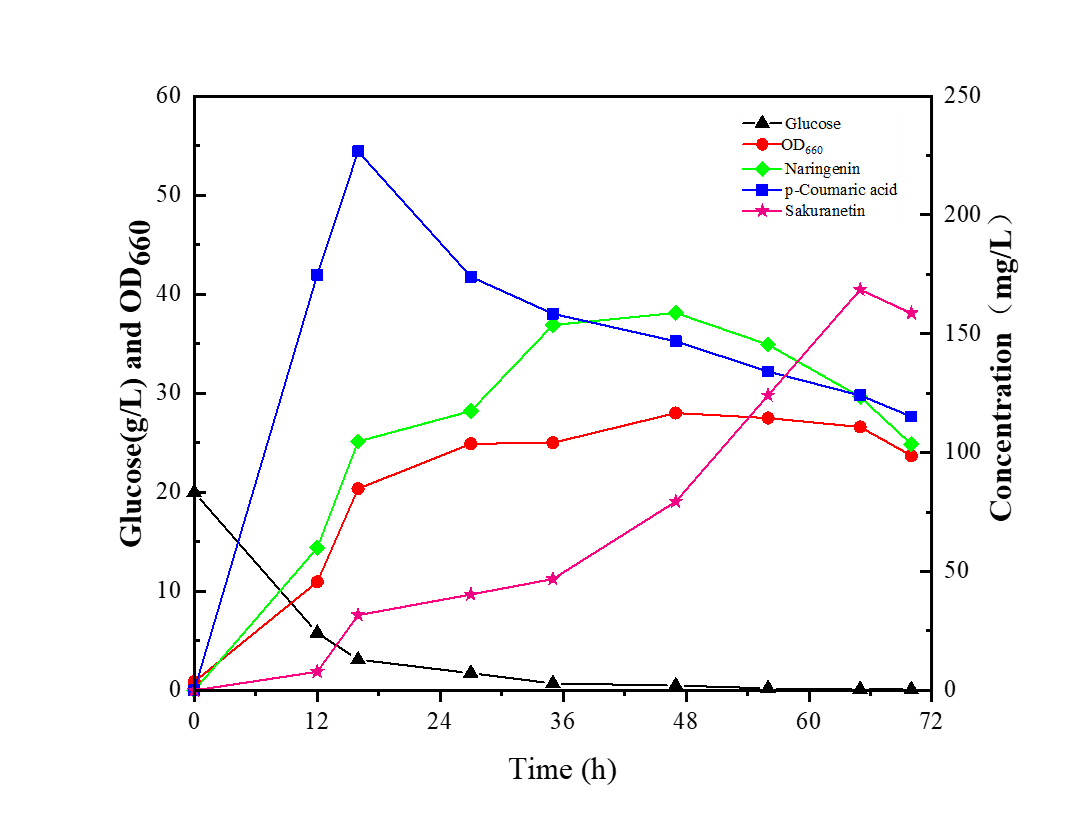


**Fig. 2.** Productin ofsakuranetin from glucose by *S.cerevisiae* expressing *At4CL, PhCHS, MsCHI, OsNOMT.* **(A)** The modified constitutive promoters of strain YHS01 for expression of sakuranetin pathway genes. **(B)** The modified *GAL* promoters of strain YHS02 for expression of sakuranetin pathway genes. **(C)** HPLC analysis of sakuranetin, naringenin, p-coumaric acid and sample of strain YHS02. **(D)** Mass spectra of sakuranetin standard and in 72h fermentation sample strain of YHS02. **(E)** Production of sakuranetin, naringenin, and *p*-coumaric acid in YHS01 and YHS02 after 72h culturing. All strains were grown following the procedures described in “Materials and methods” section.



**Fig. 3. (A)** Production of sakuranetin by overexpressing the genes of *AtPAL2*, *HaTAL*, *PhCHS* and *OsNOMT* after 72h after culturing. **(B)** Optimizing the synthetic pathway of aromatic amino acids（L-Phe/L-Tyr）increases sakuranetin production after 72h culturing. YHS09 was constructed by overexpression of *ARO4K229L* and *ARO7 G141S*; YHS11 was constructed by deletion of *ARO10* and *PDC5*. YHS16 was constructed by overexpressing genes of *ARO2*, *ARO1*, *PHA2*, *MtPDH1* and *EcaroL* in YHS11 to introduce more carbon flux into downstream pathway.



**Fig. 4.** The effect of malonyl-CoA supply on sakuranetin synthesis. YHS17 was constructed by knockout of *YPL062W* gene in YHS16*.* YHS18 was constructed by overexpression of a double-point mutant acetyl-CoA carboxylase ACC1S659A, S1157A in YHS17.

**Fig. 5.** Sakuranetin production and OD660 in YHS16 with 1-L bioreactor fermentation.

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