Metabolic engineering of Saccharomyces cerevisiae for enhanced production of caffeic acid

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Research

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Abstract

Background

As a natural phenolic acid product of plant source, caffeic acid displays diverse biological activities and acts as an important precursor for the synthesis of other valuable compounds. Limitations in chemical synthesis or plant extraction of caffeic acid trigger interest in its microbial biosynthesis. Recently, *Saccharomyces cerevisiae* has been reported sporadically for biosynthesis of caffeic acid via free plasmid-mediated pathway assembly. However, the production was far from satisfactory and even relied on the addition of precursor.

Results

In this study, we first established a controllable caffeic acid pathway by employing a modified *GAL* regulatory system in *S. cerevisiae* and realized *de novo* biosynthesis of 313.8 mg/L caffeic acid from glucose. Combinatorial engineering strategies including eliminating the tyrosine-induced feedback inhibition, deleting genes involved in competing pathways and overexpressing rate-limiting enzymes led to about 2.5-fold improvement in the caffeic acid production, reaching up to 769.3 mg/L in shake-flask cultures. To our knowledge, this is the highest ever reported titer of caffeic acid *de novo* synthesized by engineered yeast.

Conclusions

Caffeic acid production in *S. cerevisiae* strain was successfully improved by adopting a glucose-regulated *GAL* system and comprehensive metabolic engineering strategies. This work showed the prospect for microbial biosynthesis of caffeic acid and laid the foundation for constructing biosynthetic pathways of its derived metabolites.

Background

Caffeic acid, also known as 3,4-dihydroxy cinnamic acid, has attracted increasing attention due to its antioxidant [1], antivirus [2], anticancer [3, 4] and anti-inflammatory biological properties [5]. Moreover, caffeic acid is an important precursor of plant-originated aromatic chemicals like rosmarinic acid, chlorogenic acid and caffeic acid phenethyl ester [6–8]. Therefore, it shows great potential in nutritional, pharmaceutical and cosmetics industries [9]. Considering the environmental and economic benefits, biosynthesis of caffeic acid via engineering model microbes such as *Escherichia coli* and *Saccharomyces cerevisiae* provides a promising alternative to chemical synthesis or plants extraction [10].

The biosynthesis of caffeic acid starts from L-phenylalanine or L-tyrosine through the endogenous shikimate pathway [11]. In plant, the deamination of L-phenylalanine is catalyzed by phenylalanine ammonia lyase (PAL) to produce cinnamic acid. The sequential two-step hydroxylation at the 4- and 3-positions of the benzyl ring of cinnamic acid is executed by two cytochrome P450 monooxygenases, cinnamate-4-hydroxylase (C4H) and *p*-coumarate 3-hydroxylase (Coum3H) [12], forming caffeic acid through *p*-coumaric acid. In recent years, reports have sporadically emerged regarding metabolic engineering for heterogeneous caffeic acid production in *E. coli*. However, the plant-originated P450 enzymes are difficult to express in microbial systems [13]. Alternatively, tyrosine containing a 4-hydroxyl group could be directly converted to *p*-coumaric acid by microbial tyrosine ammonia lyase (TAL) [14]. For further hydroxylation of *p*-coumaric acid, the *sam5*-encoded Coum3H from the actinomycete *Saccharothrix espanaensis* [15, 16] or the cytochrome P450 CYP199A2 from the bacteria *Rhodopseudomonas palustris* [17, 18] could be used, enabling caffeic acid formation in *E. coli*. By introducing *RgTAL* from *Rhodotorula glutinis* into *E. coli* together with expressing endogenous 4-hydroxyphenylacetate 3-hydroxylase (4HPA3H) and increasing the intracellular supply of tyrosine by overexpression of PEP synthase (encoded by ppsA) and transketolase (encoded by tktA) and feedback inhibition resistant 3-deoxy-*D*-arabino-heptulosonate-7-phosphate synthase (encoded by $aroG^{fbr}$) and chorismate mutase-prephenate dehydrogenase (encoded by $tyrA^{fbr}$), the highest caffeic acid production reached 766.7 mg/L from simple carbon sources in shake flasks [19]. However, the cell growth and caffeic acid production still relied on phenylalanine supplement.

S. cerevisiae as GRAS (generally regarded as safe) organism with well-characterized genetic background, superior stress tolerance and excellent fermentation properties becomes an attractive microbial host for caffeic acid production. However, neither Coum3H nor CYP199A2 could enable caffeic acid biosynthesis in yeast [11]. The bacterial 4-hydroxyphenylacetate 3-hydroxylases (4HPA3H) complex encoded by *HpaB* and *HpaC* was also found to effectively catalyze p-coumaric acid to caffeic acid (Fig. 1) [13]. Expression of HpaB and HpaC from *E. coli* in *S. cerevisiae* led to caffeic acid production [11], and replacement of the *E. coli* enzymes with the combination of HpaB from *Pseudomonas aeruginosa* and HpaC from *Salmonella enterica* significantly improved the caffeic acid yield by 45.9-fold, leading to the highest production of caffeic acid (about 289.4 mg/L) in yeast [11]. However, this process still relied on feeding of exogenous L-tyrosine as the precursor. Li Y *et al* reported that simultaneous expression of RcTAL from *Rhodobacter capsulatus* and the P450-dependent monooxygenase C3H together with its associated cytochrome P450 reductase CPR1 from *Arabidopsis thaliana* could enable *de novo* biosynthesis of caffeic acid from glucose in *S. cerevisiae*. However, low caffeic acid production (about 11.432 mg/L) was obtained, ascribed to the low activity of C3H [20]. In both studies, episomal vectors were used for expression of caffeic acid pathway genes in *S. cerevisiae*. Considering that the yeast transformants harboring several plasmids are genetically unstable, integrating the caffeic acid pathway into the yeast genome may create a more stable cell factory.

As found with other tyrosine-derived products [21–23], the shortage of precursor supply may be another limiting factor of caffeic acid biosynthesis in *S. cerevisiae*. The critical step of shikimate pathway is the

condensation of two starter units named phosphoenolpyruvate (PEP) and 4-erythritol phosphate (E4P) by isoenzymes Aro3 and Aro4 to produce 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP) [23, 24]. In addition, chorismite, the last common intermediate for three aromatic amino acids, is transformed by the chorismate mutase Aro7 to generate prephenate, which is further divided into two branches, one towards L-phenylalanine and the other towards L-tyrosine (Fig. 1). In this pathway, the activity of Aro4 and Aro7 are feedback-inhibited by the end product tyrosine. Feedback-insensitive variant $Aro4^{K229L}$ and $Aro7^{G141S}$ have been created by rational design [25–27]. Either individual overexpression $Aro4^{K229L}$ or simultaneous expression of $Aro4^{K229L}$ and $Aro7^{G141S}$ could effectively increase intracellular shikimate, phenylalanine and tyrosine concentrations [24, 27]. On the other hand, the prephenate dehydrogenase (Tyr1) which catalyzes prephenate to α-keto acid 4-hydroxyphenylpyruvate (4HPP), the direct precursor of L-tyrosine, is transcriptionally inhibited by phenylalanine. Replacement of the native Tyr1 promoter with a constitutive one or expression of the feedback-insensitive cyclohexadienyl dehydrogenase TyrC from *Zymomonas mobilis* could both improve the production of tyrosine and its derivatives [28, 29]. Meanwhile, decarboxylation of 4HPP catalyzed by phenylpyruvate decarboxylases (encoded by *Aro10*, *Pdc1*, *Pdc5*, and *Pdc6*) would decrease the flux towards tyrosine, among which Pdc5 and Aro10 showed stronger decarboxylation activity than the others [30]. Deletion of *Aro10* and *Pdc5* increased the intracellular tyrosine production by 5.7 folds [21] .

Taken together, alleviation of feedback inhibition and removal of competitive branches could improve the precursor supply and thus contribute to further enhancement of caffeic acid synthesis. In this study, we first constructed a controllable caffeic acid biosynthetic pathway in *S. cerevisiae* by employing a modified *GAL* system. The precursor supply was then strengthened by eliminating the feedback inhibition of aromatic amino acid and down-regulating the competitive pathways, and the rate-limiting enzyme TyrC was overexpressed to enhance the flux towards caffeic acid production.

Results And Discussion

Construction of a glucose-regulated caffeic acid biosynthetic pathway in S. cerevisiae

Biosynthesis of caffeic acid could be derived from tyrosine, while *S. cerevisiae* cells do not possess the pathway downstream of tyrosine. For heterologous biosynthesis of caffeic acid, *RgTAL* (KF765779.1) from *R. glutinis*, *HpaB* (PHSS01000001.1) from *Pseudomonas aeruginosa* and *HpaC* from *S. enterica* reported with excellent performance [11, 31] were chosen as the target genes for pathway construction. To avoid the expensive galactose addition, the modified *GAL* system constructed by knocking out *GAL80* (encoding a repressor of Gal4, which confers repression in the absence of galactose) was employed herein, leading to dynamic expression of the target genes in response to glucose concentration (Fig. 2b). The codon-optimized *OHpaB* together with *HpaC* under *GAL1*/*GAL10* promoters were integrated into *GAL1-7* genomic loci of the *GAL80* deletion strain YXWP-113 [32] while the codon- optimized *ORgTAL* was localized in *DPP1* site. The metabolites of the resulting strain YCA113-2B were analyzed by high performance liquid chromatography (HPLC). A new peak with the same retention time as the caffeic acid standard was observed when comparing the spectra to those of the starting strain YXWP-113 (Fig. 2a).

Time courses of YCA113-2B showed that caffeic acid production started upon depletion of glucose and finally accumulated to 313.8 mg/L (38.5 mg/g DCW) in the medium after 72 h shake-flask culture (Fig. 2c). This yield was slightly higher than the previous highest production (about 289.4 mg/L) reported in *S. cerevisiae* by employing same species originated HpaB and HpaC with this study [11] and much higher than the production reported by Li Ye *et al* [20]. The differences in promoters, terminators, functional genes and fermentation media between the study of Li Y *et al* [20] and ours might be responsible for the different caffeic acid production in *S. cerevisiae*.

Elimination of feedback inhibition of DAHP synthases and chorismate mutase

The biosynthesis of tyrosine, the direct precursor of caffeic acid, is strictly regulated in *S. cerevisiae*. Therefore, relieving feedback inhibition of tyrosine may lead to improved caffeic acid production. After deletion of *Aro3* gene inhibited by phenylalanine, little effect on caffeic acid accumulation and cell growth was observed (Fig. 3). Thus, *Aro3* can be used as a target locus for gene integration. Subsequently, tyrosine feedback inhibition insensitive mutant $Aro4^{K229L}$ under *GAL1* promoter was integrated into *Aro3* site by using the linearized PUMRI-Δ*Aro3*- $Aro4^{K229L}$ vector. The resulting strain YCA113-4B produced 537.7 mg/L of caffeic acid, which was about 79.5% higher before $Aro4^{K229L}$ overexpression (Fig. 3). However, the biomass of YCA113-4B decreased by 14.7% compared with YCA113-3B.

In addition, chorismate mutase Aro7 is another key enzyme feedback-inhibited by tyrosine. Therefore, $Aro7^{G141S}$ which abolished the tyrosine feedback inhibition properties was overexpressed in YCA113-4B strain, which slightly increased the caffeic acid level in the resulting strain YCA113-5B (551.8 mg/L). The production improvement after alleviation of feedback inhibition regulation was not as significant as anticipated, which may be ascribed to existing downstream bottlenecks.

Deletion of competing pathway genes and overexpression of rate-limiting caffeic acid synthetic enzyme

To further improve the production of caffeic acid, the rate-limiting steps in the synthetic pathway were eliminated by overexpression of the corresponding enzymes, and the carbon flux was redirected to the target pathway by deletion of the competitive pathways. Phenylpyruvate decarboxylase (Aro10) is a key enzyme responsible for channeling tyrosine flux to tyrosine degradation pathway. Thus, *Aro10* gene was deleted in YCA113-5B strain to reduce the competitive consumption of 4-HPP. The resulting strain YCA113-6B produced about 625.0 mg/L of caffeic acid, which was improved by 13.3% compared with YCA113-5B, suggesting that 4-HPP is indeed a key intermediate in the caffeic acid biosynthetic pathway. The transcription of the native *Tyr1* gene is tightly regulated by phenylalanine concentration [28], which limited the conversion of prephenate to 4-HPP. Therefore, feedback-insensitive TyrC from *Z. mobilis* was integrated into the *Aro10* genomic loci of YCA113-5B to deregulate this reaction. The resulting strain YCA113-8B produced 769.3 mg/L caffeic acid, which was 23.1% and 145.2% higher than those of YCA113-6B strain and YCA113-2B, respectively (Fig. 4). In addition, we also tested the impact of deleting the 4-HPP-consuming pyruvate decarboxylase (encoded by *Pdc5*) in strain YCA113-8. However, no

significant production improvement was observed in the resulting strain YCA113-10. which might be resulted from the insufficient downstream pathway in charge of precursor conversion to caffeic acid. Therefore, strengthened downstream pathway is required for further improvement of caffeic acid production.

Conclusion

In this work, *S. cerevisiae* was engineered to *de novo* biosynthesis of caffeic acid from glucose. The introduction of codon-optimized *ORgTAL* from *R. glutinis* together with the combination of *OHpaB* from *P. aeruginosa* and *HpaC* from *S. enterica* under modified *GAL* regulation system led to glucose-regulated production of 313.8 mg/L caffeic acid. Further optimizations combining the relief of tyrosine feedback inhibition, reduction of carbon flux diversion into competing pathways and overexpression of rate-limiting pathway enzyme improved the caffeic acid production to 769.3 mg/L, which was 2.7-fold higher than the highest ever reported caffeic acid production by engineered *S. cerevisiae* strain. The yeast strains obtained in this work would lay a foundation for microbial hyperproduction of caffeic acid and its derivatives. Future efforts toward enhanced caffeic acid production may include cascade assembly of downstream pathway enzymes for enhanced catalytic efficiency and engineering of heterogeneous enzymes toward improved activity.

Materials And Methods

Strain, media and reagents

E. coli DH5α (Novagen, USA) was used for propagation of recombinant plasmids. Luria-Bertani broth (LB) medium (0.5% Bacto yeast extract, 1% Bacto-tryptone [Difco Laboratories], 1% NaCl, pH 7.0) containing 50 μg/mL of kanamycin or 100 μg/mL of ampicillin was used for culturing *E. coli* carrying transformed vectors. *S. cerevisiae* strain BY4741 was used as the host for constructing the caffeic acid biosynthetic pathway. YPD medium (1% yeast extract, 2% peptone and 2% glucose) was used for cultivation of *S. cerevisiae* strains. Geneticin (G418, 200 μg/mL) was supplemented in YPD agar for selection of engineered yeast strain. SD (synthetic complete drop-out medium, 20 g/L D-glucose, 5 g/L ammonium sulfate, 1.7 g/L yeast nitrogen base) supplemented with 100 mg/L uracil, 100 mg/L L-histidine-HCL, 100 mg/L L-methionine, 150 mg/L L-leucine, 150 mg/L L-lysine-HCL and 1 mg/ml of 5-Fluoroorotic acid was used for selection of recombinants with *KanMX-URA-PRB322ori* marker excision.

Deletion of Aro3, Aro10 **and** Pdc5 **genes**

Aro3-UP homologous arm of *Aro3* was amplified with the primers *Aro3-upF* & *Aro3-upR*. Downstream homologous arm of *Aro3* was amplified with the primers *Aro3-DnF* & *Aro3-DnR*, generating *Aro3-Down*. The *Aro3* homologous arms were obtained by overlap extension PCR using *Aro3-upF* and *Aro3-DnR* as the primers and *Aro3-UP* and *Aro3-Down* as the templates. The resulting segment was fused with plasmid backbone amplified from pUMRI-11 (KM216413) [33]with the primers *p21-F1* and *p21-R1* by *in vitro*

homologous recombination, generating pUMRI-Δ*Aro3*. Similarly, pUMRI-Δ*Aro10* and pUMRI-Δ*Pdc5* plasmids containing the homologous arms of *Aro10* and *Pdc5* respectively were constructed. The recombinant plasmids were digested with *Sfi*I and integrated into the corresponding loci to delete *Aro3*, *Aro10* or *Pdc5* respectively. The primers are listed in Table S1.

Genes Amplification And Recombinant Plasmids Construction

For construction of the caffeic acid synthetic pathway, *RgTAL* from *R. glutinis*, *HpaB* from *P. aeruginosa* and *TyrC* from *Z. mobilis* were codon-optimized according to the preferred codon usage of *S. cerevisiae* using JCAT tools (http://www.jcat.de/) and synthesized by Generay Biotech (Shanghai, China). *HpaC* was amplified from *S. enterica* C50336 using HpaC-F(*EcoR* I) and HpaC-R (*Bgl* II) primers. The detailed sequences of *ORgTAL*, *OHpaB*, *HpaC* and *OTyrC* are shown in the supporting information. *Aro4* and *Aro7* were amplified from the genome of *S. cerevisiae*. The series of pUMRI plasmids were used to integrate the pathway genes into different genomic loci of *S. cerevisiae.* All the primers and detailed information of plasmids used in this study are listed in Table S1 and Table 1.

Table 1
Plasmids constructed in this study

Plasmids[a]	Genotype/ Description	Reference
pUMRI-11	*loxp-KanMX-URA3-pbr322ori-loxp*, T_{ADH1}-*MCS1*-P_{GAL10}-P_{GAL1}-*MCS2*-T_{CYC1}, *DPP1* homologous arm	KM216413
pUMRI-13	*loxp-KanMX-URA3-pbr322ori-loxp*, T_{ADH1}-*MCS1*-P_{GAL10}-P_{GAL1}-*MCS2*-T_{CYC1}, *GAL1-7* homologous arm	KM216415
PUMRI-13-*OHpaB-HpaC*	*loxp-KanMX-URA3-pbr322ori-loxp*, T_{ADH1}-*HpaC*-P_{GAL10}-P_{GAL1}-*OHpaB*-T_{CYC1}, *GAL1-7* homologous arm	This study
PUMRI-11-*ORgTAL*	*loxp-KanMX-URA3-pbr322ori-loxp*, T_{ADH1}-*MCS1*-P_{GAL10}-P_{GAL1}-*ORgTAL* -T_{CYC1}, *DPP1* homologous arm	This study
pUMRI-Δ*Aro3*	*loxp-KanMX-URA3-pbr322ori-loxp*, T_{ADH1}-*MCS1*-P_{GAL10}-P_{GAL1}-*MCS2*-T_{CYC1}, *Aro3* homologous arm	This study
pUMRI-Δ*Aro10*	*loxp-KanMX-URA3-pbr322ori-loxp*, T_{ADH1}-*MCS1*-P_{GAL10}-P_{GAL1}-*MCS2*-T_{CYC1}, *Aro10* homologous arm	This study
pUMRI-Δ*Aro3*-Aro4^{K229L}	*loxp-KanMX-URA3-pbr322ori-loxp*, T_{ADH1}-*MCS1*-P_{GAL10}-P_{GAL1}-Aro4^{K229L}-T_{CYC1}, *Aro3* homologous arm	This study
pUMRI-Δ*Aro3*-*Aro4*^{K229L} - *Aro7*^{G141S}	*loxp-KanMX-URA3-pbr322ori-loxp*, T_{ADH1}-Aro7^{G141S}-P_{GAL10}-P_{GAL1}- Aro4^{K229L}-T_{CYC1}, *Aro3* homologous arm	This study
pUMRI-Δ*Aro10*-*OTyrC*	*loxp-KanMX-URA3-pbr322ori-loxp*, T_{ADH1}-*OTyrC*-P_{GAL10}-P_{GAL1}-*MCS2* -T_{CYC1}, *Aro10* homologous arm	This study
pUMRI-Δ*Pdc5*	*loxp-KanMX-URA3-pbr322ori-loxp*, T_{ADH1}-*MCS1*-P_{GAL10}-P_{GAL1}-*MCS2*-T_{CYC1}, *Pdc5* homologous arm	This study

Construction Of Caffeic Acid-producing Yeast Strains

The starting strain YXWP-113 was constructed in previous work by knocking out the *GAL80* gene of BY4741 via homologous recombination and using the *LEU2* marker for auxotroph selection [34]. The recombinant pUMRI plasmids carrying pathway genes linearized with *Sfi* I were sequentially transformed into YXWP-113 using the LiAc/SS carrier DNA/PEG method [35] and the resulting strains were selected by G418. The genes were integrated into different genomic loci, such as *GAL1-7* (ChrII 274632–279536), *DPP1* (ChrIV 1031419 – 1030550), *Aro3* (Chr IV 521939–522785), *Aro10* (Chr IV1233993-1235961) and *Pdc5* (chr XII 410723–412414). The detailed information of the yeast strains are shown in Table 2.

Table 2
Strains used in this study

Strain	Genotype/ Description	Reference
BY4741	*MAT*a, *his3*Δ*1,leu2*Δ*0, met15*Δ*0, ura3*Δ*0*	[36]
YXWP-113	BY4741, Δ*gal80*::*LEU2*	[32]
YCA113-2B	YXWP-113, Δ*gal1-7*::T_{ADH1}-*HpaC*-P_{GAL10}-P_{GAL1}-*OHpaB*-T_{CYC1}, Δ*dpp1*:: T_{ADH1}-*MCS1*-P_{GAL10}-P_{GAL1}-*ORgTAL*-T_{CYC1}	This study
YCA113-3B	YCA113-2B, *ΔAro3*::T_{ADH1}-*MCS1*-P_{GAL10}-P_{GAL1}-*MCS2*-T_{CYC1}	This study
YCA113-4B	YCA113-3B, *ΔAro3*::T_{ADH1}-*MCS1*-P_{GAL10}-P_{GAL1}- $Aro4^{K229L}$-T_{CYC1}	This study
YCA113-5B	YCA113-4B, *ΔAro3*::T_{ADH1}-$Aro7^{G141S}$-P_{GAL10}-P_{GAL1}- $Aro4^{K229L}$-T_{CYC1}	This study
YCA113-6B	YCA113-5B, *ΔAro10*:: T_{ADH1}-*MCS1*-P_{GAL10}-P_{GAL1}-*MCS2*-T_{CYC1}	This study
YCA113-8B	YCA113-5B, *ΔAro10*:: T_{ADH1}-*OTyrC*-P_{GAL10}-P_{GAL1}-*MCS2*-T_{CYC1}	This study
YCA113-10B	YCA113-8B, *ΔPdc5*:: T_{ADH1}-*MCS1*-P_{GAL10}-P_{GAL1}-*MCS2*-T_{CYC1}	This study

Cultivation In Shaking Flasks

Single colonies were picked from YPD agar plate, inoculated into 5 ml YPD culture tubes and incubated overnight at 30 °C in a rotary shaker (220 rpm). The seed cultures were then transferred into 50 ml fresh YPD medium to an initial OD_{600} of 0.05 and grown under the same conditions for 72 h. For measurement of dry cell weight, 2 mL cultures were harvested by centrifugation at 12000 rpm for 2 min, the yeast cells were washed twice, and then dried at 95℃ to a constant weight the.

Analysis Of Caffeic Acid

For caffeic acid quantification, the supernatant medium was collected after fermentation by centrifugation at 12000 rpm for 5 min. The samples were diluted with ultrapure water and filtered before analysis on an HPLC (Agilent 1200) system equipped with Pntulips QS-C18 PLUS column (4.6 × 250 mm, 5 μm, puningtech). Gradient program was performed with solvent A (water containing 0.1% (v/v) formic acid) and solvent B (acetonitrile) as the mobile phase. The program started with 90% of solvent A and 10% of solvent B. The concentration of solvent B subsequently increased to 30% within 15 min, continued

up to 50% at 30 min. The flow rate was 1 mL/min with constant column temperature at 35℃ and caffeic acid was detected at 320 nm.

Abbreviations

PAL: phenylalanine ammonia lyase; C4H:cinnamate-4-hydroxylase; Coum3H:*p*-coumarate 3-hydroxylase; TAL:tyrosine ammonia lyase; 4HPA3H:4-hydroxyphenylacetate 3-hydroxylases; GRAS:generally regarded as safe; PEP:phosphoenolpyruvate; E4P:4-erythritol phosphate; DAHP:DAHP:3-deoxy-D-arabino-heptulosonate-7-phosphate;; EPSP:5-enolpyruvyl-3-shikimate phosphate; Tyr1:Prephenate dehydrogenase; 4HPP:4-hydroxyphenylpyruvate; Aro10:Phenylpyruvate decarboxylase; TyrC:cyclohexadienyl dehydrogenase from *Zymomonas mobilis*; HPLC:High-pressure liquid chromatography.

Declarations

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Authors' contributions

PPZ conceived and designed the experiments. PPZ and CLY performed the experiments. BS, YD and NNX assisted in experiments. PPZ and CLY wrote and edited the manuscript. LDY contributed materials and revised the manuscript. All authors read and approved the final manuscript

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Availability of date and materials

All date generated or analyzed during this study are included in this article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

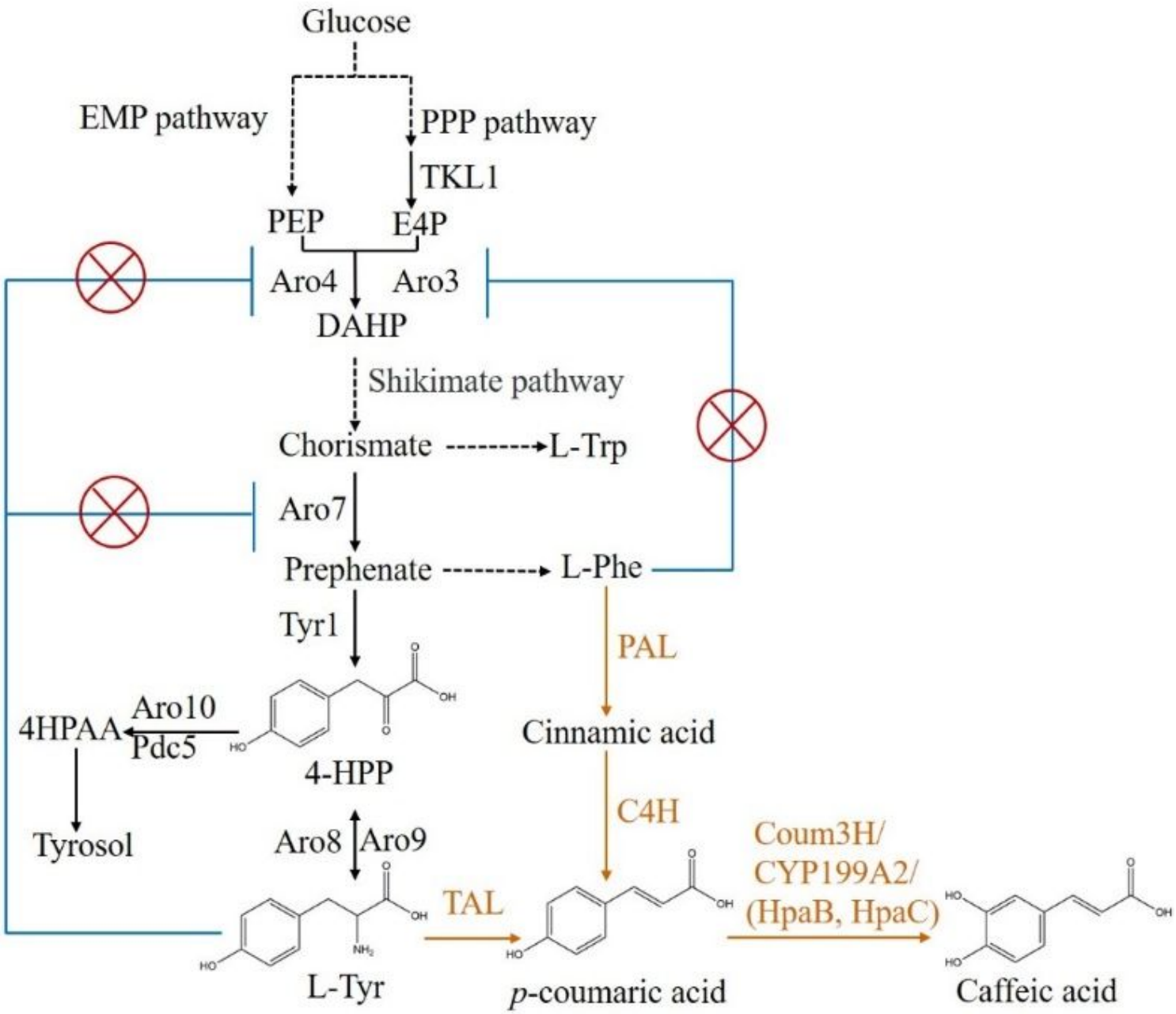

Figure 1

De novo biosynthetic pathway of caffeic acid from glucose in S. cerevisiae. EMP pathway, Embden-Meyerhof-Parnas pathway; PPP pathway, pentose phosphate pathway; PEP, phosphoenolpyruvate; E4P, 4-erythritol phosphate; DAHP, 3-deoxy-D-arabino-heptulosonate-7-phosphate; L-Trp, tryptophan; L-Phe, Phenylalanine; L-Tyr, tyrosine; 4-HPP, p-hydroxyphenylpyruvate; 4HPAA, 4-hydroxyphenylacetaldehyde; Dotted lines indicated multi-enzyme reactions.

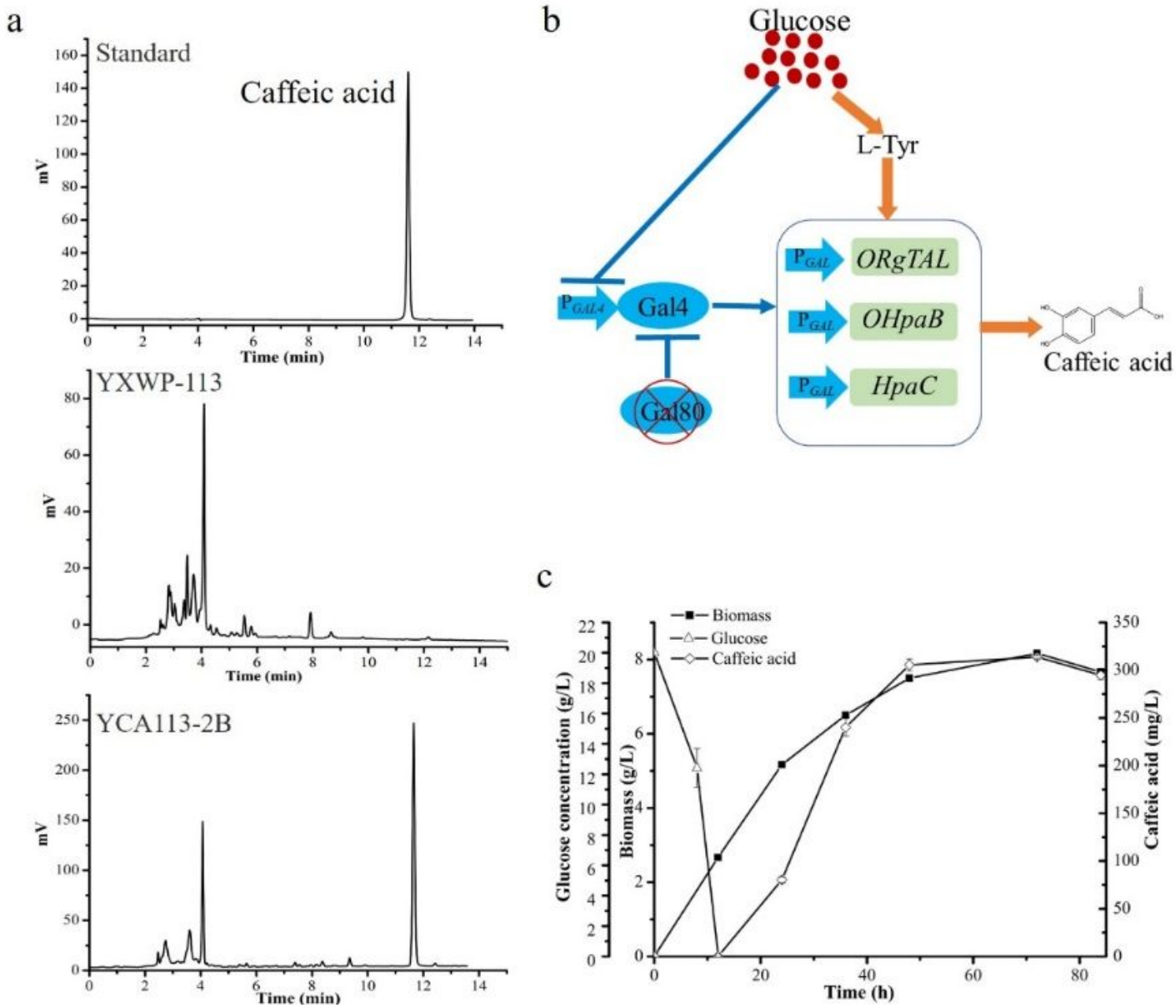

Figure 2

Caffeic acid production of S. cerevisiae by introducing ORgTAL, OHpaB and HpaC genes. a, HPLC analysis of the caffeic acid standard and the culture of the starting strain YXWP-113 and YCA113-2B. b, The modified GAL regulation system for expression control of heterogeneous caffeic acid pathway genes. c, Time courses of cell growth, glucose consumption and caffeic acid production of YCA113-2B. Error bars represent standard deviations from three independent experiments.

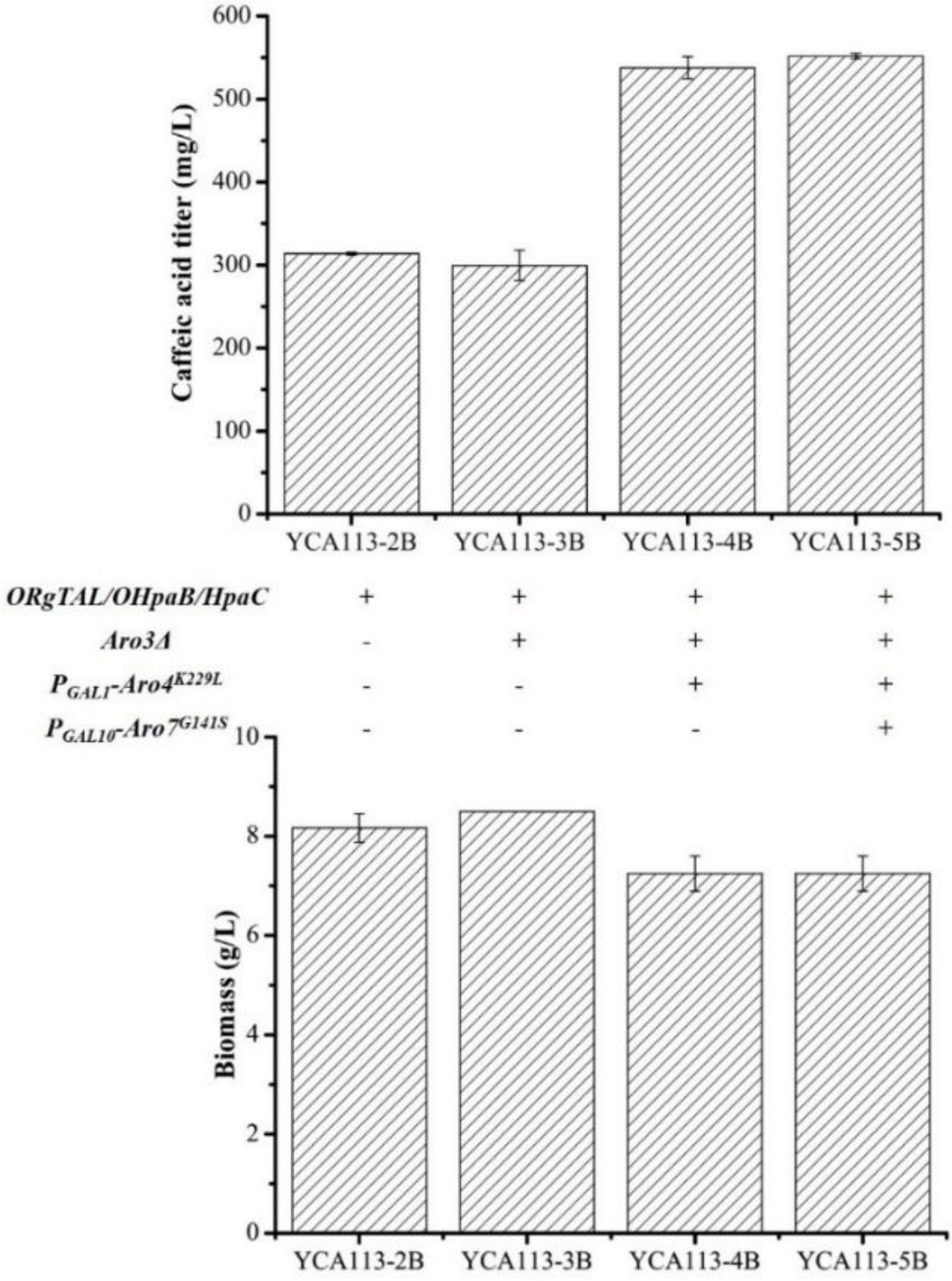

Figure 3

The effect of reliving the tyrosine feedback inhibition on the biomass and caffeic acid production. YCA113-3B was constructed by knockout of Aro3 gene in YCA113-2B; YCA113-4B was constructed by overexpression of tyrosine feedback-insensitive ARO4K229L in YCA113-3B; YCA-5B was constructed by overexpression of tyrosine feedback-insensitive Aro7G141S in YCA113-4B.

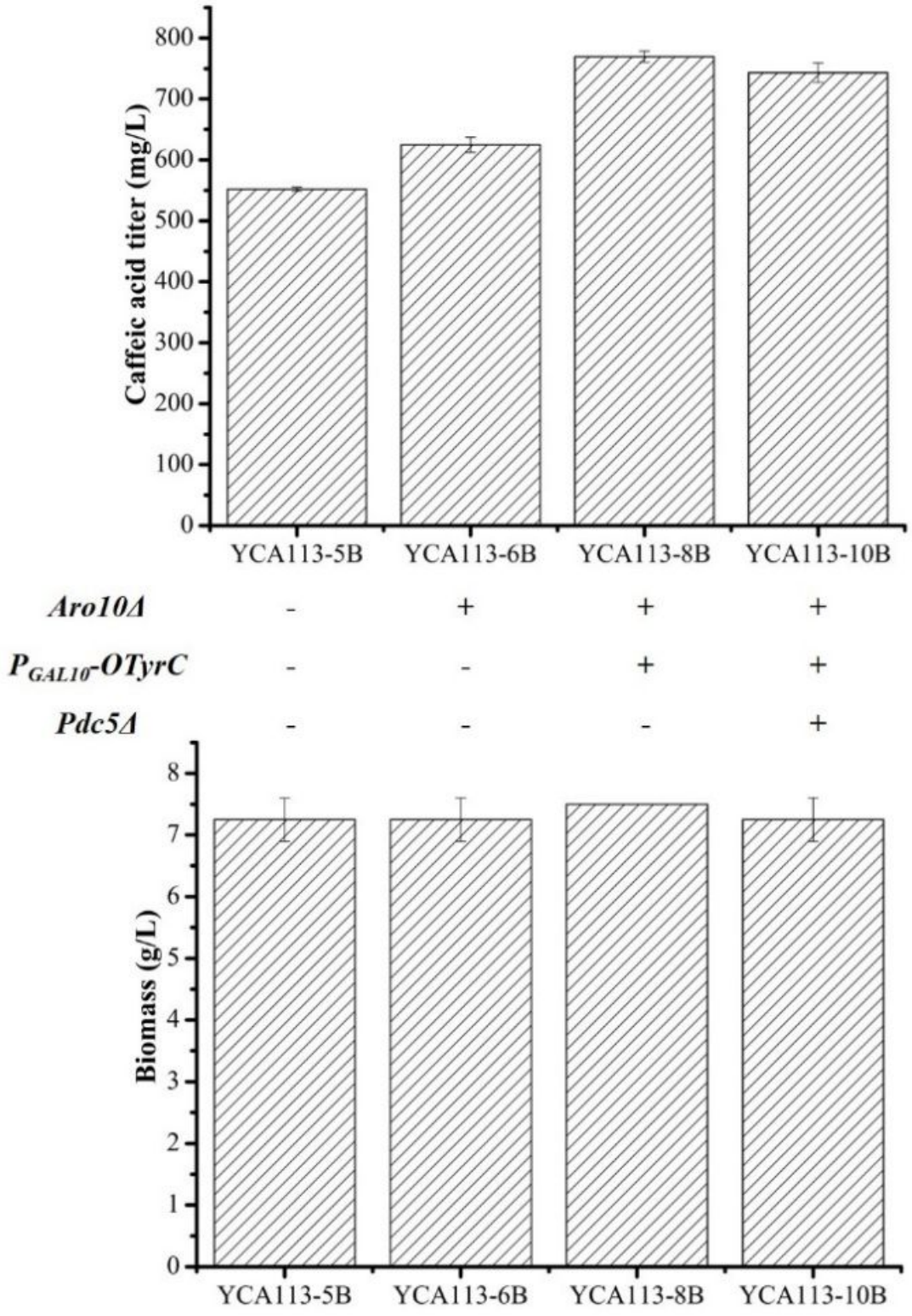

Figure 4

The effect of deleting competing pathway genes and overexpressing rate-limiting enzymes on the biomass and caffeic acid production. YCA113-6B was constructed by knockout of Aro10 gene in YCA113-5B; YCA113-8B was constructed by overexpression of codon-optimized TyrC gene from Z. mobilis in YCA113-6B; YCA-10B, deletion of Pdc5 gene in YCA113-8B.

Supplementary Files

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