Improvement of Xylose Utilization and L-ornithine Production by Metabolic Engineering of *Corynebacterium glutamicum*

Ge Gao
East China University of Science and Technology
Yan Zhang
East China University of Science and Technology
Ying Zhou (zhouying@ecust.edu.cn)
East China University of Science and Technology https://orcid.org/0000-0001-9241-1293
Bang-Ce Ye
East China University of Science and Technology

Research

Keywords: L-ornithine, MCorynebacterium glutamicum, Xylose, Metabolic engineering

Posted Date: September 22nd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-81006/v1

Abstract

Background: L-ornithine is a basic amino acid, which shows significant value in food and medicine industries. Xylose is the most important alternative carbon source of glucose in lignocellulosic hydrolysate. It is urgent to develop a high-efficiency cell factory for L-ornithine production with glucose and xylose.

Results: In this study, the genes enconding xylose isomerase and xylulose kinase were introduced into *Corynebacterium glutamicum* S9114 to establish xylose metabolism pathway, and then xylose became a substitute carbon source of glucose. In addition, the optimization and overexpression of phosphoenolpyruvate carboxylase and pentose transporter had been conducted to promote the synthesis of L-ornithine for the first time. Furthermore, though optimizing the concentration ratio of glucose and xylose (7:3), adding biotin and thiamine hydrochloride, we arrived at the highest L-ornithine yield 41.5g/L in shaking flask fermentation so far.

Conclusions: Our results demonstrate that the combination of metabolic engineering and the optimization of fermentation process can make great potential for L-ornithine production by lignocellulose hydrolysate.

Background

L-ornithine is a non-proteinogenic amino acid, which is an important intermediate product in the urea cycle. In this cycle, L-ornithine is a key precursor for the production of L-citrulline, L-proline and L-arginine, and the downstream product of L-ornithine is glutamate. L-ornithine has been attracted great attention for its biological functions [1] such as improving visual function [2], regulating the secretion of hormones [3], and is widely used in daily health and medical care such as the positive effect on the protection and vitality recovery of human liver and heart [4–6]. In addition, a recent report demonstrates that L-ornithine has a great prospect for the development of new anticancer drugs as raw material [7].

Corynebacterium glutamicum is the most important and mature strain to produce amino acids such as glutamate, L-alanine, L-serine, L-arginine, and L-proline [8–12] in the past forty years. Although *E. coli* and *Saccharomyces cerevisiae* strains had ever been engineered to be the producer of L-ornithine [13–15], *C. glutamicum*, which produces large amounts of glutamate, the precursor of L-ornithine, still to be the preferred dominant strain [16]. Because the concentration of intracellular glutamate is an important driving force for L-ornithine production in microbial strains. Random mutation together with genetic and metabolic engineering had been the common strategies in the breeding process for L-ornithine production by *C. glutamicum*. However, the random mutation breeding has some disadvantages, including the complex screening process, low success rate and high probability of reverse-mutation. Those made directed genetic metabolic engineering to be the priority option. Consequently, a series of measures were taken to increase the L-ornithine production by genetic engineering modification [17]. The general strategy of genetic engineering focused primarily on the main L-ornithine synthesis pathway [18–23, 12]; the

transportation of L-ornithine amino acids [19]; glycolysis, acetic acid metabolism and pentose phosphate pathway [24, 25], together with the tricarboxylic acid cycle and glucose utilization pathway [26–31]. In addition to rational modification, adaptive evolution strategies combined with transcriptional levels analysis provides another strategy to develop a strain with high performance [32]. Jensen et al. constructed *C. glutamicum* ORN6 by knocking out *argF*, *argR* and *argG*, attenuating the expression of *pgi* and increasing the copy number of the arginine operon argCJB$^{A49V, M54V}$D on the chromosome, the L-ornithine yield reached 0.52 g/g [12]. Hwang et al. knocked out *ncgl2053*, *ncgl0281* and *ncgl2582* that encoding $NADP^+$-dependent oxidoreductase, which resulted in the loss of glucose dehydrogenase activity and the improvement of 6-phosphate gluconate dehydrogenase activity, the production of L-ornithine was 66.3% higher than that of the starting strain [33]. Shu et al.deleted *proB* and *argF* to block the branch of the L- ornithine synthesis pathway, mutated *ArgB* and expressed heterologous *argA* and *argE* to introduce an artificial linear transacetylation pathway, which had increased the production of L-ornithine up to 40.4 g/L in 5-L bioreactor [34]. Zhang et al adopted a series of genetic engineering modifications to achieved the maximum L- ornithine yield of 43.6 g/L in fed batch fermentation by far [26]. Although many strategies have been adapted to increase the production of L-ornithine, how to build a more efficient industrial strain with practical applications is still a long way off.

In order to take full advantage of abundant renewable resources, many studies have been focused on how to construct an efficient microbial cell factory utilizing xylose and glucose as mixed carbon sources in the past fifteen years [35]. Thanks to the weak carbon catabolite repression, *C. glutamicum* was regarded as a major industrial force with great potential in recent years [36, 37]. However, due to lacking of xylose isomerase (XylA), *C. glutamicum* could not grow in medium containing xylose as the sole carbon source. Buschke et al. and Gopinath et al. used the exogenous xylose isomerase (XylA) and xylulose kinase (XylB) to establish the isomerase pathway in *C. glutamicum* [38, 39]. Five copies of *xylAB* operon from *E. coli* were integrated to *C. glutamicum* R chromosome to generate the strain X5C1 which could consume 40 g/L glucose and 20 g/L xylose in 12 hours [40]. In addition, several other strategies were adopted to improve the xylose utilization, including the introduction of arabinose transporter [41, 42], overexpressing of TAL/TKT in the pentose phosphate pathway [43]. Meiswinkel et al. constructed an engineered strain *C. glutamicum* PUT21 by introducing *xylA* from *X. campestris*, *xylB* from *C. glutamicum* and *argBAD* operon from *E. coli* to produce 2.59 g/L of L-ornithine, and the volumetric ornithine productivity was 43.2 mg/ mg/(L·h) [44]. In addition to xylose isomerase metabolic pathway, Christian et al. introduced the *xylXABCD* operon from *Caulobacter crescentus* into *C. glutamicum* ATCC13032 to establish the Weinberg pathway [42]. Although the utilization of xylose by *C. glutamicum* has already been realized, the utilization rate of xylose is still unsatisfied. More modified strategies are needed to improve the utilization rate of xylose and the production of L-ornithine., which opened the door to the efficient utilization of lignocellulose.

In our previous studies, we have successfully constructed the *C. glutamicum* SO26 with high L-ornithine yield [19, 26]. In this study, we attempted to utilize the most abundant carbon source in lignocellulose

hydrolysate - glucose and xylose. The approaches of metabolic engineering and fermentation process control were adopted to accelerate the xylose consumption rate and the yield of L-ornithine. Firstly, a more efficient *xylAB* operon was screened out from different strains, and the arabinose transporter *araE* from *Bacillus subtilis* was knocked into the *iolR* locus under the promoter P_{eftu}. Secondly, the acetylation of phosphoenolpyruvate carboxylase (PEPC) was reduced to release the feedback inhibition of aspartic acid, and a strong constitutive promoter P_{H36} was introduced in the upstream of *pepc*. The strain after a series of modulations was named *C. glutamicum* XAB03. After the optimization of fermentation process, we have found that the optimal concentration ratio of glucose and xylose(7:3) and the addition of coenzyme (biotin 0.9 μM and thiamine-HCl 15 μM) could reach up to the highest yield of L-ornithine 41.5 g/L in shaking flask fermentation up to date. The metabolic engineering processes had been illustrated in Fig. 1. Schematic diagram.

Results And Discussion

Comparison with the xylose utilization capacity of xylose isomerases and xylulose kinases from different carbon sources

Xylose isomerase (*xylA*) and xylulose kinase (*xylB*) exist in the form of gene clusters. The xylose isomerase metabolic pathway from different sources established in *C. glutamicum* have different xylose utilization capacities, this might be the result of the genetic codon preference between the *xylAB* source strain and *C. glutamicum* [44]. The *xylAB* genes derived from *E. coli* MG1655 and *X. campestris* were expressed under IPTG induced vector pXMJ19, in which the P_{tac} is the promoter for gene expression. The resulting expression plasmids were labeled $p19P_{lac}$-EcoAB and $p19P_{tac}$-XcaAB. The two plasmids were transformed into *C. glutamicum* SO26 to obtain the *C. glutamicum* EAB and XAB. Figure 2a showed the growth curve and xylose consumption of strain EAB and XAB during 72 hours fermentation. The results indicated that *xylAB* from *X. campestris* achieved higher xylose consumption (45.1 g/L) in comparison with *xylAB* from *E. coli* MG1655 (25.0 g/L). The growth OD_{600} value increased from 12.05 (EAB) to 12.95 (XAB). The L-ornithine concentration in the supernatant of fermentation was determined as shown in Fig. 2b. The L-ornithine production titer of the strain XAB (21.6 ± 0.19 g/L) was 18.7% higher than strain EAB (18.2 ± 0.35 g/L), and the corresponding xylose yield of XAB was 0.48 g/g. The *C. glutamicum* XAB had been performed the better capacity of xylose utilization and L-ornithine synthesis than strain EAB.

Knocking-in the pentose transporter demonstrates the promotion of xylose utilization and L-ornithine production

In order to increase the rate of xylose consumption, the pentose transporter gene (*araE*) from *Bacillus subtilis* was integrated into the genome locus of *iolR* with the strong P_{eftu} promoter, to generate the strain termed as XAB01. The pentose transporter AraE is not only extremely significant for the arabinose transportation, but also promotes the transportation of xylose to cells [45]. As expected, the results showed that strain XAB01 demonstrated superior xylose consumption and L-ornithine synthesis than strain XAB (Fig. 3a and 3b). strain XAB01 consumed 47.9 g/L xylose after 72 h fermentation, with an

average consumption rate of 0.665 g/(L·h). Through the rapid utilization of xylose, the growth and L-ornithine yield of strain were also improved. The output of L-ornithine increased by 12.5% (24.3 ± 0.23 g/L) compared with strain XAB, and the xylose yield was 0.51 g/g.

Effect of modification of phosphoenolpyruvate carboxylase on the production of L-ornithine

Phosphoenolpyruvate carboxylase (PEPC) is an enzyme in the glycolysis pathway of *C. glutamicum* and plays an important role in the regulation of the TCA cycle. Phosphoenolpyruvate and carbon dioxide synthesize oxaloacetic acid under the catalysis of PEPC, and then oxaloacetic acid enters the TCA cycle for further metabolism. The expression and activity of PEPC affects the synthesis of glutamic acid in *C. glutamicum* [46, 47]. Lysine at position 653 (K653) is essential for the regulation of PEPC acetylation. Megumi ed al. found that acetylation of PEPC at K653 could decrease enzymatic activity and glutamate production, which refer to the K653-acetylation could regulate PEPC activity negatively. Mutated K653 into arginine could decrease the level of acetylation on PEPC, which correspondingly improve its activity [47]. In addition, PEPC would be feedback suppressed by aspartic acid when it was overexpressed in *C. glutamicum*. The inhibitory effect could be effectively reduced when the aspartic acid at position 299 was mutated to asparagine with a similar structure [46]. In our study, we attempted to combine the weakening acetylation by K653R and the attenuating feedback inhibition by D299N of PEPC to enhance the activity of this enzyme, leading to promote the glutamate synthesis and the L-ornithine yield. The preferred CGC and AAC codons in *C. glutamicum* S9114 were chosen to mutate K653 and D299. At the same time, the *pepc* gene was overexpressed by adding a strong constitutive promoter H36 [48]. Multiple fragments had been fused into a complete fragment by PCR overlapping amplification technology. After two-point mutations in *pepc*, strain XAB03 was constructed. The results of shaking flask fermentation of strain XAB03 have showed that the production of L-ornithine was 27.1 ± 0.32 g/L, which was 11.5% higher than that of strain XAB01 (Fig. 4b). Moreover, this strain had a positive effect on the growth of *C. glutamicum* (Fig. 4a). These results indicated the importance of PEPC in L-ornithine synthesis, and the modification strategies were available and effective for the synthesis of L-glutamate, L-citrulline and L-arginine.

Addition of biotin and thiamine hydrochloride accelerates the synthesis of L-ornithine

In addition to the modification of key enzymes in metabolic pathways, the addition of key coenzymes is also an important method to promote the synthesis of products [49]. Biotin and thiamine hydrochloride are the coenzymes of carboxylase in the metabolic process. Biotin plays an important role in the metabolism of bacterial proteins, which could change the content of cell membrane components and permeability. Different concentrations of biotin affect the transcription levels of enzymes and promote the synthesis of glutamate [49, 50]. In order to further optimize the fermentation process, we attempted to adding the coenzyme during the L-ornithine production. The optimal concentration of biotin and thiamine hydrochloride in the fermentation medium was 0.9 μM and 15 μM [51], and the L-ornithine production increased to 33.4 g/L compared with no adding coenzyme fermentation (27.1 g/L) (Fig. 5). During the fermentation period from 0–48 hours in shake flasks, the strain XAB03 had been grown with abundant nutrients, the energy distribution mainly distributed on the growth of the bacteria while the production of

L-ornithine was weak. From 48–72 hours, the growth was tended to be stable and the L-ornithine was synthesized rapidly. The results provided a research direction for the promotion of certain target products through the addition of coenzymes and some small molecules.

Effects of a combination of glucose and xylose on L-ornithine

Metabolism of different sugars is an important basement for the lignocellulose utilization. Glucose and xylose are the most important six- and five-carbon sugars in the hydrolysis of lignocellulose. We compared L-ornithine yield in different ratios of glucose and xylose in the case of the total sugar concentration was constant. On the premise that the total sugar concentration was 100 g/L, seven groups of different glucose concentration gradients were chosen (Fig. 5). The mixed carbon sources showed the advantage of L-ornithine yield compared with using glucose or xylose as the sole carbon source. The results showed that the maximum L-ornithine production could be obtained (41.5 ± 0.02 g/L) when glucose was 70 g/L and xylose was 30 g/L (Fig. 5b, c).

Based on all the above conditions, the OD_{600} and L-ornithine production of strain XAB03 could reach to 16.8 ± 0.19 and 41.5 g/L (Fig. 6c) respectively after shake flask fermentation for 72 hours, which was the highest titer so far to the best of our knowledge (Table 2), 7.8% higher than Zhang et al. had reported, and increased by 43.4% compared with the sole xylose carbon source [26]. The 2:1 ratio was close to the ratio of glucose to xylose after lignocellulose hydrolysis. This result lays a foundation for the feasibility and superiority of L-ornithine synthesis from lignocellulose hydrolysate.

Table 2
Synthesis of L-ornithine by *C. glutamicum*

Strains	L-ornithine concentration ($g \cdot L^{-1}$)/yield	Cultivation	Sugar source	Modulations	References
C. glutamicum SJC8514	12.48/ND	Shake flask; batch	Glucose	Overexpression of *ncgl0452* and *argCJBD*mut	[22]
C. glutamicum SJC8039	14/ND	Shake flask; batch	Glucose	Deletion of *argF*, *argR*, and *proB*; Blocking gluconate biosynthesis	[33]
C. glutamicum YW06	51.5/0.240	Bioreactor; fed-batch	Glucose	Deletion of *argF*, *argR* and *proB*; Reinforcement of the PPP pathway flux; The use of a feedback-resistant enzyme	[25]
C. glutamicum ORN1 (pVWEx1-*araBAD*)	25.8/0.78	Shake flask	Glucose, arabinose	Deletion of *argF*, *argR*; in-frame deletion of *argF* and *argR*, auxotrophic for l-arginine	[53]
C. glutamicum ΔAPE6937R42	24.1/0.298	Bioreactor; batch	Glucose	Deletion of *argF*, *argR*, and *proB*; Adaptive evolution in presence of L-ornithine	[32]
C. glutamicum Δ*APE::rocG*	14.8/ ND	Shake flask; batch	Glucose	Deletion of *argF*, *proB*, *speE*::Ptac-M-*rocG*, *argR*::Ptac-M-*gapC*	[23]
C. glutamicum ORN6	20.96/0.524	Shake flask; batch	Glucose	Deletion of *argF*, *argR*, and *argG*; overexpression of *argBM*; attenuation of *pgi*	[12]
C. glutamicum 1006 *ΔargR-argJ*	31.6/0.396	Shake flask; batch	Glucose	Deletion of *argR*; overexpression of *argJ*	[20]
C. glutamicum XAB03	41.5/ND	Shake flask; batch	Glucose, xylose	*C. glutamicum* SO26 with *xylAB* from *X. campestris*, expression of *araE*, overexpression of *pepc*	This study

Conclusions

In this study, *xylAB* operon was introduced into *C. glutamicum* SO26 to achieve the consumption of xylose for the production of L-ornithine. And then, we verified the AraE, the reduction in the degree of acetylation and the release of feedback inhibition of aspartic acid of PEPC, the addition of biotin and thiamine hydrochloride, the resulting strain *C. glutamicum* XAB03 has reached 41.5 g/L shaker flask output from glucose and xylose. This work also shows the possibility of making full use of lignocellulose for the synthesis of L-ornithine and lays the foundation for the further realization of industrialized strain production.

Methods

Bacterial strains, plasmids and primers

The strain SO26 originated from *C. glutamicum* S9114 with a series of modifications (deletion of *argF*, *ncgl1221*, *argR*, *putP*, *iolR*, and *mscCG2*; attenuation of *odhA*, *proB*, *pta*, *cat*, and *ncgl2228*; and overexpression of *lysE*, *gdh*, *gdh2*, *cg3035*, *pfkA*, *pyk*, *glt*, *tkt*, *argCJBD*, and *iolT1*) was used as a starting strain for further metabolic engineering in this study. *E. coli* DH5α was used as host for rapid replication of recombinant plasmids. Xylose isomerase (*xylA*) and xylulose kinase (*xylB*) were amplified from *E. coli* K-12 MG1655 and *X. campestris*, respectively. The arabinose transporter (*araE*) were derived from *Bacillus subtilis*. All the strains and plasmids used in this study were presented in Table 1.

Table 1
Strains and plasmids

Strains/plasmids	Relevant characteristics	Source
Strains		
E. coli DH5	Clone host strain	Transgen
E. coli K-12 MG1655	The source of Eco-*xylAB* genes	Lab stock
X. campestris	The source of Xca-*xylAB* genes	Lab stock
C. glutamicum SO26	Deletion of *argF*, *ncgl1221*, *argR*, *putP*, *iolR*, and *mscCG2*; attenuation of *odhA*, *proB*, *pta*, *cat*, and *ncgl2228*; and overexpression of *lysE*, *gdh*, *gdh2*, *cg3035*, *pfkA*, *pyk*, *glt*, *tkt*, and *iolT1*	Lab stock
Bacillus subtilis	The source of pentose transporter *araE* gene	Lab stock
C. glutamicum pX	*C. glutamicum* SO26 with pXMJ19	This study
C. glutamicum EAB	*C. glutamicum* SO26 with p19P_{lac}-EcoAB	This study
C. glutamicum XAB	*C. glutamicum* SO26 with p19P_{lac}-XcaAB	This study
C. glutamicum XAB01	XAB with the knocking of *araE* gene into the locus of *iolR*	This study
C. glutamicum XAB02	XAB01with P_{H36} promoter in the upstream of *pepc* gene and 1957–1959 bp CGC replaces AAG in *pepc* gene	This study
C. glutamicum XAB03	XAB02 with 895–897 bp AAC replaces GAT in *pepc* gene	This study
Plasmids		
pK18mobsacB	The suicide vector containing the *B. subtilis sacB* gene; kan^R, allows for selection of double crossover in *C. glutamicum*	Lab stock
pXMJ19	High copy expression vector,Cm^RLaclq promotertac promoter	Lab stock
p19P_{lac}-EcoAB	A derivative of pXMJ19, harboring the *xylAB* operon from *E. coli* MG1655	This study
p19P_{lac}-XcaAB	A derivative of pXMJ19, harboring the *xylAB* operon from *X. campestris*	This study

Strains/plasmids	Relevant characteristics	Source
pk18-P_{eftu}-△iolR::araE	Using pK18*mobsacB* and *araE* to connect through the upper and lower homology arms of △*iolR*, and add P_{eftu} promoter in the upstream of *araE* gene	This study
pk18-P_{H36}-pepcTI	A originated from pK18*mobsacB*, add P_{H36} promoter in the upstream of pepc gene and 1957–1959 bp CGC replaces AAG in *pepc* gene	This study
pk18-P_{H36}-pepcT2	On the basis of pk18-P_{H36}-pepcTI, harboring 895–897 bp AAC replaces GAT in *pepc* gene	This study

Construction of plasmids and strains

The basic DNA manipulation and strain construction were operated according to the standard molecular cloning manual. All the primers used in this study were presented in Table S1 (Supplementary Information). The suicide vector pK18*mobsacB* containing the sucrose lethal gene *sacB* was used to delete or integrate gene on genome.

The *xylAB* gene clusters from *E. coli* and *X. campestris* were amplified by primers EAB-F/EAB-R and XAB-F/XAB-R respectively, the inserted restriction sites were *Hind*III/*EcoR*I and *Hind*III/*Sac*I of pXMJ19. The plasmids pXMJ19, p19P_{lac}-EcoAB, and p19P_{lac}-XcaAB were transformed into *C. glutamicum* SO26 to produce strains *C. glutamicum* pX□EAB□XAB. *AraE* from *B. subtilis* with a constitutive P_{eftu} promoter were amplified by primers araE-F/araE-R and up-eftu-F/araE-eftu-R respectively. The products of fusion PCR were inserted into the *EcoR*V site of plasmid pk18-△*iolR* by Gibson assembly, and then generated the recombinant plasmid pk18- P_{eftu}-△iolR::araE and corresponding strain XAB01.

To increase the expression of PEPC by decreasing its acylation, a synthesized strong promoter H36 was inserted in the upstream of *pepc* gene [48], and then the AAG bases from 1957 bp to 1959 bp were mutated to CGC by overlap PCR, the primer up-HA-F1 / up-HA-H36-R2 amplified *pepc* upstream homology arm sequence, primer up-HA-H36-F3 / pepc-H36-R4 amplified H36 promoter, primer mutant-(KR)-F / down-HA-pepc-R8 realized the base mutation of K653R, pepc-H36-F5 / mutant-(KR)-R amplifies the *pepc* upstream homology arm sequence, primer down-HA-pepc-F9 / down-HA-R10 amplified the downstream homology arm sequence of *pepc*. Finally, the entire fragment with the primers up-HA-F1/down-HA-R10 and linerazed plasmid were combined by Gibson assembly, resulted in the recombinant plasmid pk18-P_{H36}-*pepc*T1. Furthermore, to reduce the feedback inhibition of aspartic acid on PEPC, the same operation has been done at 895–897 bp AAC to replace GAT, amplification using primers pepc-H36-F5 / D299N-R6 and D299N-F7 / down-HA-pepc-R8 with pk18-P_{H36}-*pepc*T1 as a template could obtain base mutation of D299N, forming the pk18-P_{H36}-*pepc*T2, resulted in the strain *C. glutamicum* XAB03. Primers pXMJ19-F/pXMJ19-R and M13 fwd/M13 rev were verification primers for pXMJ19 and pK18*mobsacB*, respectively.

All the recombinant plasmids were constructed in *E. coli* DH5α and transformed into *C. glutamicum* by electroporation. The mutant strains were screened through two rounds of homologous recombination, and further confirmed by colony PCR and sequencing.

Cultivation medium and conditions

Luria–Bertani (LB) medium containing NaCl 10 g/L, tryptone 10 g/L and yeast extract 5 g/L were used for cultivation of *C. glutamicum* and *E. coli* strains. Antibiotic was added to the medium for mutants screening when needed: 50 μg/mL kanamycin or 30 μg/mL chloramphenicol for *E. coli*, 10 μg/mL kanamycin or 15 μg/mL chloramphenicol for *C. glutamicum*.

For the shaking flask fermentation experiment, the correct monoclonal strains were activated twice on the LB medium for 36 hours, and then the appropriate amount of bacterial seed was inoculated into a 100 mL flask containing 10 mL of seed solution. The seed medium contains glucose 30 g/L, corn steep liquor 10 g/L, yeast extract 10 g/L, $(NH_4)_2SO_4$ 15 g/L, $MgSO_4$ 2.5 g/L, KH_2PO_4 1 g/L, K_2HPO_4 0.5 g/L, $Na_2HPO_4{\cdot}12H_2O$ 1.26 g/L and $CaCO_3$ 10 g/L, the pH was adjusted to 6.7 (Supplementary Information: Figure S1). The monoclonal strains were incubated at 30 °C, 220 rpm for 11 hours. Take an appropriate amount of seed solution and inoculate it in a 250 mL Erlenmeyer flask containing 20 mL of fermentation medium so that the OD_{600} value of the initial fermentation solution reach up to 1.0. The fermentation medium contains xylose 100 g/L or glucose 70 g/L and xylose 30 g/L, yeast extract 6 g/L, $(NH_4)_2SO_4$ 50 g/L, $MgSO_4$ 2.5 g/L, KH_2PO_4 1 g/L, K_2HPO_4 0.5 g/L, $Na_2HPO_4{\cdot}12H_2O$ 1.26 g/L, $MnSO_4{\cdot}H_2O$ 0.02 g/L, $FeSO_4{\cdot}7H_2O$ 0.02 g/L, biotin 0.9 μM, thiamine-HCl 15 μM and $CaCO_3$ 10 g/L, while the pH was adjusted to 6.7. The strains in fermentation medium were incubated at 31.5 °C, 250 rpm for 72 hours.

Analytical methods of cell growth and fermentation products

Three parallel samples (100 μL) were taken to monitor the bacteria density, consumption of glucose and xylose, and L-ornithine production for every 12 hours interval. The OD_{600} value was detected to assess the cell growth by a microplate reader (BioTek Instruments, Winooski, VT, USA) after adding 0.125 mol/L HCl to dissolve $CaCO_3$ [26]. The samples were centrifuged to obtain fermentation supernatant for subsequent analysis. The glucose concentration was analyzed by SBA-40E biosensor analyzer (Institute of Biology, Shandong Academy of Sciences, Jinan, Shandong, China). The level of xylose consumption was determined by high-performance liquid chromatography (HPLC) [48, 52]. The content of L-ornithine was determined by ninhydrin colorimetry [16]. All experiments had triple parallels, and data had been presented as mean and standard deviation (SD).

Declarations

Ethics approval and consent to participate Not applicable

Consent for publication Not applicable

Availability of data and materials All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests The authors declare that they have no competing interests

Funding This research was supported and funded by the National Key Research and Development Project (Grant no. 2018YFA0900404), the National Natural Science Foundation of China (Grant no. 31730004) and the Shanghai Municipal Science and Technology Commission Project (18JC1410802).

Authors' contributions G.G., Y.Z., acquisition, analysis, and interpretation of data, and manuscript preparation. These two authors contributed to the work equally and should be regarded as co-first authors. Y.Z., B.-C.Y., study conception and design, data analysis, and final approval of the manuscript. All authors read and approved the final manuscript.

Acknowledgements We would like to thank Dr. Li-Ming Liu for providing us with *Corynebacterium glutamine* S9114.

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Figures

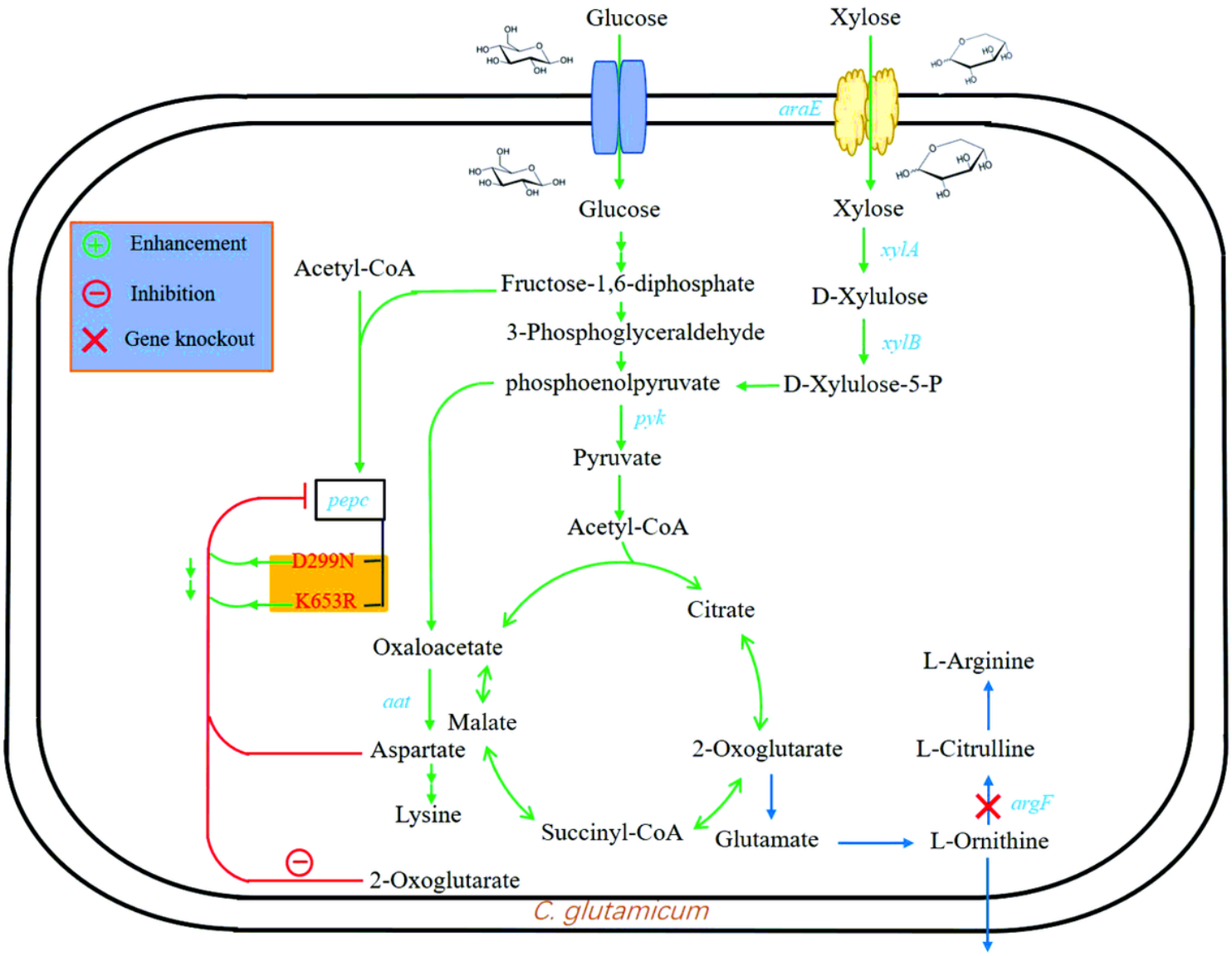

Figure 1

Schematic diagram of the metabolic engineering process of C. glutamicum. pepc encodes phosphoenolpyruvate carboxylase; pyk encodes pyruvate kinase; xylA encodes xylose isomerase; xylB encodes xylulose kinase; aat encodes aspartate aminotransferase; araE encodes pentose transporter; argF encodes N-ornithine carbamoyl transferase.

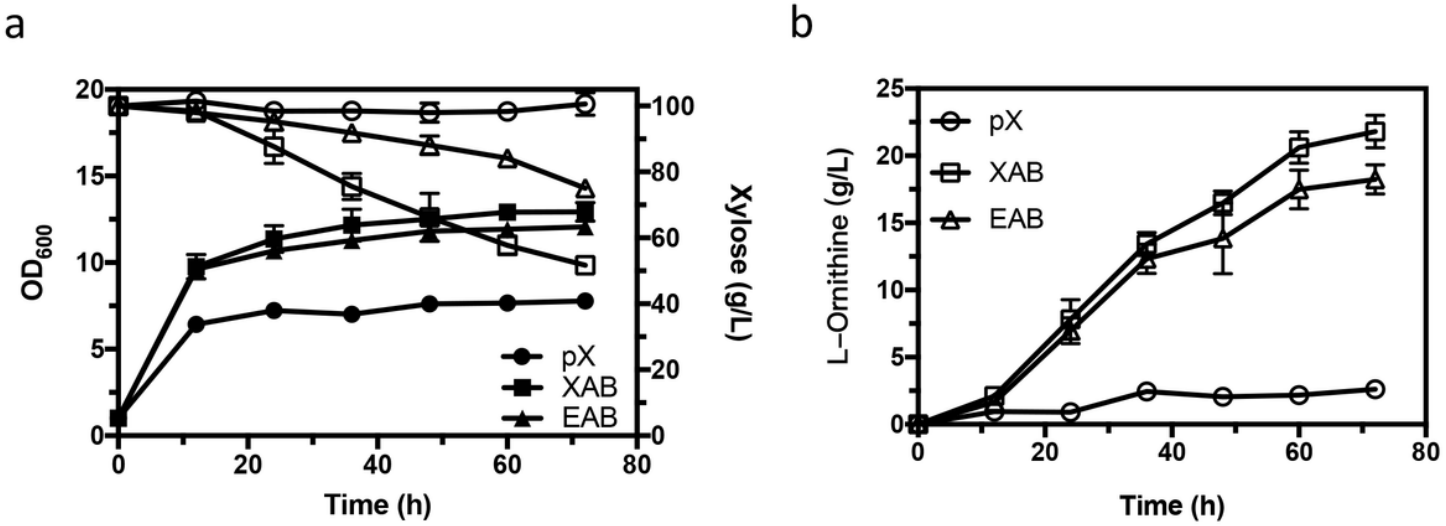

Figure 2

Comparison of the effects of xylAB genes from different strains. a Growth curve (solid) and xylose consumption (hollow) curves. b L-Ornithine production curves. Strain pX (hollow circular) is a control strain containing pXMJ19, Strain XAB (hollow square) overexpresses xylAB from E. coil, Strain EAB (hollow upper triangle) overexpresses xylAB from X. campestris.

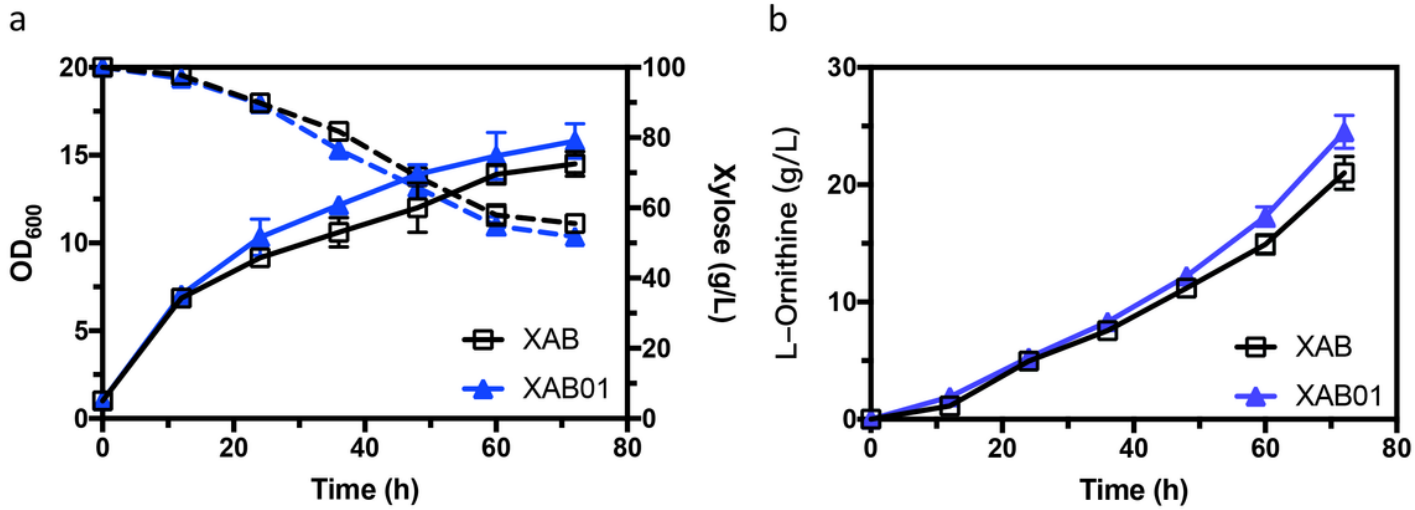

Figure 3

The promotion of araE on xylose and the effect on L-ornithine production. a: Growth and xylose consumption curves. b: L-Ornithine production curves. Strain XAB01 (blue solid triangle) integrated the araE gene from B. subtilis with a strong promoter Peftu, and XAB (black hollow square) is the control strain.

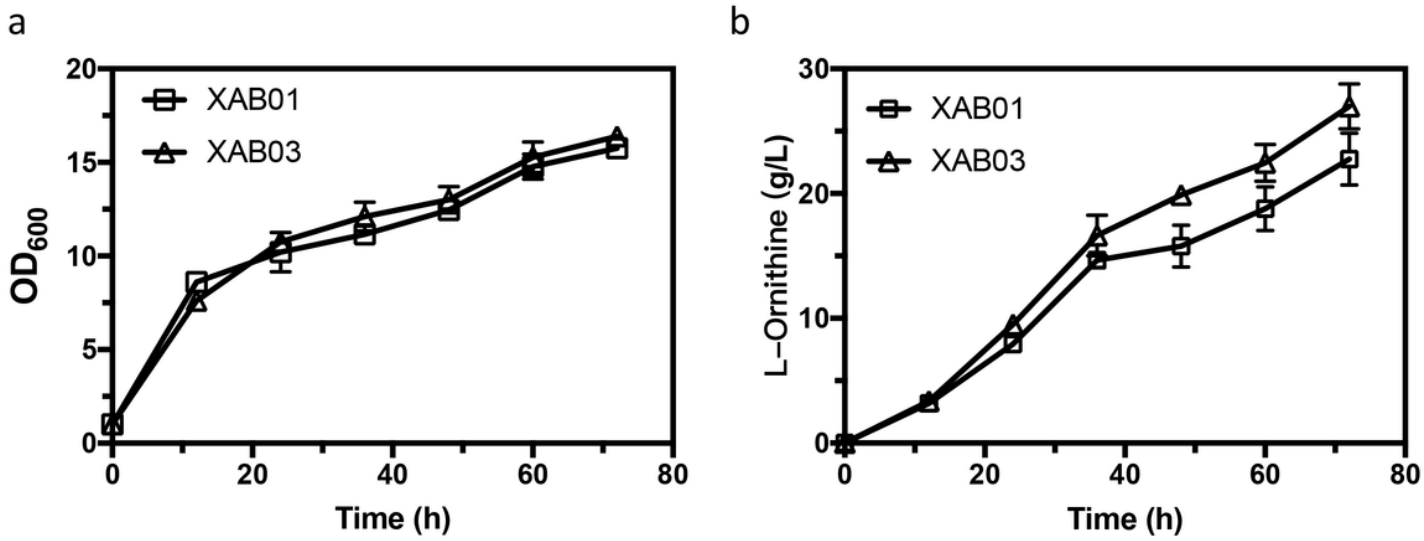

Figure 4

The PEPC modification reinforces the production of L-ornithine. a: Growth curves. b: L-Ornithine production curves. XAB01 (blue solid triangle) integrated the araE gene from B. subtilis with a strong promoter Peftu. XAB03 (hollow upper triangle) modified pepc on the basis of XAB01.

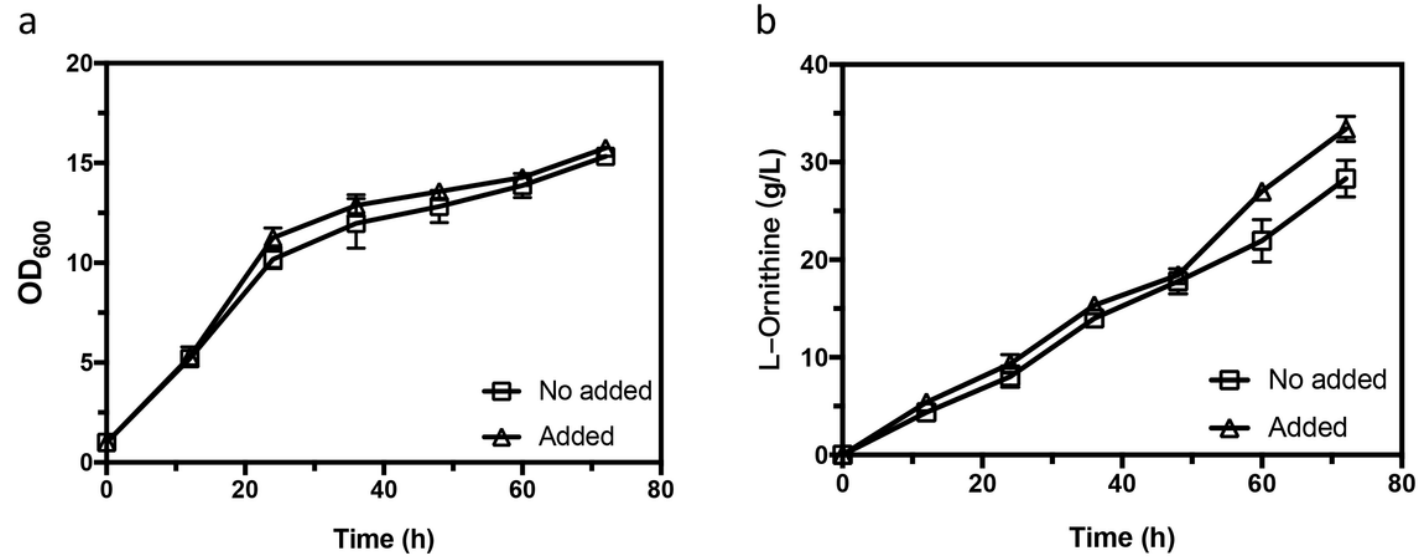

Figure 5

The effect of adding biotin and thiamine hydrochloride on L-ornithine production. a: Growth curves for XAB03. b: L-Ornithine production curves for XAB03. Added (Biotin and thiamine hydrochloride, hollow upper triangle), No added (hollow square).

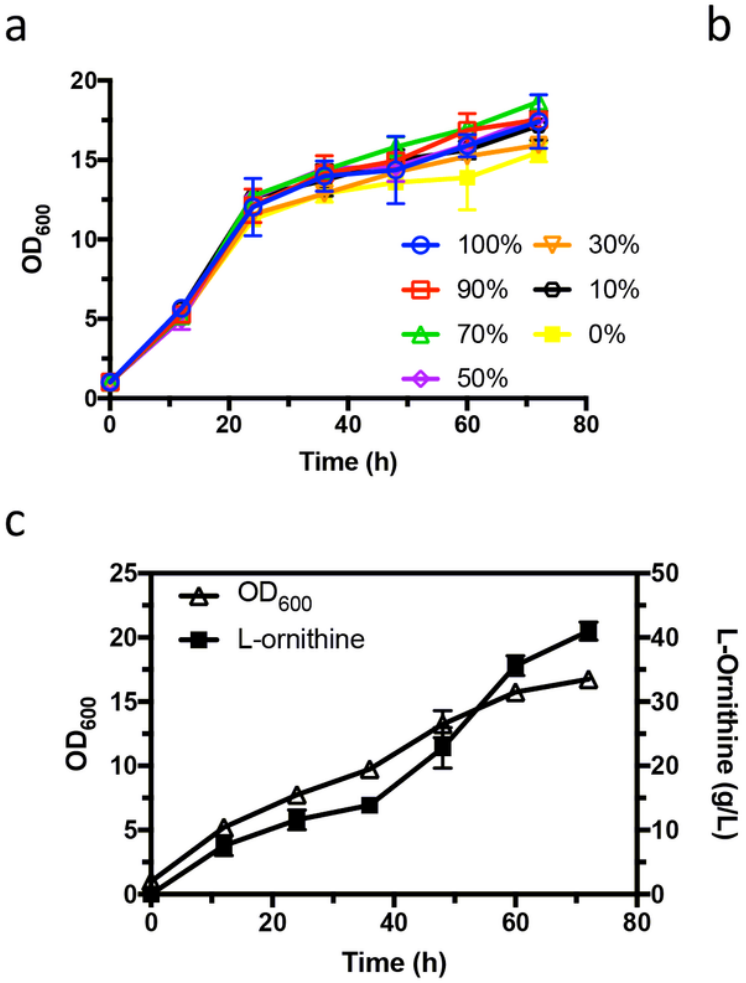

Figure 6

The effect of different ratios of glucose and xylose on L-ornithine production a: Growth curves for C. glutamicum XAB03 at different glucose concentrations ratios (100%, blue circle; 90%, red hollow square; 70%, green upper triangle; 50%, purple diamond; 30%, orange lower triangle; 10%, black regular hexagon; 0%, yellow solid square.). b: L-Ornithine production curves. c: Optimal shake flask fermentation experiment of C. glutamicum XAB03 in OD600 (hollow upper triangle) and L-ornithine production (solid square) in glucose 70 g/L and xylose 30 g/L.

Supplementary Files

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- TableS1.pdf
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