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High Production of γ-Aminobutyric Acid by Activating the *xyl* Operon of *Lactobacillus brevis*

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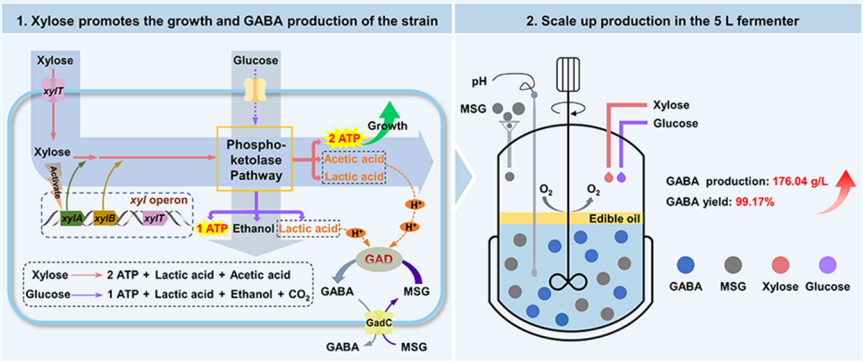

ABSTRACT: γ-Aminobutyric acid (GABA) is an inhibitory neurotransmitter with important physiological functions such as sleep assistance and anti-depression. In this study, we developed a fermentation process for the high-efficiency production of GABA by *Lactobacillus brevis* (*Lb. brevis*) CE701. First, xylose was found as the optimal carbon source that could improve the GABA production and OD_{600} in shake flasks to 40.35 g/L and 8.64, respectively, which were 1.78-fold and 1.67-fold of the glucose. Subsequently, the analysis of the carbon source metabolic pathway indicated that xylose activated the expression of the *xyl* operon, and xylose metabolism produced more ATP and organic acids than glucose, which significantly promoted the growth and GABA production of *Lb. brevis* CE701. Then, an efficient GABA fermentation process was developed by optimizing the medium components using response surface methodology. Finally, the production of GABA reached 176.04 g/L in a 5 L fermenter, which was 336% higher than that in a shake flask. This work enables the efficient synthesis of GABA using xylose, which will provide guidance for the industrial production of GABA.

1. INTRODUCTION

γ-Aminobutyric acid (GABA) is a non-protein amino acid commonly distributed in animals, plants, and microorganisms.[1−3] GABA is an essential inhibitory neurotransmitter in the brain and possesses various physiological functions, such as anti-hypertension,[4,5] anti-depression,[6] anti-diabetes,[7,8] and immunity improvement.[9] The demand for GABA is increasing because of its accelerated development as a dietary supplement and functional medicine treating certain neurological disorders.[10,11] Currently, GABA is mainly produced by chemical synthesis,[12] plant enrichment,[13] and microbial fermentation.[14−16] The chemical synthesis of GABA has severe health risks, such as using toxic chemical reagents, resulting in a prohibition of its usage as a food additive.[17] GABA produced by plant enrichment has the disadvantages of low yield, difficult extraction, and high costs.[18] As a mild, efficient, and safe production method, microbial fermentation is extremely attractive and promising for the green and efficient production of GABA.[19]

Recent studies have concentrated on the production of GABA by lactic acid bacteria (LAB) fermentation[16,18,20] because LAB is generally regarded as a safe (GRAS) organism,[21−23] which is suitable for the production of food-grade GABA.[24,25] As a LAB species, *Lactobacillus brevis* (*Lb. brevis*) has been considered a critical strain for GABA production due to its high GABA-producing capacity, such as *Lb. brevis* NPS-QW-145,[26] *Lb. brevis* NCL912,[27] *Lb. brevis* TCCC 13007,[28] *Lb. brevis* CRL 1942,[29] etc. GABA synthesis by *Lb. brevis* is achieved through the glutamate decarboxylase (GAD, EC 4.1.1.15) system under acidic conditions.[30] The GAD system consists of the GAD and glutamate/GABA antiporter (GadC).[31] Glutamate is transported into the cytosol via GadC and performs an irreversible decarboxylation reaction catalyzed by GAD to produce GABA, and the GABA is then

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released into the extracellular environment via GadC.[32,33] In recent years, several measures have been taken to improve GABA production by *Lb. brevis*, including regulating the GAD system,[34,35] optimizing the fermentation process,[36,37] developing co-culture systems,[38] etc. Previously, some researchers have pointed out that GABA production was related to cell density.[39] However, how to achieve efficient GABA production by increasing the cell density of *Lb. brevis* has rarely been investigated.

The components of the medium, especially the carbon source, are important factors influencing bacterial growth.[16,40] *Lb. brevis* is a heterofermentative LAB that catabolizes carbohydrates through the phosphoketolase (PK) pathway.[41,42] Glucose was usually used as a carbon source for producing GABA in LAB,[18] but most heterofermentative LAB grow poorly in glucose.[43,44] The optimal carbon source for strain growth varied among *Lb. brevis*. Previous studies suggested that *Lb. brevis* grows better in xylose, ribose, arabinose, or fructose than in glucose.[45,46] The *xyl* operon is activated when fermented with xylose.[47] The *xyl* operon has three components: xylose isomerase (XylA, 50.47 kDa), xylulose kinase (XylB, 55.20 kDa), and xylose transporter (XylT, 49.2 kDa).[48] In previous work, we screened out a strain of *Lb. brevis* CE701 with GABA production capacity.[49] But the poor growth of the strain limited the high-efficiency production of GABA. To increase the cell density of *Lb. brevis* CE701 for improving GABA production, the fermentation process needs to be further explored.

Herein, we screened the growth-promoting factors of *Lb. brevis* CE701. Xylose was found to significantly promote the growth of the strain and increase GABA production. Then, based on the analysis of the composition and expression regulation mechanisms of the *xyl* operon, we deeply explored the carbon source metabolic pathway of *Lb. brevis* CE701 to investigate the reason for xylose promoting the growth and GABA production of the strain. Further, the medium components were optimized by response surface methodology to develop an efficient GABA fermentation process. Finally, the green and efficient production of GABA was achieved by *Lb. brevis* CE701 in a 5 L fermenter under optimal fermentation conditions, which might have an impetus for the industrial production of GABA.

2. MATERIALS AND METHODS

2.1. Reagents, Strain, Medium, and Cultivation Conditions in Flasks. Glucose, fructose, xylose, mannose, D-galactose, maltose, sucrose, lactose, cornstarch, potato starch, yeast extract, peptone, soy peptone, and tryptone were supplied by Dingguo Biotechnology Co., Ltd. (Tianjin, China). Arabinose, pyridoxal-5′-phosphate (PLP), and Tween 80 were provided by Aladdin (Shanghai, China). Beef extract, corn steep liquor, urea, diammonium citrate, and triammonium citrate were purchased from Heowns Biochemical Technology Co., Ltd. (Tianjin, China). Monosodium glutamate (MSG) was purchased from Meihua Holdings Group Co., Ltd. (Hebei, China). All other reagents were obtained from Jiangtian Chemical Technology Co., Ltd. (Tianjin, China).

Lb. brevis CE701, isolated from pickles by our previous work,[49] was used in this study. The DeMan, Rogosa, and Sharpe (MRS) medium and Glucose-Yeast-Peptone (GYP) seed medium were used for the activation and seed culture of *Lb. brevis* CE701, respectively, and their components were described in detail in our previous report.[50] The fermentation mediums (FM1, FM2, FM3, FM4, FM5, FM6, and FM7) listed in Table S1 were used for GABA production.

The *Lb. brevis* CE701 was first activated at 35 °C and 200 rpm for 24 h in a 5 mL MRS medium. After that, a volume of 250 μL (0.5% v/v) of activated strain was subcultured into a 250 mL flask containing 50 ml GYP seed medium and cultured for 24 h at 35 °C without shaking as the seed culture. The seed culture was then inoculated at 2% (v/v) into a 50 ml fermentation medium in a 250 mL flask and fermented at 35 °C for 96 h without shaking. Samples were taken every 12 h.

2.2. Screening of Growth-Promoting Factors for *Lb. brevis* CE701. To screen the optimal growth-promoting factors of *Lb. brevis* CE701, the effects of different carbon sources (fructose, xylose, arabinose, mannose, D-galactose, maltose, sucrose, lactose, cornstarch, and potato starch), nitrogen sources (yeast extract, peptone, soy peptone, tryptone, beef extract, corn steep liquor, and urea), and inorganic salts (K_2HPO_4, KH_2PO_4, Na_2HPO_4, NaH_2PO_4, disammonium citrate, and triammonium citrate) in FM1 on the growth and GABA production were investigated. After screening xylose as the optimum carbon source, the effect of xylose concentrations (0−32 g/L) on GABA production was further investigated.

Furthermore, to explore the effect of mixed sugar (glucose and xylose) on the fermentation process, the concentration of xylose in FM2 was fixed at 24 g/L, and the effect of glucose addition amount (from 10 to 100 g/L) on GABA production and cell density of *Lb. brevis* CE701 was investigated. Culture and fermentation were conducted as described in 2.1.

2.3. Quantitative Real-Time PCR of Xylose Operon Genes. Quantitative real-time PCR (qRT-PCR) was used to detect the relative transcription expression of the *xyl* operon in FM3, FM4, FM5, and FM6. Cells in different fermentation mediums were harvested, and the method of SDS/phenol was used to extract total RNA, as described previously.[32] The mRNAs were reverse-transcribed to cDNA and then quantified via real-time PCR using TransScript Green Two-Step qRT-PCR SuperMix (TransGen Biotech Co., Ltd., Beijing). The primers used in qRT-PCR are listed in Table S2. The relative expression of *xyl* genes was calculated utilizing the comparative critical threshold method ($2^{-\Delta\Delta Ct}$), and the 16S rRNA of the strain was taken as a housekeeping gene.

2.4. Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) Analysis. Each of the 100 μL fermentation broth in FM3, FM4, FM5, and FM6 was collected. The wet cells were gathered by centrifuging at 10,000*g* for 5 min, followed by washing twice with sterile water. Then, the wet cells were resuspended in 20 μL loading buffer and boiled in water for 10 min. The samples were then cooled to room temperature and analyzed by SDS-PAGE.

2.5. Optimization of Fermentation Medium Components by Response Surface Methodology. The components of FM2 were optimized by response surface methodology (RSM). First, the significant factors affecting GABA production were determined by Plackett−Burman (PB) design, and the variables and experimental results are listed in Tables S3 and S4, respectively. Then, the central composite design (CCD) based on the four significant factors (xylose, yeast extract, $MnSO_4{\cdot}4H_2O$, and MSG) and five levels was applied to determine the optimal concentrations of the fermentation medium components. The variables and the experimental design in the CCD are listed in Tables S5 and S6, respectively. The average GABA generated from three

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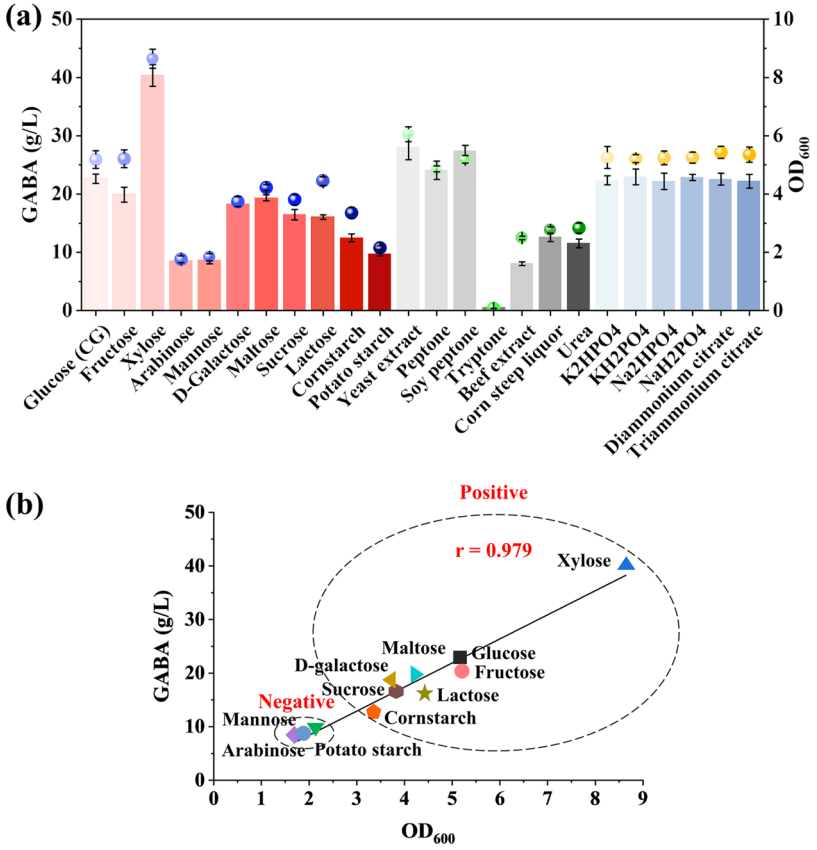

Figure 1. Screening of growth-promoting factors for *Lb. brevis* CE701. (a) Effect of different carbon sources, nitrogen sources, and inorganic salts on GABA production (histogram) and OD_{600} (scatter plot) of *Lb. brevis* CE701. (b) Correlation analysis between GABA production and cell density (OD_{600}).

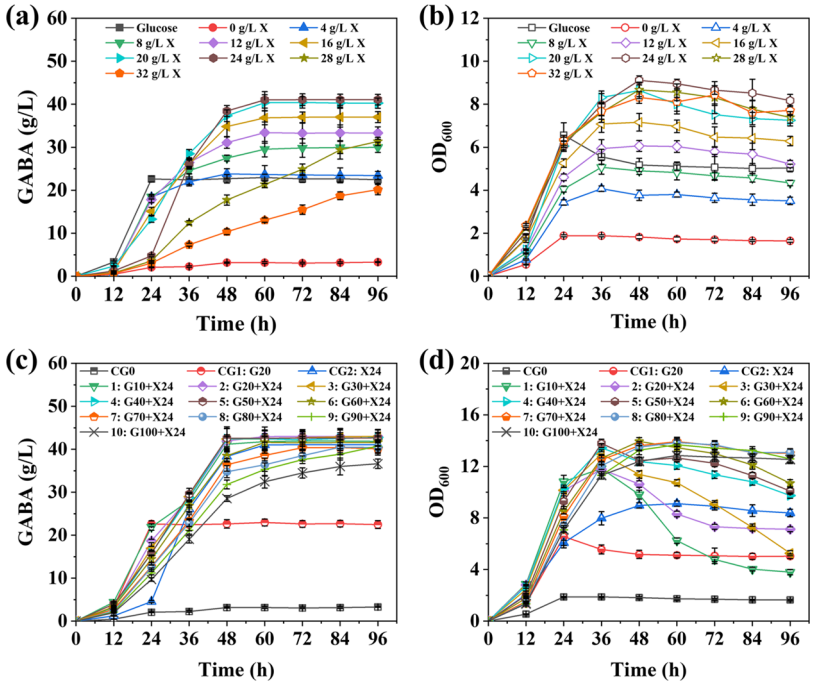

Figure 2. Effects of xylose concentration on GABA production (a) and OD_{600} (b) and the effects of glucose additions in mixed sugar fermentation on GABA production (c) and OD_{600} (d). "X" and "G" are the abbreviations of xylose and glucose, respectively. "CG0" was a control group without any carbon source; "CG1" was a control group with 20 g/L glucose; and "CG2" was a control group with 24 g/L xylose. The abbreviation "G10+X24" indicates the mixed sugar of 10 g/L glucose and 24 g/L xylose, and the other abbreviations represent similar meanings.

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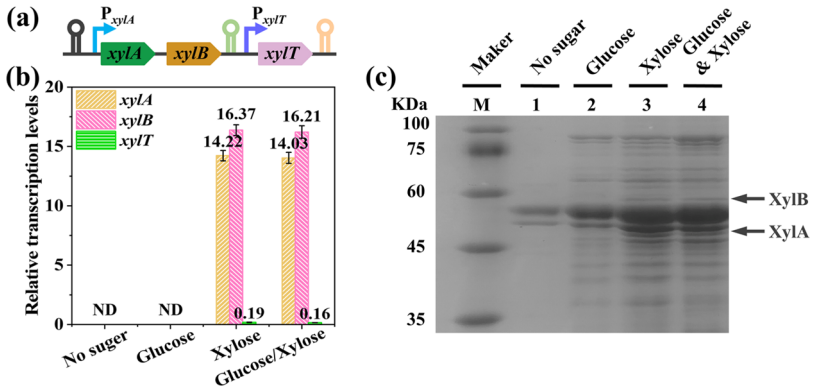

Figure 3. Structure analysis of the *xyl* operon in *Lb. brevis* CE701 and its transcription and expression when fermented with different carbon sources. (a) Structure of the *xyl* operon. (b) Relative transcription levels of the *xyl* operon when fermented with different carbon sources in *Lb. brevis* CE701. "ND" indicates not detected. (c) SDS-PAGE profiles of *Lb. brevis* CE701 when fermented with different carbon sources. M, maker; lanes 1, 2, 3, and 4 show the expression of the *xyl* operon when fermented with no sugar, glucose, xylose, and mixed sugar, respectively.

replicates of each experiment was taken as the response value. Experimental design, data analysis, and graphical plotting were carried out with Design Expert software (version 12.0.3).

2.6. Cultivation and Production of GABA in a 5 L Fermenter. The fermenter was filled with 3 L of fermentation medium (FM7) inoculated with 10% (v/v) seed culture and then covered with edible oil to create anaerobic conditions. The initial fermentation conditions were set at 35 °C and pH = 6.2, and the fermentation conditions were then adjusted to 40 °C and pH = 4.8 after 24 h. Glucose (400 g/L, 375 mL) was added to the flow between 0 and 24 h, and 262 g of solid MSG (without sterilization) was added directly to the 5 L fermenter through the feed inlet at 12, 24, 36, and 48 h, respectively. The pH of the fermentation broth was adjusted by H_2SO_4 (3 M) and NaOH (3 M). Samples were collected every 12 h to determine cell density and the concentrations of GABA, MSG, xylose, and glucose.

2.7. Analytical Procedures. The concentrations of GABA and MSG in the fermentation of broth samples were analyzed by HPLC as previously described.[50] The method for detecting the contents of lactic acid, acetic acid, ethanol, xylose, and glucose was the same as that described by Wu et al.[32] The cell density (OD_{600}) was detected by a spectrophotometer at a wavelength of 600 nm (UV spectrophotometer 721G, Precision Scientific Instruments Co., Ltd., Shanghai, China). The GABA yield was defined as the following equation:

$$\text{GABA yield} = \frac{C_{GABA} V_{broth}}{w_{MSG}} \times \frac{MW_{MSG}}{MW_{GABA}} \times 100\% \quad (1)$$

where C_{GABA} is the GABA concentration (in g/L), V_{broth} is the fermentation broth volume (in L), w_{MSG} is the total MSG weight (in g), and MW_{MSG} and MW_{GABA} are the molecular weights of MSG and GABA, respectively.

2.8. Statistical Analysis. All data presented in the bars and tables were means ± standard deviations (SD). The correlation analysis was performed using IBM SPSS Statistics 20.0 version.

3. RESULTS AND DISCUSSION

3.1. Screening of Growth-Promoting Factors for *Lb. brevis* CE701. The effects of carbon sources, nitrogen sources, and inorganic salts on the growth and GABA production of *Lb. brevis* CE701 are shown in Figure 1a. When *Lb. brevis* CE701 was fermented with xylose, the OD_{600} (8.64) and GABA production (40.35 g/L) were the highest, which were 1.67-fold and 1.78-fold of the control group (CG), respectively. Among the nitrogen sources investigated (Figure 1a), the yeast extract had a relatively significant promotion of OD_{600} (6.05) and GABA production (28.03 g/L). The addition of inorganic salts had few effects on the growth and GABA production of *Lb. brevis* CE701.The correlation analysis between GABA production and cell density showed a positive correlation coefficient (r = 0.979), as shown in Figure 1b. Therefore, increasing the cell density of *Lb. brevis* CE701 was essential to promote GABA production.

Table 1. Measured Metabolites of *Lb. brevis* CE701 When Fermented with Different Carbon Sources

carbon source	lactic acid (g/L)	acetic acid (g/L)	ethanol (g/L)	pH
glucose	12.10 ± 0.41	ND[a]	6.65 ± 0.37	4.42 ± 0.13
xylose	14.72 ± 0.54	9.08 ± 0.37	ND	3.61 ± 0.15
glucose and xylose	25.63 ± 0.83	9.26 ± 0.52	6.23 ± 0.21	3.36 ± 0.13

[a]ND, not detected.

Xylose was chosen as the optimal carbon source, and the effect of xylose concentration on the fermentation process was further investigated, as shown in Figure 2a,b. The GABA production increased gradually with increasing xylose concentration, with the highest GABA production of 41.00 g/L (60 h) at 24 g/L xylose. Meanwhile, the OD_{600} also reached a maximum value of 9.11 at 60 h, which was the main reason for the high GABA production. However, the fermentation period of xylose (60 h) was longer than that of glucose (24 h), as shown in Figure 2a, which may be due to the requirement for induced expression of the *xyl* operon before catabolizing xylose.[47] Therefore, we developed a process of mixed sugar fermentation using glucose and xylose to investigate its effect on the fermentation period. Compared to the CG2, the glucose supplement from 10 to 50 g/L maintained high GABA productivity and cell density at 48 h (Figure 2c,d) and shortened the fermentation period by 12 h (Table S7). In particular, when adding 50 g/L glucose, the OD_{600} and GABA production reached a maximum of 12.6 and 42.54 g/L, which were 1.37-fold and 1.04-fold of the CG2, respectively. Meanwhile, glucose and xylose were depleted by *Lb. brevis* CE701 at the addition of 50 g/L glucose (Figure S1). Thus, the mixed sugar fermentation process with 24 g/L xylose and

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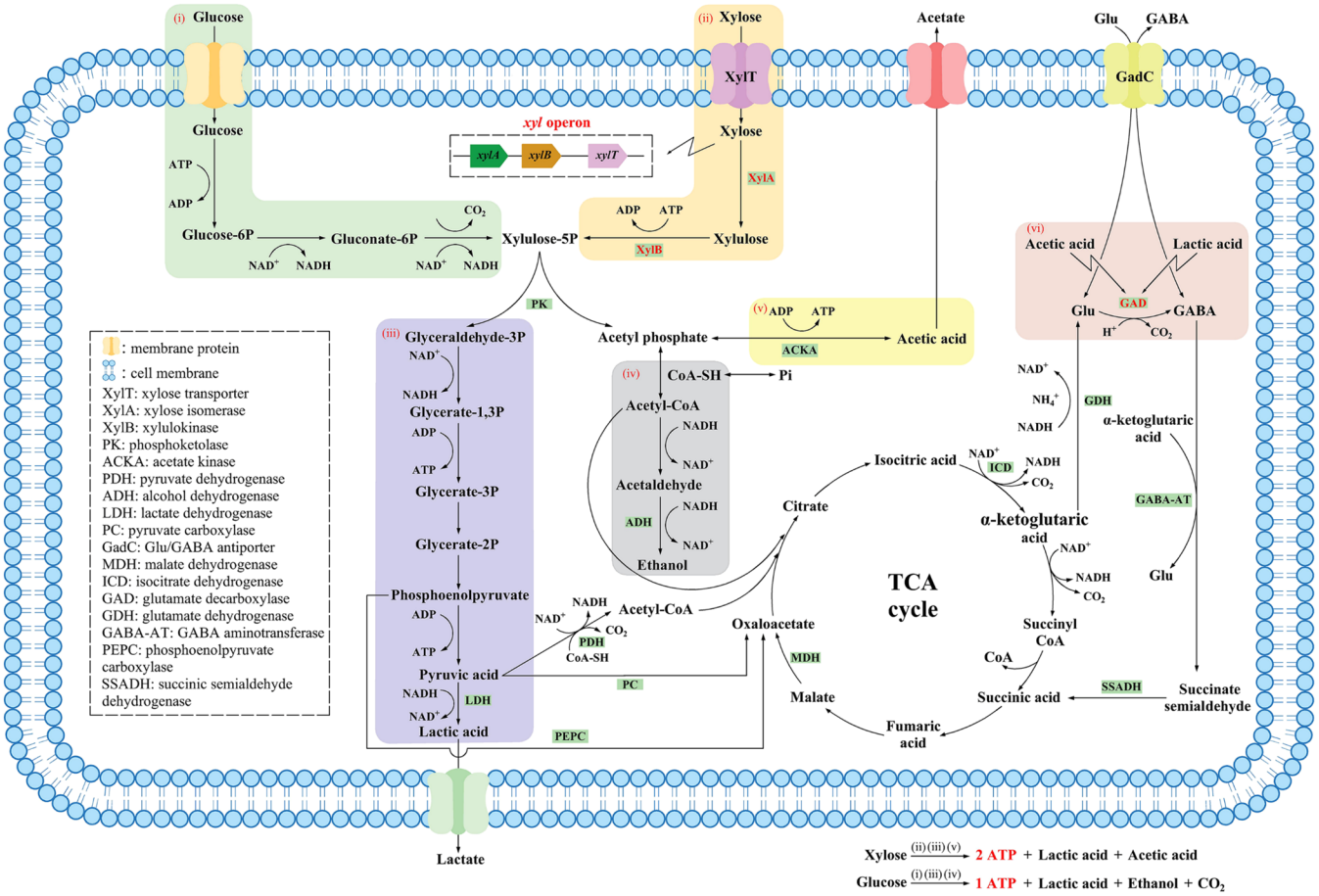

Figure 4. Schematic diagram of metabolic pathways of glucose and xylose in *Lb. brevis* CE701.

Table 2. ANOVA of the Quadratic Model Established by CCD[a]

source[b]	sum of squares	degree of freedom	mean square	*F*-value	*P*-value
model	745.62	14	53.26	27.66	<0.0001
X_1	177.72	1	177.72	92.31	<0.0001
X_2	38.94	1	38.94	20.22	0.0004
X_3	2.63	1	2.63	1.37	0.2604
X_4	23.07	1	23.07	11.98	0.0035
X_1 X_2	0.07	1	0.07	0.03	0.8553
X_1 X_3	0.01	1	0.01	0.01	0.9336
X_1 X_4	1.97	1	1.97	1.02	0.3282
X_2 X_3	0.06	1	0.06	0.03	0.8636
X_2 X_4	2.23	1	2.23	1.16	0.2991
X_3 X_4	0.10	1	0.10	0.05	0.8248
X_1^2	470.14	1	470.14	244.19	<0.0001
X_2^2	40.08	1	40.08	20.82	0.0004
X_3^2	17.95	1	17.95	9.32	0.0081
X_4^2	58.68	1	58.68	30.48	<0.0001
residual	28.88	15	1.93		
lack of fit	25.83	10	2.58	4.24	0.0621

[a] R^2 = 0.9627, adjusted R^2 = 0.9279, predicted R^2 = 0.8022. [b] X_1: Xylose (g/L), X_2: Yeast extract (g/L), X_3: $MnSO_4 \cdot 4H_2O$ (g/L), X_4: MSG (g/L).

50 g/L glucose was determined and applied to the fermentation in a 5 L fermenter.

3.2. Analysis of Carbon Source Metabolism in *Lb. brevis* CE701. *Lb. brevis* CE701 could effectively use xylose to promote strain growth and GABA production, which was associated with carbon source metabolism. Therefore, we deeply explored the character of the carbon source metabolism in *Lb. brevis* CE701 based on the analysis of the composition and expression regulation mechanisms of the *xyl* operon.

The *xyl* operon in *Lb. brevis* CE701 consisted of *xylA*, *xylB*, and *xylT* (Figure S2 and Table S8), and they were sequentially arranged on the genome of *Lb. brevis* CE701 to form the *xylABT* gene cluster shown in Figure 3a. As observed in Figure 3b, the *xyl* operon did not transcribe when fermented with glucose, but its relative transcript levels were significantly upregulated with xylose or mixed sugar (glucose and xylose) as the carbon source. Correspondingly, as shown in Figure 3c, two bands with molecular weights close to 50 KDa (XylA) and 55 KDa (XylB) were observed in lane 3 (xylose) and lane 4 (mixed sugar). It is indicated that the expression of the *xyl* operon was activated by xylose in *Lb. brevis* CE701, which was consistent with Kim[47] and Chaillou et al.[48] Notably, the transcription (Figure 3b) and expression (Table S9) of the *xyl* operon were not affected when co-utilized glucose and xylose compared to using xylose alone, which indicated that there was a more relaxed control of xylose utilization in mixed sugar fermentation of *Lb. brevis* CE701.

The measured metabolic products of *Lb. brevis* CE701 using different carbon sources are listed in Table 1. The measured metabolites of glucose were lactic acid and ethanol, while the measured metabolites of xylose were lactic acid and acetic acid. The differences in measured metabolites between glucose and xylose were related to the carbon source metabolic pathway of

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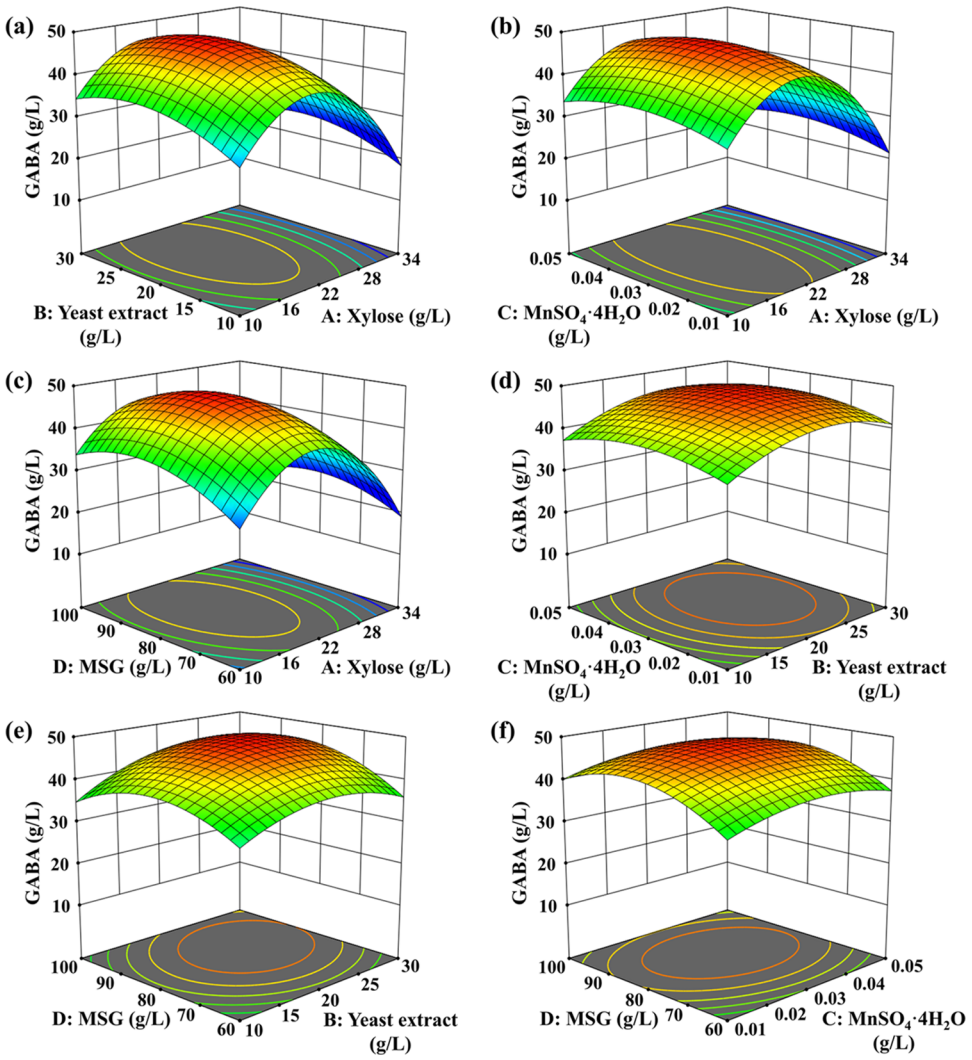

Figure 5. 3D response surface curve of the interactive effect between different factors on GABA production. (a) Effect of xylose and yeast extract on GABA production. (b) Effect of xylose and $MnSO_4·4H_2O$ on GABA production. (c) Effect of xylose and MSG on GABA production. (d) Effect of yeast extract and $MnSO_4·4H_2O$ on GABA production. (e) Effect of yeast extract and MSG on GABA production. (f) Effect of $MnSO_4·4H_2O$ and MSG on GABA production.

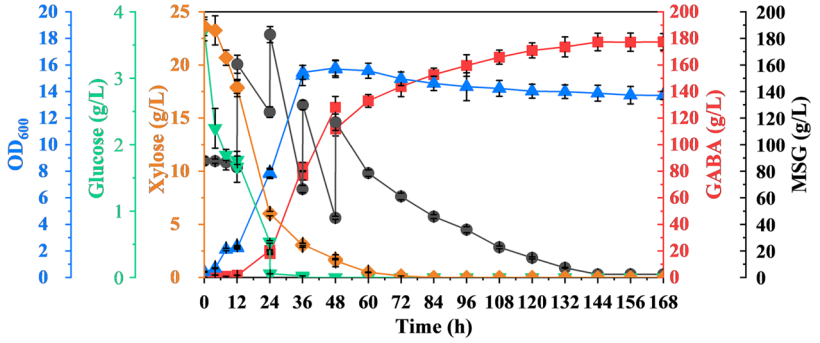

Figure 6. High production of GABA by *Lb. brevis* CE701 in the 5 L fermenter. Symbols: OD_{600} (▲), residual glucose (▼), residual xylose (♦), GABA production (■), and residual MSG (●).

Lb. brevis CE701. Based on the carbon source metabolic pathway of *Lb. brevis* ATCC 367 in the Kyoto Encyclopedia of Genes and Genomes (KEGG), we inferred the carbon source metabolic pathway in *Lb. brevis* CE701, as shown in Figure 4. The PK pathway in *Lb. brevis* CE701 were divided into five modules, (i), (ii), (iii), (iv), and (v). *Lb. brevis* CE701 metabolized glucose via modules (i), (iii), and (iv), and the metabolites were lactic acid and ethanol. The NAD^+ produced in module (iv) compensated for the NAD^+ consumed in module (i). When fermented with xylose, the *xyl* operon in modules (ii) was activated for catabolizing xylose to xylulose-5-phosphate and then generated lactic acid and acetic acid via

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modules (iii) and (v), respectively. Ethanol was not produced during xylose metabolism because the catabolism of xylose did not require the regeneration of additional amounts of NAD^+ via module (iv).[46,51] As a result, xylose fermentation produced twice as much ATP as glucose (Figure 4), which may be the main reason for the higher OD_{600} of *Lb. brevis* CE701. Moreover, xylose metabolism produced more organic acids (lactic acid and acetic acid) to decrease the pH of the medium (Table 1), which could enhance the activity of GAD in module (vi) to increase GABA production.

3.3. Optimization of Fermentation Medium Components by RSM. The components of the fermentation medium (FM2) were optimized by RSM to determine the optimum proportions of each component for the efficient production of GABA. Xylose, yeast extract, $MnSO_4{\cdot}4H_2O$, and MSG were identified as significant factors ($P < 0.05$) affecting GABA production by PB design (Table S10). Then, the optimal combination of the four significant factors was optimized by CCD, and the results of the analysis of variance (ANOVA) are listed in Table 2. The $F = 27.66$ and $P < 0.0001$ implied that the quadratic model was significant. In addition, the high R^2 (0.9627), adjusted R^2 (0.9279), and predicted R^2 (0.8022) confirmed the good consistency between experimental data and predicted data. The following second-order polynomial formula was developed to explain the GABA production by multiple regression analysis of the experimental data:

$$\begin{aligned} Y = {} & -132.4121 + 5.1023X_1 + 1.6028X_2 + 545.9167X_3 \\ & + 2.4410X_4 - 0.0021X_1X_2 + 0.4896X_1X_3 \\ & - 0.0058X_1X_4 + 1.2125X_2X_3 + 0.0075X_2X_4 \\ & - 0.7813X_3X_4 - 0.1150X_1^2 - 0.0484X_2^2 \\ & - 8088.5417X_3^2 - 0.0146X_4^2 \end{aligned} \quad (2)$$

where Y represents the GABA production; X_1, X_2, X_3, and X_4 are the concentrations of xylose, yeast extract, $MnSO_4{\cdot}4H_2O$, and MSG, respectively.

The three-dimensional (3D) response surface curve showed the interaction of two different factors on GABA production (Figure 5). The variation of the GABA production in each 3D response surface curve presented a convex surface, which meant that there was a maximum GABA production in the investigated concentration range. Thus, by optimizing the solution of the quadratic model eq 2, the highest GABA production of 48.85 g/L was predicted when the concentrations of xylose, yeast extract, $MnSO_4{\cdot}4H_2O$, and MSG were 19.70, 22.61, 0.03, and 87.36 g/L, respectively (i.e., FM7). Then, the fermentation was performed in FM7, and the actual GABA production of 49.36 g/L was close to the predicted value, which verified the model's applicability. In conclusion, the optimal fermentation medium (FM7) for GABA production was obtained via RSM.

3.4. High Production of GABA in the 5 L Fermenter. The time course of fermentation by *Lb. brevis* CE701 in the 5 L fermenter is shown in Figure 6. With the consumption of xylose and glucose, *Lb. brevis* CE701 grew rapidly from 12 to 36 h, and the OD_{600} reached a maximum value of 15.77 at 48 h. The increase in OD_{600} was accompanied by an increase in GABA production, and the final GABA concentration reached 176.04 g/L at 144 h, which was 4.36-fold of that in shake flasks. Meanwhile, the MSG was consumed quickly, and few MSG remained. The volume of the fermentation broth increased to 4.5 L due to inoculation, feed, glucose, and acid supplementation. Finally, 1310 g MSG was converted into 792.18 g of GABA, with a yield of 99.17%. To conclude, xylose activated the *xyl* operon and enabled efficient utilization of xylose through the PK pathway, which greatly facilitated the growth of *Lb. brevis* CE701 and increased GABA production.

4. CONCLUSIONS

This study confirmed that xylose could significantly promote the growth and GABA production of *Lb. brevis* CE701. Then, further analysis showed that xylose activated the expression of the *xyl* operon and produced twice as much ATP and more organic acids than glucose via the PK pathway, which promoted the growth of the strain and GABA production. After optimizing the medium components by RSM, the high GABA production of 176.04 g/L with a yield of 99.17% in the 5 L fermenter was obtained. The efficient synthesis of GABA using xylose by *Lb. brevis* CE701 provides support for the industrial production of GABA.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c08272.

Fermentation medium used for GABA production (Table S1); primers used for PCR and RT-qPCR analysis (Table S2); the variables and levels in the PB design (Table S3); the experimental design and results of PB design (Table S4); the variables and levels in CCD (Table S5); the experimental design and results of CCD (Table S6); the effect of glucose addition on GABA productivity, GABA production, and OD_{600} during the mixed sugar fermentation process (Table S7); the statistical table of gene sequences with high similarity to the *xyl* genes of *Lb. brevis* CE701 (Table S8); the expression levels of the *xyl* operon in *Lb. brevis* CE701 when fermented with different carbon sources (Table S9); the effects of the factors and statistical analysis of the PB design (Table S10); the strategy for mixed sugar fermentation using glucose and xylose (Figure S1); electrophoretic profile of the *xyl* genes (Figure S2) (PDF)

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Notes

The authors declare no competing financial interest.

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