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Probiotic attributes, antioxidant and neuromodulatory effects of GABA-Producing *Lactiplantibacillus plantarum* SY1 and optimization of GABA production

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Abstract

Γ-aminobutyric acid (GABA), a major inhibitory neurotransmitter in the central nervous system, has been shown to alleviate various physiological disorders including insomnia, hypertension, depression, and memory loss. Lactic acid bacteria (LAB), recognized as safe GABA producers, have attracted increasing attention. This study aimed to screen GABA-producing LAB from naturally fermented dairy products and evaluate their probiotic potential, antioxidant and neuromodulatory activities, while optimizing GABA production. GABA-producing LAB were screened using the Berthelot method and thin-layer chromatography. The safety of *Lactiplantibacillus plantarum* SY1 was assessed through hemolysin production and drug sensitivity tests. *L. plantarum* SY1 demonstrated high tolerance to acidic conditions and low bile salt concentrations, along with significant antioxidant capacity (49 ± 0.2% DPPH radical scavenging rate, 86.1 ± 0.14% hydroxyl radical scavenging rate, and 32.7 ± 1.6% superoxide radical anion scavenging rate). In vivo experiments revealed that *L. plantarum* SY1 extended the lifespan of *C. elegans* N2, enhanced oxidative stress resistance, and delayed paralysis in transgenic *C. elegans* (CL4176) by 23.53%. Through OFAT strategy and RSM optimization, GABA production reached 1.49 g/L under optimal conditions (37℃, pH 4.44, 96 h fermentation, and 4.16% inoculum). These findings demonstrate that *L. plantarum* SY1 is a promising GABA-producing strain with antioxidant and neuromodulatory effects, suggesting its potential as an anti-aging and neuroprotective probiotic.

Keywords Gamma-aminobutyric acid, *Lactiplantibacillus plantarum*, Probiotic, Antioxidant, Neuromodulatory

Introduction

Gamma-aminobutyric acid (GABA), a non-protein amino acid, serves as a key inhibitory neurotransmitter in the central nervous system [1]. Extensive research has demonstrated its biological functions, including amelioration of Alzheimer's disease, anxiolytic effects, improvement of depression, and relief of insomnia [2]. Given these neuroprotective benefits, GABA's potential in treating neurological disorders has garnered significant attention [3]. Although orally administered exogenous GABA cannot cross the blood–brain barrier, emerging evidence

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suggests that GABA synthesized by intestinal microbiota may influence brain function through the enteric nervous system [4–6]. GABA biosynthesis in microorganisms is mediated by glutamate decarboxylase (GAD) enzymes, which catalyse the irreversible decarboxylation of L-glutamate to form GABA. A wide variety of microbial species have been shown to produce GABA, including members of the genera *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, *Carnobacterium*, *Enterococcus*, *Oenococcus*, *Tetragenococcus*, *Vagococcus* and *Weissella*, as well as certain *Bacteroides* and *Bifidobacterium* species. Compared to chemical synthesis, microbial GABA production offers the following advantages: (1) lower production costs, (2) environmentally sustainable processes and (3) direct in situ synthesis in fermented food matrices [7].

In this study, we characterize *Lactiplantibacillus plantarum* SY1, a novel multifunctional strain isolated from traditional fermented dairy products in Inner Mongolia. The strain exhibits three remarkable properties: (1) efficient γ-aminobutyric acid (GABA) biosynthesis, (2) potent antioxidant capacity, and (3) demonstrable neuromodulatory effects in *Caenorhabditis elegans*, distinguishing it from conventional GABA-producing probiotics. Through systematic optimization involving single-factor experiments and response surface methodology, we established optimal culture conditions for maximal GABA production. These findings position *L. plantarum* SY1 as a promising psychobiotic candidate with potential anti-aging and neuroprotective applications.

Material and methods

Isolation of GABA-Producing lactic acid bacteria

Natural fermented milk samples were collected from herders' homes in Hulunbuir, northeastern Inner Mongolia. Each sample was diluted in sterile water, plated on MRS agar, and incubated at 37 °C for 24–48 h to obtain single colonies. Suspected lactic acid bacteria colonies were repeatedly streaked on MRS plates for purification. The isolated strains were cultured in liquid MRS medium at 37 °C for 72 h. Following centrifugation at 6000 rpm for 5 min, the supernatant was collected. GABA production by selected strains was determined using the Berthelot method and thin-layer chromatography (TLC).

For thin-layer chromatography (TLC) analysis of GABA, 5 µL aliquots of cell-free supernatant were spotted onto silica gel 60 F254 aluminum-backed plates (Merck, Darmstadt, Germany). Chromatographic separation was performed using a mobile phase consisting of n-butanol:glacial acetic acid:deionized water (5:3:2, v/v/v). The presence of GABA compound in the sample was observed by red color spots on plate after addition of 0.2% of ninhydrine [8].

16S rRNA sequencing

The universal primer set 27 F (5'- AGAGTTTGATCC TGGCTCAG −3') and 1492R (5'- GGTTACCTTGTT ACGACTT −3') were used to identify the GABA-producing strain. The PCR cycling conditions were: initial denaturation at 95℃ for 5 min, cycles of denaturation at 95℃ for 30 s, annealing at 55℃ for 30 s, and extension at 72℃ for 45 s, a final extension at 72℃ for 10 min and 10 °C until halted by user. DNA sequencing was performed at Biozeron (Shanghai, China).

Probiotic properties of the *L. plantarum* SY1

Hemolytic test

The colonies were streaked onto blood agar plates containing 5% sheep defibrinated blood and incubated at 37 °C to assess hemolytic activity. Hemolytic reactions were recorded after 24–48 h of incubation [9].

Antibiotic susceptibility test

The drug sensitivity of the candidate strain was evaluated using the Kirby-Bauer antibiotic susceptibility test (K-B test). The strain was inoculated into liquid medium and cultured overnight at 37 °C. Subsequently, 200 µL of the bacterial suspension was added to 100 mL of MRS medium. After pouring the plates, antibiotic disks were placed on the MRS agar surface. The diameter of the inhibition zones was measured following overnight incubation [10].

Acid and bile salt resistance assay

Overnight bacterial cultures were inoculated into liquid media with varying pH values and incubated at 37 °C for 24 h to assess bacterial growth. Similarly, overnight cultures were inoculated into liquid media containing different concentrations of bile salts and incubated at 37 °C for 24 h to evaluate bacterial tolerance [11].

In Vitro free radical scavenging activities

The bacterial liquid culture was centrifuged at 6000 g for 5 min, and the supernatant was collected as cell-free culture supernatant (CFCS) [12].

DPPH Radical Scavenging Activity: 0.4 mL CFCS was mixed with 1.6 mL of freshly prepared 0.2 mM DPPH absolute ethanol solution to reaction under light protection for 25 min, the same volume of blank liquid medium was used instead of CFCS as a control. After completion of the reaction, the value of OD_{517} was measured. Where A_1 was the OD_{517} value of blank and A_0 was the OD_{517} value of sample, DPPH radical scavenging activity was calculated as:

$$\text{Scavenging effect} = (A_1 - A_0)/A_0 \times 100\%$$

Hydroxyl Radical Scavenging Assay: The reaction system included sodium salicylate solution (5 mM), ferrous sulfate solution (5 mM), and hydrogen peroxide solution (3 mM). 1 mL of each system was mixed with 1 mL CFCS. The control group was incubated with the same amount of blank liquid Instead of CFCS. After reaction for 50 min and centrifugation at 8000 g for 1 min, the value of OD_{510} was measured. The scavenging rate of superoxide anion radicals was calculated as follows, where A_1 represents the experimental group and A_0 represents the control group.

$$\text{Scavenging effect} = (A_1 - A_0)/A_0 \times 100\%$$

Superoxide Radical Anion Scavenging Assay: 1 mL of CFCS and 2.8 mL of Tris–HCl solution (0.1 M, pH8.0) were mixed and 0.1 mL Pyrogallol (0.05 M) solution was added to the mixture, the reaction was kept from light for 5 min. For the blank, CFCS was replaced with an equal volume of water. The OD_{325} value was recorded at 30 s and 300 s. where A represents the experimental group and B represents the control group.

$$\text{Scavenging effect} = [(B_{300s} - B_{30s}) - (A_{300s} - A_{30s})]/(B_{300s} - B_{30s}) \times 100\%$$

Lifespan assay

Age-synchronized *C. elegans* eggs were cultured on modified nematode growth medium (mNGM) plates until reaching the L4 larval stage. Fresh liquid cultures of 100 µL *E. coli* OP50, *L. plantarum* SY1, and GABA solution (1 g/L) were plated on mNGM plates supplemented with 100 µM FUDR (to prevent worm reproduction) and incubated overnight at 37 °C. Twenty L4-stage *C. elegans* (N2-Bristol strain) were transferred to each plate. Worm mortality was recorded daily under a microscope until all worms died. Each experiment was performed in triplicate [13].

In vivo antioxidant test

Fresh liquid cultures of 100 µL *E. coli* OP50, *L. plantarum* SY1, and GABA standard solution were plated on NGM plates and incubated overnight at 37 °C. L4-stage *C. elegans* were transferred to the prepared plates. After 5 days of feeding, approximately 20 worms from each group were transferred to blank mNGM plates containing 2 mM hydrogen peroxide. Worm mortality was recorded under a microscope every two hours until all worms died. Each experiment was performed in triplicate [14].

Paralysis assay

Fresh liquid cultures of *E. coli* OP50, *L. plantarum* SY1, and a GABA standard solution, each with a volume of 100 µL, were evenly spread onto NGM agar plates and incubated overnight at 37 °C. Synchronized L1-stage *C. elegans* of the CL4176 strain were then transferred onto these prepared plates. Following a 3-day feeding period at 20 °C, Aβ protein expression was induced by shifting the incubation temperature to 25 °C to initiate paralysis assays. The number of paralyzed worms was meticulously recorded at 4-h intervals until complete paralysis of all worms was observed. To ensure statistical reliability, each experimental condition was replicated three times [15].

Quantification of GABA Content

The bacteria were cultured in liquid MRS broth medium at 37 °C for 72 h. The fermentation broth was centrifuged at 8000 ×g for 10 min to separate the supernatant. Subsequently, 0.5 mL of the supernatant was mixed with 0.2 mL of 0.2 M borate buffer and 1.0 mL of 6% phenol, using the culture medium as a blank control. The mixture was thoroughly vortexed and then incubated in an ice bath for 5 min. Following this, 0.4 mL of NaClO solution (containing available chlorine) was added, and the mixture was returned to the ice bath for an additional 5 min. The solution was then heated in a boiling water bath for 10 min and allowed to cool to room temperature. The absorbance of the resulting blue-green solution was measured at 630 nm using a spectrophotometer. To establish a GABA concentration–response curve, the same reaction conditions were applied to a series of solutions containing known concentrations of GABA, and their absorbance values were recorded accordingly.

The reactions were performed under identical conditions using aqueous solutions of GABA at varying concentrations (0.2 g/L, 0.4 g/L, 0.6 g/L, 0.8 g/L, and 1.0 g/L) to construct a standard curve for GABA quantification. Each concentration was treated as described previously: mixed with borate buffer, phenol, and NaClO solution, followed by incubation in an ice bath, heating in a boiling water bath, and cooling to room temperature. The absorbance of the resulting solutions was measured at 630 nm, and the data were plotted to generate the standard GABA concentration–response curve. This curve was then used to determine the GABA content in the experimental samples based on their absorbance values.

Optimization of fermentation conditions

The optimal process conditions were initially identified using a one-factor-at-a-time (OFAT) strategy (initial pH, fermentation time, substrate, temperature and inoculum size). Based on the findings from the OFAT approach, a Box-Behnken design (BBD) was employed to further optimize the process, incorporating three independent variables. The experimental runs were designed, and the resulting data were analyzed using the statistical software Stat-Ease Design Expert 11.1.1.0. This response surface methodology allowed for the evaluation of interactions between variables and the identification of optimal conditions with greater precision and efficiency [16].

Results

Isolation and identification of GABA-producing LAB

Potential GABA-producing strains were isolated from naturally fermented milk products collected from Inner Mongolia. Nine strains were isolated and purified from various dairy products using the Berthelot method to determine their GABA production capacity. One of the strains with the highest yield was selected for subsequent experiments. TLC was used to further confirm GABA production (Fig. 1). The selected strain was further characterized using 16S rRNA gene sequencing to determine its taxonomic classification. BLAST analysis of the 16S rRNA sequence revealed 100% similarity with *Lactiplantibacillus plantarum* based on the NCBI database. The sequence was subsequently deposited in the NCBI GenBank under the accession number PV645098, and the strain was designated as *Lactiplantibacillus plantarum* SY1.

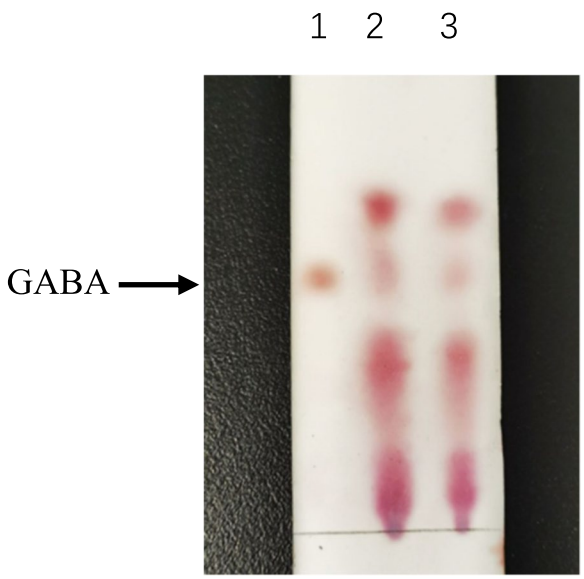

Fig. 1 Thin-layer chromatography of the GABA standard (line 1) and fermentation supernatant (line 2,3)

Hemolytic test

The hemolytic test results indicated that *L. plantarum* SY1 exhibited no hemolytic activity, confirming its safety profile. Based on this finding, the strain was deemed suitable for further probiotic evaluation and was selected for subsequent studies.

Antibiotic susceptibility testing

L. plantarum SY1 demonstrated sensitivity to ampicillin and chloramphenicol, while exhibiting resistance to cephalin, ceftazidime, vancomycin, rifampicin, gentamicin, clindamycin, norfloxacin, and enrofloxacin, as detailed in Table 1. These antibiotic susceptibility profiles provide valuable insights into the strain's potential applications and safety considerations.

Acid resistance and bile salt resistance assay

The acid tolerance test revealed that *L. plantarum* SY1 exhibited a 30% survival rate at pH 3.0 and a 59% survival rate at pH 3.5 after overnight incubation (Fig. 2), indicating its strong adaptability to acidic environments. In terms of bile salt tolerance, the strain demonstrated a 66% survival rate at a low bile salt concentration (0.10%). However, its survival rate decreased significantly to approximately 17% under medium and high bile salt concentrations. These results suggest that *L. plantarum* SY1 possesses robust acid tolerance but limited bile salt tolerance at higher concentrations.

In vitro Free Radical Scavenging activities

As shown in Table 2, *L. plantarum* SY1 demonstrated significant free radical scavenging activity, with a DPPH

Table 1 The antibiotic susceptibility of *L. plantarum* SY1

Antibiotics	diameters of zone of inhibitions(mm)	susceptibility
Tetracycline	15	I
Cephalin	6	R
Ceftazidime	0	R
Ampicillin	19	S
Vancomycin	0	R
Rifampicin	7	R
Erythromycin	15	I
Gentamicin	0	R
Clindamycin	0	R
Chloramphenicol	23	S
Norfloxacin	0	R
Enrofloxacin	6	R

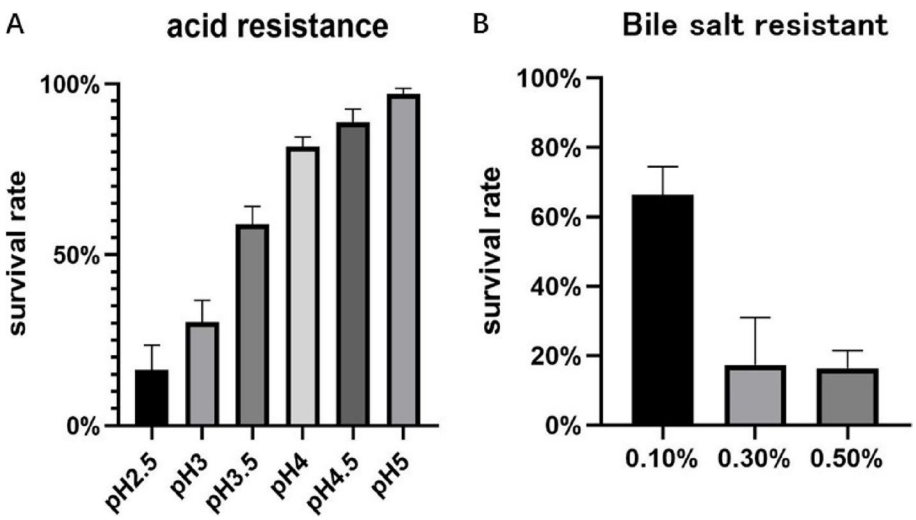

Fig. 2 Acid resistance and bile salt resistance of *L. plantarum* SY1. **A** *L. plantarum* SY1 were cultured overnight at pH 2.5, 3.0, 3.5, 4, 4.5, 5, 6(control) **B** *L. plantarum* SY1 were cultured in MRS liquid medium containing 0% (control), 0.1%, 0.3% and 0.5% bile salts

Table 2 In vitro free radical scavenging ability determination experiment

In vitro antioxidant assays	Free radical scavenging rate
DPPH radical scavenging activity	49 ± 0.2%
Hydroxyl radical scavenging activity	86.1 ± 0.14%
Superoxide radical anion scavenging activity	32.7 ± 1.6%

radical scavenging rate of 49 ± 0.2%, a hydroxyl radical scavenging rate of 86.1 ± 0.14%, and a superoxide radical anion scavenging rate of 32.7 ± 1.6%. These results indicate that the strain effectively scavenges DPPH radicals, hydroxyl radicals, and superoxide radicals, highlighting its potent free radical degradation capabilities. The findings underscore the potential of *L. plantarum* SY1 as a strain with notable antioxidant properties.

Lifespan assay

As illustrated in Fig. 3, the mean lifespan of the control group was 8.24 ± 0.483 days, while the GABA group and *L. plantarum* SY1 group exhibited mean lifespans of 9.22 ± 0.59 days and 10.37 ± 0.15 days, respectively. Compared to the control group, the mean lifespan increased by 11.9% in the GABA group and by 25.85% in the *L. plantarum* SY1 group. Throughout the observation period, nematodes in the *L. plantarum* SY1 group consistently demonstrated better survival rates than those in the control group, with their lifespan extended by over two days. These results indicate that *L. plantarum* SY1 significantly enhances

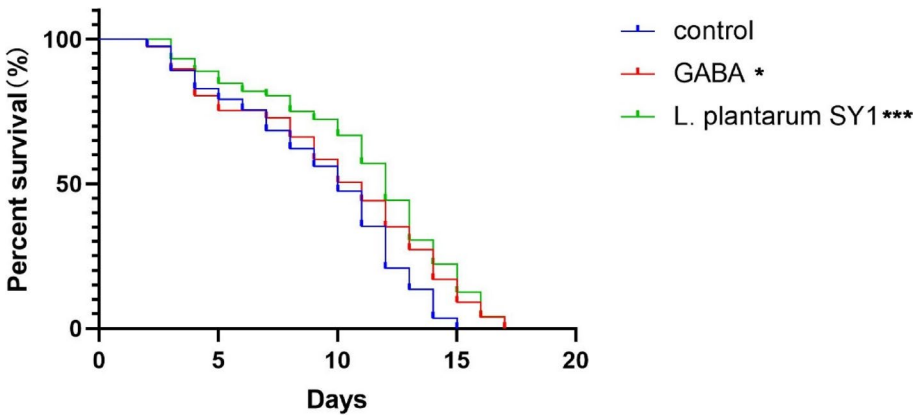

Fig. 3 Survival curves of *C. elegans* fed with different food sources

the lifespan of nematodes, highlighting its potential as a probiotic with lifespan-extending properties.

In vivo antioxidant test

Under oxidative stress conditions induced by hydrogen peroxide, *C. elegans* fed with *L. plantarum* SY1 exhibited significantly improved survival compared to the control group. The mean lifespan of the control group was 9.26 ± 0.48 h. In contrast, the GABA group showed a mean lifespan of 8.62 ± 0.426 h, representing a 6.91% reduction compared to the control group. Meanwhile, the *L. plantarum* SY1 group demonstrated a mean lifespan of 11 ± 0.533 h, reflecting an 18.8% increase in survival time (Fig. 4). After the induction of oxidative stress, worms in all groups began to die progressively after 4 h, and no surviving worms were observed in the control group by 16 h. These findings suggest that *L. plantarum* SY1 significantly enhances the antioxidant capacity of *C. elegans*, whereas no notable improvement was observed in the GABA group compared to the control.

Paralysis assay

In the Aβ-induced paralysis assay, worm paralysis began to manifest at the 20 th hour of observation. By the 60 th hour, all worms in the control group had succumbed to paralysis. In contrast, 48% of the worms in the GABA group and 26% of the worms in the *L. plantarum* SY1 group remained unparalyzed at the same time point (Fig. 5). These results demonstrate that both GABA and *L. plantarum* SY1 significantly delayed the onset of paralysis in the CL4176 strain of *C. elegans*, highlighting their potential neuroprotective effects.

Optimization of GABA production

The fermentation process was optimized using a OFAT approach, and the Berthelot method was employed to determine the optimal culture conditions for GABA production. The initial pH of the fermentation medium was optimized to 4.5, yielding a GABA production of 1.17 g/L. Deviations from this pH value, either higher or lower, resulted in reduced GABA production. The optimal fermentation duration was identified as 96 h, during which GABA production increased steadily, reaching its

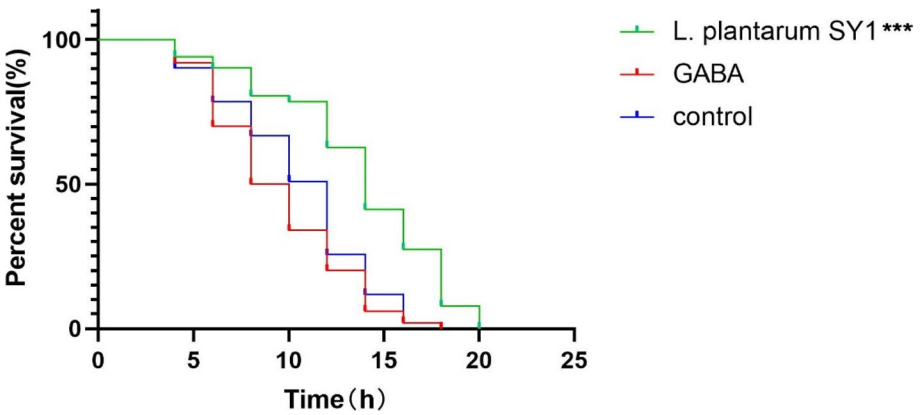

Fig. 4 Survival curves of *C. elegans* under oxidative stress

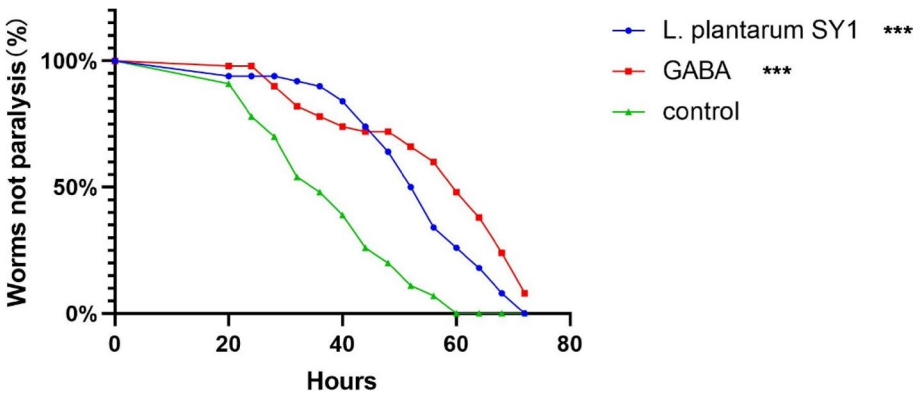

Fig. 5 Paralysis of CL4176 was delayed by feeding GABA and *L. plantarum* SY1

peak concentration at 96 h. Beyond this point, GABA yield began to decline, likely due to partial degradation of GABA over time.

As illustrated in Fig. 6, the addition of the substrate (glutamate) had minimal impact on GABA production. This may be attributed to the fact that the endogenous substrate produced by the bacteria was sufficient, and exogenous substrate addition could potentially inhibit enzyme activity. Regarding fermentation temperature, the highest GABA production (1.19 g/L) was achieved at 37 °C, although production levels remained relatively stable between 31 °C and 37 °C. The optimal inoculation dose was determined to be 4% and 6%, both yielding a GABA production of 1.02 g/L (Fig. 6E). Additionally, the growth curve of the bacteria and the corresponding GABA production curve were analyzed (Fig. 6F-G), providing further insights into the relationship between bacterial growth and GABA yield.

Based on the results of the single-factor experiments, three critical variables—initial pH, fermentation time,

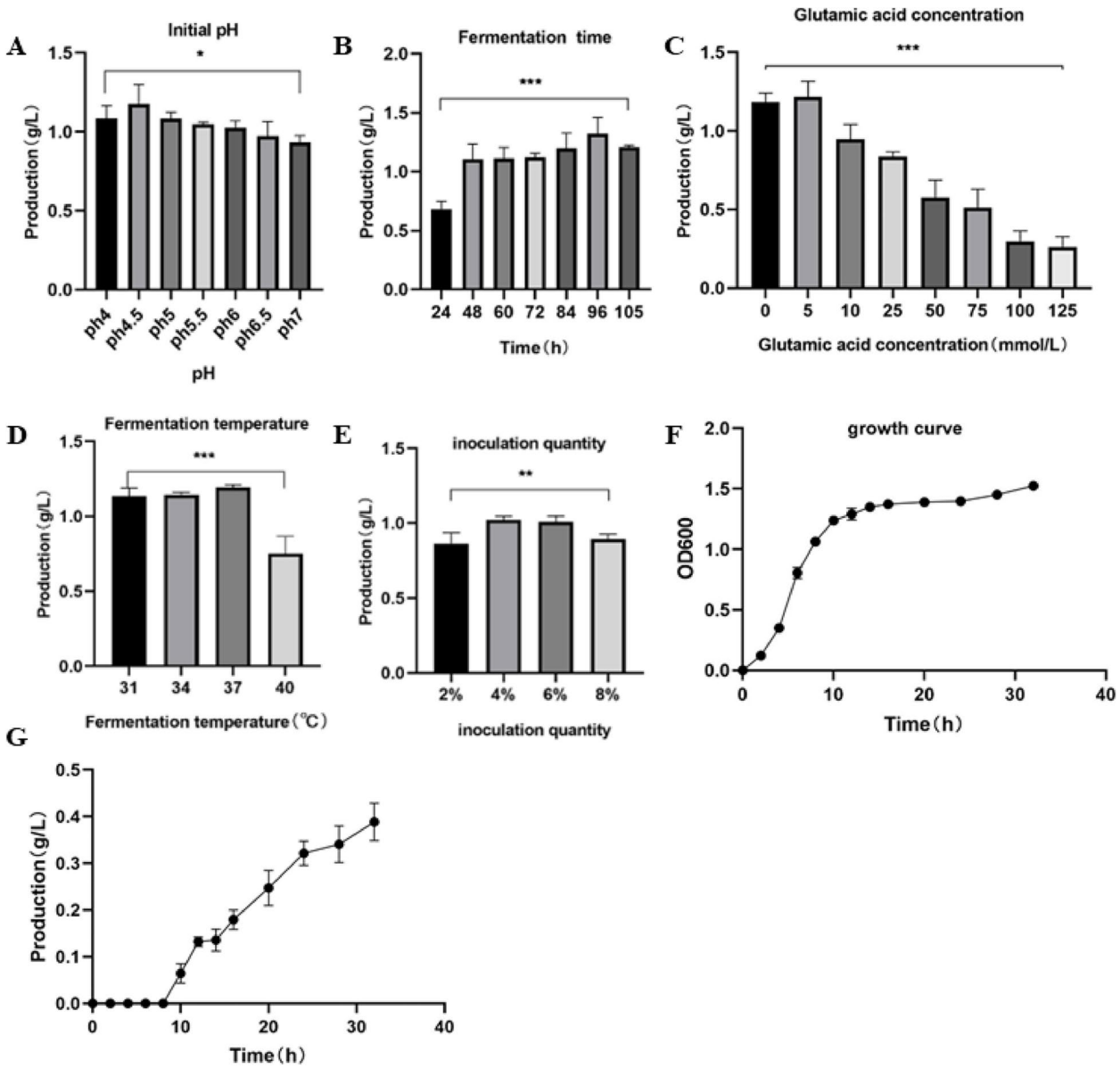

Fig. 6 Optimization of GABA Production by OFAT Strategy. **A** To determine the optimal initial pH for fermentation. **B** The optimal fermentation time was determined. **C** The amount of substrate added was determined. **D** Determination of the optimal temperature. **E** Establishment of inoculum size. **F** growth curve for *L. plantarum* SY1. **G** Determination of GABA production with growth curves

and inoculum size—were selected for the response surface methodology (RSM) design. Three levels for each variable were chosen, and the experiments were conducted in triplicate. The data were analyzed using Design-Expert 11.1.1.0 software, and the analysis of variance (ANOVA) for the response surface quadratic model revealed that the regression model was statistically significant, while the lack of fit was not (Table 3). The coefficient of determination (R^2) and adjusted R^2 (Adj R^2) for the model were 89.17% and 75.16%, respectively, indicating that the model was suitable for analyzing and predicting GABA production.

The quadratic regression model equation and response surface graphs were used to determine the optimal fermentation conditions: an initial pH of 4.44, a fermentation time of 96 h, and an inoculum size of 4.16% (Fig. 7). The regression equation derived from the model is as follows:

$$Y = -85.21095+16.19684A+6.75835B+0.769275C-0.451429AB-0.059393AC-0.035587BC-0.955353A^2-0.162136B^2-0.001917C^2$$

where Y represents GABA production, A is the initial pH, B is the fermentation time, and C is the inoculum size. This model provides a reliable framework for optimizing GABA production under the specified conditions.

Discussion

Gamma-aminobutyric acid (GABA) is a key inhibitory neurotransmitter in the central nervous system, synthesized from glutamate through the action of glutamate decarboxylase (GAD) [17]. In this study, *L. plantarum* SY1 was isolated from naturally fermented milk products obtained from herders' homes in Hulunbuir. *L. plantarum* SY1 demonstrated no hemolytic activity, susceptibility to ampicillin and chloramphenicol, and the ability to survive under low pH and high bile salt conditions. Additionally, *L. plantarum* SY1 exhibited notable antioxidant capacity. Lactic acid bacteria (LAB) are recognized for their probiotic properties and are easily utilized by the human body, making them ideal candidates for GABA production. Numerous studies have focused on isolating GABA-producing LAB from fermented products. For example, *L. plantarum* L42 g was isolated from fermented beef [18], *L. buchneri* from mukeunjee kimchi showed high GABA production [19], and *L. brevis* HYE1 was also isolated from kimchi [20]. The screening of GABA-producing LAB holds significant importance for the food and pharmaceutical industries, as these strains can be employed as food additives or functional supplements to develop GABA-enriched products [21–23]. For instance, in a previous study, eleven strains isolated from kimchi were selected as starter candidates for GABA production during sausage fermentation [24]. Similarly, another study investigated eighteen lactobacilli strains for GABA production in amaranth and wheat flour liquid sourdoughs to produce GABA-enriched bread [25]. These findings underscore the potential of *L. plantarum* SY1 and other GABA-producing LAB strains in developing functional foods and supplements with enhanced health benefits.

The results of the *C. elegans* lifespan extension experiment demonstrated that feeding *L. plantarum* SY1

Table 3 ANOVE for quadratic model

Source	Sum of Squares	df	Mean Square	F-value	*P*-value	
Model	0.5065	9	0.0563	6.38	0.0116	significant
A-pH	0.0059	1	0.0059	0.6738	0.4388	
B-Inoculum	0.0015	1	0.0015	0.1684	0.6938	
C-time	0.0202	1	0.0202	2.29	0.1744	
AB	0.0734	1	0.0734	8.31	0.0235	
AC	0.0457	1	0.0457	5.18	0.0570	
BC	0.1824	1	0.1824	20.67	0.0026	
A^2	0.0311	1	0.0311	3.53	0.1024	
B^2	0.1107	1	0.1107	12.54	0.0094	
C^2	0.0200	1	0.0200	2.27	0.1754	
Residual	0.0618	7	0.0088			
Lack of Fit	0.0466	3	0.0155	4.11	0.1027	not significant
Pure Error	0.0151	4	0.0038			
Cor Total	0.5682	16				

A, B and C represents pH value, inoculum and fermentation time

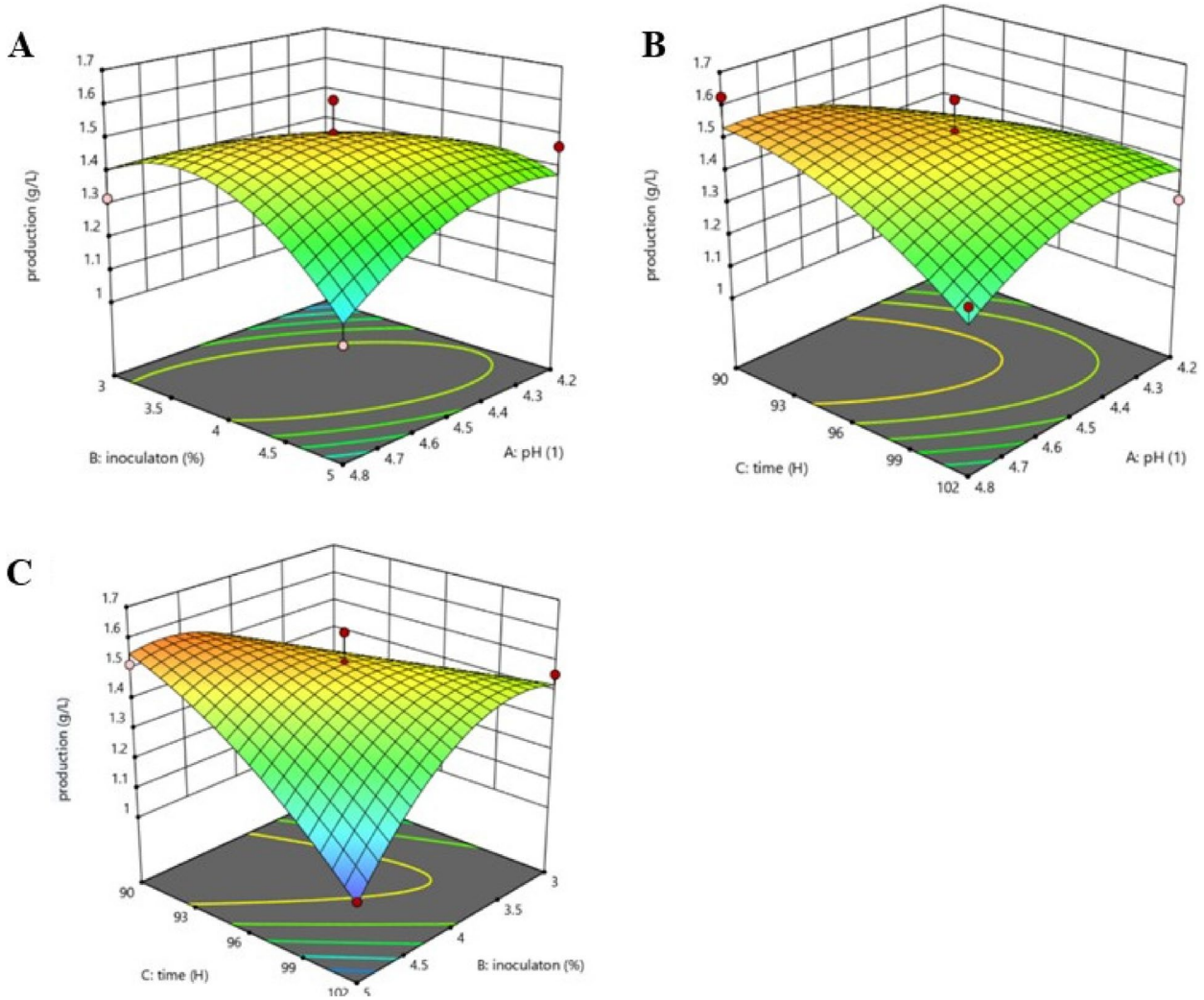

Fig. 7 Three-dimensional response surface plots indicating the interaction of each variable on GABA production by *L. plantarum* SY1. **A** GABA production by the interaction between initial pH and inoculum (**B**) GABA production by the interaction between fermentation time and initial pH value. **C** GABA production by the interaction between fermentation time and inoculum

significantly prolonged the lifespan of the worms compared to the control group. Under oxidative stress conditions induced by hydrogen peroxide, *C. elegans* N2 fed with *L. plantarum* SY1 exhibited an 18.8% improvement in survival rate compared to the control group. These findings align with previous studies showing that lactic acid bacteria (LAB) can extend the lifespan of *C. elegans*. For instance, feeding *L. rhamnosus* R4 increased the mean lifespan of *C. elegans* by 36.1% compared to the control [26].

Additionally, a paralysis assay was conducted to evaluate the potential alleviation of Aβ-induced toxicity. The results revealed that feeding *L. plantarum* SY1 significantly delayed the onset of paralysis in the worms, indicating its potential to mitigate Aβ toxicity. These findings highlight the beneficial effects of *L. plantarum* SY1 in enhancing lifespan, improving oxidative stress resistance, and reducing Aβ-induced paralysis in *C. elegans*, further supporting its potential as a probiotic with neuroprotective and anti-aging properties. It has been shown that increasing GABA synthesis and transport prevents seizure-like behaviour in *C. elegans* [27].

The optimal conditions for efficient GABA production by *L. plantarum* SY1 under various environmental and nutritional conditions were determined. In the single-factor experiments, key parameters such as initial pH, fermentation temperature, fermentation time, substrate addition, and inoculum size were evaluated. The results indicated that the optimal initial pH was 4.5, the optimal fermentation temperature was 37

°C, and the optimal inoculum size was 4%, selected based on cost–benefit principles. GABA accumulation peaked at 96 h of fermentation. Interestingly, when the glutamate concentration was low (5 mmol/L), GABA production slightly increased, but further increases in substrate concentration led to a decline in GABA yield. These findings are consistent with previous studies, such as one that optimized GABA production in *Lactobacillus brevis* FBT215 using OFAT, achieving a yield of 1688.65 ± 14.29 μg/mL by focusing on medium composition, initial pH, fermentation temperature, and fermentation time [28]. However, due to the limitations of the OFAT method, which cannot account for interactions between factors, RSM was employed for further optimization. Based on OFAT results, initial pH, fermentation time, and inoculum size were chosen for RSM using Design-Expert 11.1.1.0 software. The optimal conditions (pH 4.44, 96 h, 4.16% inoculum) yielded a predicted GABA production of 1.49 g/L. This approach aligns with other studies, such as the optimization of *L. brevis* FBT215, which achieved a GABA yield of 1812.16 ± 23.16 μg/mL using RSM [28], and *L. brevis* HYE1, whose GABA production increased from 14.64 mM to 18.76 mM after RSM optimization [20]. These results underscore the effectiveness of RSM in enhancing GABA production by accounting for factor interactions and optimizing multiple variables simultaneously.

However, further research is necessary to elucidate the neuroprotective mechanisms of *L. plantarum* SY1 and to investigate whether GABA-producing bacteria can play a role in mitigating nervous system diseases in mammalian models. Such studies will provide deeper insights into the therapeutic potential of this strain and its applicability in functional foods and pharmaceutical developments.

Conclusion

This study successfully isolated a GABA-producing strain, *Lactiplantibacillus plantarum* SY1, which demonstrated excellent probiotic properties, including acid and bile salt tolerance, non-hemolytic activity, and antibiotic susceptibility. Additionally, *L. plantarum* SY1 exhibited significant potential in neuroprotection and antioxidant activities, as evidenced by its ability to extend the lifespan of *C. elegans*, enhance oxidative stress resistance, and alleviate Aβ-induced paralysis. The optimization of GABA production using both the OFAT method and RSM yielded a maximum GABA production of 1.49 g/L. These findings highlight *L. plantarum* SY1 as a promising GABA-producing strain with potential applications as an anti-aging and neuroprotective probiotic.

Abbreviations

GABA	γ-Aminobutyric acid
LAB	Lactic acid bacteria
TLC	Thin-layer chromatography
CFCS	Cell-free culture supernatant
OFAT	One-factor-at-a-time
RSM	Response surface methodology

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Not applicable.

Authors' contributions

Conceptualization, W.C.; methodology, X.B. and P. S.; software, P.S.; validation, X.B.; formal analysis, P.S.; investigation, P.S. and X.B.; resources, data curation, P.S. and X.B.; writing—original draft preparation, P.S. and X.B.; writing—review and editing, visualization, W.C.; supervision, W.C.; project administration, W.C.; funding acquisition, W.C.

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Data availability

The 16S rRNA sequence of L. plantarum SY1 was deposited in the NCBI GenBank under the accession number PV645098 (https://www.ncbi.nlm.nih.gov/nuccore/PV645098). The other data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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