Experiment name: Explore the effect of different nitrogen sources on GABA synthesis

The experimental environment was room temperature 31℃.

Objective: To determine the GABA content of the samples by Berthelot colorimetric method, and to find the reagent with the strongest catalytic effect on GABA synthesis in organic and inorganic nitrogen sources.

Experimental materials:

The main reagent	specifications	place of origin
Gamma-aminobutyric acid standard	A purity of 98% or more	Shenggong Bioengineering (Shanghai) Co., LTD
phenol	Analysis of pure	Tianjin Damao Chemical Reagent Factory
Sodium tetraborate	Analysis of pure	Tianjin Damao Chemical Reagent Factory
Sodium hypochlorite	Analysis of pure	Tianjin Damao Chemical Reagent Factory
Strains of Lactobacillus longer DLF-19076		The laboratory screened and stored
MRS culture medium	Glucose 20g/L, peptone 10g/ L L, beef paste 10g/L, yeast extract 5g/L, sodium acetate 5g/L, citric acid Diammonium 2 g/L, dipotassium hydrogen phosphate 2.0g/L, 0.25 g/L manganese sulfate tetrahydrate, sulfuric acid heptahydrate Manganese 0.58 g/L, Tween-80 1 mL/L, make Adjust pH to 6.5 with 1mol/L HCL, Autoclaved at 120 ° c for 20min	This laboratory configuration and storage

Experimental process: Appropriate amount of wet thallus was taken and loaded into 4 shaking bottles, 150ml each,and the pH was adjusted to 6.5.2% yeast was added to the medium. Powder, 2% peptone, 2% urea, 2% beef paste, 0.1% ammonium sulfate, 0.1% ammonium phosphate, 0.1% ammonium chloride and 0.1% ammonium nitrate were added at 35 ℃. The samples were incubated in shaking flask at 120 r/min and fermented for 12 h.About 10g of unstratified samples in good flow state were placed in the centrifuge tube and used on the table

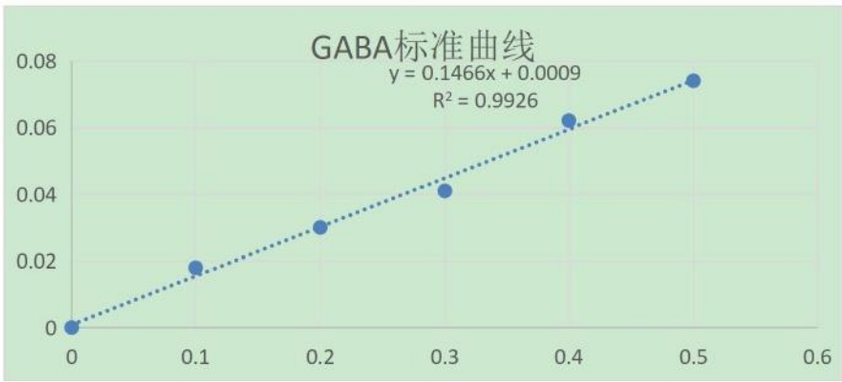
The high speed centrifuge was centrifuged at 8000r/min for 10min. After centrifugation, the supernatant was quickly separated from the sediment, and samples were taken 300μl of

supernatant was put into the test tube, and 300 μl of deionized water was taken into another test tube as a blank control experiment, and then 10mL of substance was added into the test tube. The molar concentration of sodium tetraborate solution was 0.1mol/L, the mass fraction of sodium hypochlorite solution was 600μL, the mass fraction of benzene was 400 μl, the mass fraction of benzene was 6%. The phenol solution, after shaking, was placed in a boiling water bath for 10min and an ice water bath for 5min.After the test tube solution developed color, different concentrations were measured at 645nm wavelength.The absorbance value of the standard solution, and all the samples to be tested need to carry out three parallel experiments, according to the measured absorbance value, according to the GABA standard curve.

The GABA content of the corresponding samples to be tested can be obtained.Experimental results:

Nitrogen source	First set of absorbance	Absorbance Set 2	Absorbance Set 3
Yeast powder	0.039	0.041	0.042
peptone	0.062	0.060	0.055
The urea	0.058	0.054	0.057
Beef paste	0.022	0.032	0.031
Ammonium sulfate	0.076	0.070	0.069
Ammonium phosphate	0.070	0.065	0.070
Ammonium chloride	0.063	0.070	0.068
Ammonium nitrate	0.063	0.069	0.069

Nitrogen source	Yeast powder	peptone	The urea	Beef paste	Ammonium sulfate	Ammonium phosphate	Ammonium chloride	Ammonium nitrate
Absorbance	0.041	0.059	0.056	0.028	0.072	0.068	0.067	0.067



Results Analysis: GABA synthesis concentration under nitrogen source can be obtained from GABA standard curve

Nitrogen source	GABA concentration (g/L)
Yeast powder	0.274
peptone	0.396
The urea	0.376
Beef paste	0.185
Ammonium sulfate	0.485
Ammonium phosphate	0.458
Ammonium chloride	0.451
Ammonium nitrate	0.451

The synthesis efficiency of GABA was improved under the condition of organic nitrogen peptone as compared with beef extract μ = 0.396−0.2740.274 = 44.53% under the condition of inorganic nitrogen ammonium sulfate as compared with ammonium chloride. Peptone in organic nitrogen source and ammonium sulfate in inorganic nitrogen source had the best effect on GABA synthesis.The synthesis efficiency of GABA was improved μ = 0.485−0.451 0.451= 7.54%

Studies on the preparation of γ-aminobutyric acid by Microbial fermentation

Yuemin Leng, Xu Guo, Hengxu Liu, Zeyu Liu, Zhende Wang

Abstract

γ-aminobutyric acid (GABA) is a kind of non-protein amino acid that regulates various physiological functions of organisms. It is mainly distributed in mammals, plants and microorganisms. It has been widely used in the medical field and is a kind of high polymer organic matter with great research value.In this paper, the high yield of γ-aminobutyric acid Lactobacillus brevitae DLF-19076 as the research object, the culture medium composition and fermentation conditions were optimized, and in the fermentation process, respectively, using batch fermentation and batch flow feeding fermentation process, to study the pH, strain concentration and temperature factors on the fermentation process. In order to improve the yield of γ-aminobutyric acid, γ-aminobutyric acid prepared by biological method to provide a more suitable preparation method.

Key words: Microbial fermentation;γ-aminobutyric acid;Conditions optimization

1 Overview of γ-aminobutyric acid

1.1 Physicochemical properties of γ-aminobutyric acid

γ-aminobutyric acid (GABA) has a molecular formula of C4H9NO2 and a molecular weight of 103.1 [1].GABA is widely distributed in the natural boundary and has been found to exist in animals, some plants and microorganisms.In animal cells, most of GABA exists in neural tissues, and γ-aminobutyric acid is relatively concentrated in the substantia nigra, striatum, hypothalamus and brain stem [2].The content of γ-aminobutyric acid is not high in animals and plants, and the content of γ-aminobutyric acid in animal brain tissue is only 0.1-0.6 mg/g [3].Therefore, γ-aminobutyric acid is mostly obtained from microbial fermentation process.

γ-aminobutyric acid is white crystalline powder, no optical activity, easily soluble in water, 25℃ solubility of 130 g/100mL[1], insoluble in room temperature ethanol.In aqueous solution, GABA was dominated by zteriions, with PK values of 10.56 and 4.03, and isoelectric point pI of 7.19.It can react with ninhydrin to form a purple substance with a maximum absorbance at 570 nm. This reaction can be used for the quantitative analysis of GABA.

1.2 Physiological function of γ-aminobutyric acid

GABA is an important substance that inhibits nerve information transmission and participates in a variety of physiological and metabolic activities. Its main physiological functions are as follows:

(1) Regulating blood pressure

Some studies have shown that GABA can regulate blood pressure, mainly by promoting vascular softening and dilatation, and reducing blood viscosity.GABA acts in the vasomotor center of the spinal cord, and identifies the role of GABA A receptors acting on postsynaptic GABA A receptors in dilating blood vessels and binding with GABA B receptors to inhibit sympathetic excitability, so as to effectively promote vascular dilation [3].In addition, γ-aminobutyric acid can also inhibit the secretion of antidiuretic hormone and the activity of angiotensin-converting enzyme (ACE), thereby realizing the dilatation of blood vessels, and finally achieving the effect of lowering blood pressure [3],4).

(2) Treat epilepsy

The seizure of epilepsy is related to the imbalance of GABA and glutamate. GABA receptor agonists can effectively control the abnormal conflict of neurons, and GABA receptor inhibitors can increase the content of GABA in the synaptic clef, thus effectively inhibiting the effect of epilepsy.In addition, GABA can increase the anticonvulsant threshold, which is a specific biochemical drug for the treatment of intractable epilepsy [5].

(3) To promote reproduction

GABA has the function of promoting hormone secretion, so it can indirectly affect the function of reproductive organs, mainly manifested as affecting the uterus and transfusio

1.3 Main preparation methods of γ-aminobutyric acid

The research on GABA has become a hot spot for many researchers. A series of studies have been conducted on the preparation of GABA. The current preparation methods are as follows:

(1) Chemical synthesis method

GABA can be prepared by γ-chloroprene replacing water reaction. Under high temperature conditions, γ-chloroprene can react with potassium phthalimide, and then hydrolyze with concentrated sulfuric acid, and GABA can be prepared after crystallization.This method involves a lot of chemical process, there are a lot of chemical residues in the production process, commonly used in the chemical field, can not be used in the field of medicine and food.

(2) Plant enrichment method

GABA has been found in many natural plants, and the content of GABA increased significantly under adverse factors.Therefore, under the conditions of low temperature, low oxygen and acid/salt stress, a trace amount of GABA can be enriched.Although there is no pollution in the obtained GABA, the content of the obtained GABA is very low, and it is obviously difficult to be used in large-scale production.

(3) Microbial synthesis method

Under the catalytic action of GABA, glutamic acid or sodium glutamic acid decarboxylated into GABA.However, microorganisms use it as production bacteria due to its wide distribution, fast growth and easy culture.The microbial synthesis method developed well and solved various problems existing in the other two methods.At present, many strains can be used as production bacteria, such as Escherichia coli, yeast, glutamatergic corynebacterium, and the strains suitable for GABA can be selected according to different microbial characteristics.Lactic acid bacteria, as food-safety grade microorganisms, has become the best bacteria applied in food, with excellent commercial development prospects.

2 Research status of γ-aminobutyric acid fermentation by lactic acid bacteria

As a food-safe microorganism, lactic acid bacteria have been widely used in food and medicine. At present, the production of GABA by Lactobacillus mainly includes fermentation production method and cell transformation method.So far, researchers have been from vinasse, pickled vegetables, yogurt and other traditional fermented food in screening of the strains with GABA production capacity of lactic acid bacteria, such as plant lactobacillus, rhamnose, lactobacillus, brinell lactobacillus, short lactobacillus, lactobacillus casei, pediococcus pentosaceus, thermophilic streptococcus and lactic acid milk coccus butterfat subspecies [12-14].Lu et al. screened a strain of Lactococcus lactis from kimchi, and the yield could reach 7.2 g/L after optimization [14].At present, in addition to strain screening and culture condition optimization, there are also mutagenesis methods for strains to improve GABA synthesis ability. For example, a strain of Lactobacillus brevi HJXJ-01 was screened from unsterilized fresh milk, and mutagenesis was repeated by UV and 60Co γ-rays. After 72 hours of fermentation, the yield of the mutant strain could reach 17 g/L, which was 140% higher than that of the wild-type strain [2].In summary, microbial preparation of GABA has the advantages of low cost, high safety and high yield. Therefore, how to select safe and high-yield strains from nature and how to improve the yield of GABA through optimization of fermentation process have become important factors restricting the application of microbial method into large-scale production [14]. The contraction of the oocyte tube and the acceleration of sperm transport.Zhao Hai et al. [6] studied that GABA could regulate the reproduction of decidual cells and trophoblast cells, and the results showed that GABA had a significant effect on the formation of placenta in mice.Liu Xiaoyan et al. [7] proved for the first time that GABA is involved in the regulation of follicle development and maturation by radioimmunoassay.In addition, studies have also shown that high concentration of GABA can help improve the speed of human spermatozoa, thereby enhancing the fertilization ability of spermatozoa [8].

3 Preparation of γ-aminobutyric acid by fermentation of lactic acid bacteria

3.1 Research Contents

Lactobacillus brevi DLF-19076 with high γ-aminobutyric acid yield was selected from mustard mustard in our

laboratory. The culture medium composition and fermentation conditions were optimized, and the effects of pH, dissolved oxygen and coenzyme on the fermentation process were studied, in order to improve the yield of γ-aminobutyric acid.

3.2 Experimental materials and reagents

γ-aminobutyric acid standard: Shenggong Bioengineering (Shanghai) Co., LTD ; Lactobacillus Brevis DLF-19076: Selected and preserved in our laboratory.

Hydrochloric acid, sodium hydroxide, phenol, acetone, sodium hypochlorite, sodium tetrborate, sodium citrate, Glucose standards: Tianjin Damao Chemical Reagent Factory, Urea, ammonium chloride, ammonium phosphate, ammonium sulfate, MRS medium: glucose 20 g/L, protein aged 10 g/L, beef paste 10 g/L, leaven

The mother extract was 5 g/L, anhydrous sodium acetate 5 g/L, diammonium citrate 2 g/L, dipotassium hydrogen phosphate 2.0 g/L, manganese sulfate tetrahydrate 0.25 g/L, manganese thioate heptahydrate 0.58 g/L, and Twain -80 1 mL/L

3.3 Experimental Methods

3.3.1 Strain culture

After activation, the thalli stored in the laboratory were added to MRS medium (100 mL/250 mL) and incubated at 35 ℃ for 24 h. The culture solution was centrifuged (4 ℃, 10000 R /min, 10 min). The collected strains were washed three times with 0.2 mol/L phosphate buffer salt (pH 7.2) and stored at 4 ℃ for later use.

3.3.2 Optimization of cell-catalyzed reaction conditions

(1) Effect of medium N source on the catalytic synthesis of GABA

An appropriate amount of wet thallus was put into 4 shaker bottles (150 ml each), and the pH was adjusted to 6.5.The culture medium was supplemented with 2% yeast powder, 2% peptone, 2% urea, 2% beef extract, 0.1% ammonium sulfate, 0.1% ammonium phosphate, 0.1% ammonium chloride and 0.1% ammonium nitrate, respectively. The culture medium was shaken at 35 ℃ and 120 R /min for 12 h.The content of GABA was determined.

(2) The effect of substrate concentration on the catalytic synthesis of GABA

The solid medium of 3G, 4G, 5G, 6g, 7g and 8g MRS was added to 6 conical bottles, and 150 mL deionized water was added to the volume, and the pH was adjusted to 6.5. Then the medium was put into an autoclave and sterilized at 120℃ for 20 min. The samples were incubated at 35 ℃ and 120 R /min in shaking flask and fermented for 12 h.The content of GABA was determined.

(3) Effect of pH on the catalytic synthesis of GABA

MRS medium 63.3 g was weighed and dissolved in 1000 mL water to form liquid medium. 150 mL culture base was added to 6 conical flyers and pH was adjusted to 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, respectively. The culture medium was sterilized in autoclave at 120 ℃ for 20 min, removed, inoculated with appropriate amount of bacteria, and incubated in shake flask at 35 ℃ and 120 R /min for 12 h.The content of GABA was determined.

(4) The effect of the amount of bottling liquid on the catalytic synthesis of GABA

MRS medium 63.3 g was weighed and dissolved in 1000 mL of water to form liquid medium. Then 75 mL, 100 mL, 125 mL, 150 mL, 175 mL and 200 mL of medium were added to 6 conical bottles, and the pH was adjusted to 6.5. The culture medium was sterilized in an autoclave at 120℃ for 20 min, removed, inoculated with appropriate amount of bacteria, and incubated in shaking flask at 35 ℃ and 120 R /min for 12 h.The content of GABA was determined.

(5) The effect of strain concentration on the catalytic synthesis of GABA

MRS medium 63.3 g was weighed, dissolved in 1000 mL water and configured as liquid medium. 150 mL culture base was added to 5 conical flflask, pH was adjusted to 6.5, and then put into autoclave and sterilized at 120 ℃ for 20 min. Wet-thalli were inoculated with concentrations of 50 g/L, 100 g/L, 150 g/L, 200 g/L, and 250 g/L, respectively, and incubated in shaking flask at 35℃ and 120 R /min for 12 h.The content of GABA was determined.

(6) Effect of temperature on the catalytic synthesis of GABA

MRS medium 63.3 g was weighed, dissolved in 1000 mL of water and configured as liquid medium. 150 mL of culture base

was added to four conical flaps, the pH was adjusted to 6.5, and then sterilized in an autoclave at 120 ℃ for 20 min. The culture medium was placed in a shaker at 25℃, 30℃, 35℃, and 40℃, respectively. The culture medium was shaken at 120 r/min and fermented for 12h.The content of GABA was determined.

About 10 g of unstratified samples in good flow state were placed in the centrifuge tube and centrifuged at a speed of 8000 R /min for 10 min using a desktop high-speed centrifuge. After centrifugation, the supernatant was quickly separated from the sediment, and 300 μL of the supernatant was placed in the test tube. And 300 μL deionized water was taken in another test tube as a blank control experiment, and then 10 mL sodium tetraborate solution with a mass concentration of 0.1 mol/L, 600 μL sodium hypochlorite solution with a mass fraction of 8%, and 400 μL phenol solution with a mass fraction of 6% were added to the test tube. After shaking well, After shaking, the mixture was placed in boiling water bath for 10 min and ice water bath for 5 min.After the test tube solution developed color, the absorbance values of the standard solution with different concentrations were measured at 645 nm wavelength, and all the samples to be tested needed three parallel experiments. According to the absorbance values measured, the GABA content of the corresponding samples to be tested could be obtained according to the GABA standard curve.

3.4 Experimental Results

(1) The average absorbance of each N source was obtained as follows: yeast powder: 0.041;Peptone: 0.041;Urea: 0.056;Beef paste: 0.028;Sulfuric acid

Ammonium: 0.072;Ammonium phosphate: 0.068;Ammonium chloride: 0.067;Ammonium nitrate: 0.067

The mean GABA synthesis concentration (g/L) of each N-source was obtained as follows: yeast powder: 0.274;Peptone: 0.396;Urea: 0.376;Beef paste: 0.185;Ammonium sulfate: 0.485;Ammonium phosphate: 0.458;Ammonium chloride: 0.451;Ammonium nitrate: 0.451

The GABA synthesis efficiency of organic nitrogen source peptone was 44.53% higher than that of beef extract, and the GABA synthesis efficiency of inorganic nitrogen source ammonium sulfate was 7.54% higher than that of ammonium chloride

According to GABA standard curve, peptone in organic nitrogen source and ammonium sulfate in inorganic nitrogen source had the best GABA synthesis efficiency, and the obtained GABA concentrations were 0.396 g/L and 0.485 g/L respectively.

(2) The average absorbance of each substrate concentration was 0.020 g/mL: 0.070; 0.027 g/mL: 0.075; 0.033 g/mL: 0.080; 0.040 g/mL: 0.086; 0.047 g/mL: 0.088; 0.053 g/mL, 0.044

The average GABA synthesis concentration (g/L) of each substrate concentration was 0.020 g/mL: 0.469; 0.027 g/mL: 0.503; 0.033 g/mL: 0.538; 0.040g /mL: 0.576; 0.047 g/mL: 0.594; 0.053 g/mL, 0.294

The GABA synthesis efficiency increased by 102.14% under the condition of medium concentration of 0.047g/mL compared with 0.053g/mL

According to the GABA standard curve, the optimal substrate concentration was about 0.045 g/mL, and the obtained GABA concentration was about 0.614 g/L. The substrate concentration should be determined as 0.045 g/mL when the optimal fermentation conditions were explored.

(3) The mean value of absorbance at each pH was: pH 4.5:0.051; PH 5.0:0.039; PH 5.5:0.042; PH 6.0:0.040; PH 6.5:0.076; PH 7.0:0.056

The average GABA synthesis concentration (g/L) at each pH was as follows: pH 4.5:0.344; PH 5.0:0.258; PH 5.5:0.280; PH 6.0:0.269; PH 6.5:0.512; PH 7.0:0.378

The GABA synthesis efficiency at pH 6.5 was 98.45% higher than that at pH 5.0

According to the GABA standard curve, the optimal initial pH was 6.5 and the obtained GABA concentration was 0.519 g/mL. The pH should be determined as 6.5 when the optimal fermentation conditions were explored.

(4) The average absorbance of each bottling liquid was 75 mL: 0.028; 100 mL: 0.030; 125 mL: 0.043; 150 mL: 0.052; 175 mL: 0.032; 200 mL: 0.030

The average GABA synthesis concentration (g/L) of each bottling volume was 75 mL: 0.187; 100 mL: 0.200; 125 mL: 0.287;

150 mL: 0.346; 175 mL: 0.212; 200 mL: 0.196

The GABA synthesis efficiency was increased by 85.03% when the loading volume was 150mL compared with 75mL

According to the GABA standard curve, the optimal initial bottling liquid volume was 150mL, and the GABA concentration was 0.349 g/L. After the exploration of the optimal fermentation conditions, the liquid volume of the fashion bottle should be determined as 150mL.

(5) The average absorbance of each strain concentration was 50 g/L: 0.020; 100 g/L: 0.037; 150 g/L: 0.025; 200 g/L: 0.024; 250 g/L: 0.025

The mean GABA synthesis concentration (g/L) was obtained as follows: 50 g/L: 0.130; 100 g/L: 0.246; 150 g/L: 0.164; 200 g/L: 0.158; 250 g/L: 0.171

The GABA synthesis efficiency increased by 89.23% when the strain concentration was 100 g/L compared with 50 g/L

According to the GABA standard curve, in the catalytic GABA synthesis, the amount of GABA produced by fermentation under the condition of strain concentration of 100 g/L was higher, and the GABA concentration was 0.246 g/L.

(6) The average absorbance at each temperature was obtained as follows: 25℃ : 0.030; 30 ℃ : 0.045; 35 ℃ : 0.055; 40℃ : 0.039, the average GABA synthesis concentration (g/L) at each temperature was 25℃ : 0.198; 30 ℃ : 0.300; 35 ℃ : 0.369; The synthesis efficiency of GABA at 35℃ was 83.36% higher than that at 30℃

According to the GABA standard curve, the optimal temperature was 35℃, and the obtained GABA concentration was 0.369 g/L. The temperature of the optimal fermentation condition should be determined as 35℃.

3.5 Experimental Summary

In this chapter, the effects of medium N source, substrate concentration, pH, bottling liquid amount, strain concentration and temperature on the catalytic synthesis of GABA were discussed. The optimal reaction conditions were determined as follows: organic nitrogen source: peptone;Inorganic nitrogen source: ammonium sulfate, substrate concentration of 0.045 g/mL, pH 6.5, bottling liquid volume of 150 mL, wet strain concentration of 100 g/L, temperature 35 ℃.The data obtained in this experiment can be used for experimental research, and other variables in the experiment can be referred to, so as to find better conditions for microbial fermentation to prepare γ-aminobutyric acid.

4.References

[1] Yin W Z, Zhang H S, Yu T Y, et al. [1] Yin W Z, ZHANG H S, YU T Y, et al. Screening and identification of γ-aminobutyric acid producing strains [J]. Science and Technology of Food Industry, 2015, 36(19): 142-146.

[2] LIU L. Enhancement of γ-aminobutyric acid synthesis ability of Lactobacillus [D]. Zhejiang University, 2018. (in Chinese)

[3] Li W. Study on the production of γ-aminobutyric acid (GABA) by microbial fermentation [D]. Harbin University of Commerce, 2014. (in Chinese)

[4] Lin Q L, Wang J, Chen H N. Research progress of γ-aminobutyric acid [J]. Modern Food Science and Technology. 2008, 24(5): 496-500.

[5] Lei Zhen. Research on epilepsy detection based on steady state distribution and development of online early warning system [D]. Zhejiang University, 2016. (in Chinese)

[6] Zhao H, Lu J J, Ma J, et al. V-aminobutyric acid (GABA) is involved in the formation of mouse placenta by regulating cell proliferation [J]. Biogenesis and pregnancy avoidance,2013,33(11):719-724.

[7] Liu Xiaoyan. The role of V-aminobutyric acid in follicular development [D]. Southern Medical University,2008. (in Chinese with English abstract)

[8] RoldanER S , Murase T, Shi QX. Exocytosis in spermatozoa in response to progestorenezonapellueida [J]. Science, 1994266:1578-1581.

[9] Xie Z, Xia S, Le g. gamma-aminobutyric acid improves oxidative stress and function of the thyroid in high-fat diet fed mice[J].Journal Of function foods. 2014:76-86.

[10] Samsonova N N, Smirnov S V, Novikova A E, et al. Identification of Escherichia coli K12 YdcW protein as a gamma-aminobutyraldehyde dehydrogenase[J]. FEBS Letters. 2005, 579 (19) : 4107-4112.

[11] Wang Zhichao. Study on the synthesis of γ-aminobutyric acid by mold transformation [D]. Qilu University of Technology, 2016. (in Chinese with English abstract)

[12] Komatsuzaki N, Shima J, Kawamoto S, [12] et al. Production of γ-aminobutyric acid (GABA) by Lactobacillus paracasei isolated from traditional piled foods[J]. Applied microbiology and biotechnology. 2005, 22(6): 497-504.

[13] Wang Q, Xin Y, Zhang F, et al. Enhanced gamma-aminobutyric acid-forming activity of recombinant glutamate decarboxylase (GABAA) from Escherichia coli[J]. World journal of microbiology & biotechnology, 2011, 27(3):693-700.

[14] Zhao A, hu X, Lu P, et al. Isolation and characterization of a gamma-aminobutyic acid producing strain Lactobacillus buchneri WPZ001 that could efficiently utilize xylose and corncob hydrolysate[J]. Applied microbiology and biotechnology. 2015, 99 (7) : 3191-3200.