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Plasma Mutation Breeding of High Yield γ-Aminobutyric Acid Lactic Acid Bacteria

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ABSTRACT

γ-amino butyric acid (GABA) is a non-protein amino acid widely distributed in nature. It has analgesic effect, promotes growth hormone secretion and prevents the physiological function of Alzheimer's disease.In order to improve the yield of GABA, the strain of strain HX-3-6 was tested by Atmospheric and Room Temperature Plasma (ARTP), and the strain with more than 90% lethality was selected for fermentation, and through the gradient plate according to the level of GABA concentration and colony size of the screening. Then, use the fermentation medium for re-screening and genetic stability test. The yield of the mutant strain L-120-1 was 5.828 g/L, which was 18.84% higher than that before the mutation (4.904). The mutant strain L-120-1 was used as the starting strain for ARTP mutagenesis. The yield of the stable mutant strain 90s-high was 6.178g / L, which was 16.15% higher than that before the mutation (5.319g / L). The yield of HX-3-6 strain was higher than that of GABA mutant strain 90s-high 2, which was stable and high yield GABA was 25.98%.

KEYWORDS: lactic acid bacteria; GABA; ARTP; fermentation; genetic stability

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Introduction

γ-amino butyric acid (GABA) is a non-protein amino acid widely distributed in plants and animals, and has many important physiological functions. In plants, γ-aminobutyric acid (GABA) is involved in the process of plant resistance to stress, stress response to pests and diseases. In animals, GABA acts as a major inhibitory neurotransmitter in the central nervous system of mammals, participates in the physiological activity of the brain cycle, reduces blood pressure, treats epilepsy, sedates, promotes sleep, enhances memory, controls asthma, regulates hormone secretion, promote reproductive, renal and liver function activation and other functions, is widely used in food, medicine and other industries. Therefore, the mechanism of its preparation, research has become a hotspot. At present, mainly through the fermentation of E. coli production of GABA, but the existence of E. c oli health and safety aspects of the great hidden dangers. Therefore, it is of great significance to obtain high-yield GABA-safe new strain by mutagenesis [1 - 3].

In the food and pharmaceutical industry, the use of recognized safety probiotics - lactic acid bacteria for fermentation production GABA has a broader prospect, the current industrial production of GABA production is low [4,5], so to improve the GABA production in this experiment using plasma Breeding Breeding of Lactic Acid Bacteria.

effect is mainly due to its changes in DNA structure and the formation of mutations [6]. However, UV induction has the problems of heavy breeding process and poor mutation.

Chemical mutagenesis is carried out by the use of chemical mutagenic agents which molecular structure is not stable, which causes biological damage and false repair by chemical agents, resulting in mutants [7]. But the chemical mutation breeding process is complex and has a relatively large harm to the human body operating environment.

In this study, a new microbial mutation breeding method- atmospheric and room temperature plasma (ARTP) mutagenesis breeding system was adopted. ARTP is a kind of plasma source developed in recent years, and can produce plasma jet with high activity particle concentration between 25-40 ℃ and atmospheric temperature. The

plasma has the characteristics of low temperature, high electron temperature, balance characteristics and high activity ion concentration characteristics. According to scientific research, the active particles in the plasma act on microorganisms, which can change the structure and permeability of microbial cell wall, membrane and cause gene damage, and thus make the microbial gene sequence and its metabolic network significantly change, leading to microbial production mutation [8].

ARTP mutagenesis breeding system has the advantages of safe operation, simple process, high mutation rate and economic environmental protection. Therefore, this experiment uses ARTP to mutagenize the lactobacillus producing GABA. The mutant strain with mutant GABA then the genetic stability test was carried out by using the fermentation medium to obtain the stable mutant strain of GABA.

1. Introduction

1.1. Research Status of γ-Aminobutyric Acid

GABA in food research and application began in the mid-eighties of last century, the application of products to Japan tea drink Gabaron as the representative. Gabaron tea drink with primary hypertension in mice, a few weeks found that blood pressure from 175-180 mmHg reduced to 150 mmHg, while no other physiological abnormalities of mice [9]. In 1994, Takayo et al. found that the accumulation of GABA in fermented germs was high at 200-300 mg/100 g [10] when studying the amino acid distribution of rice germs immersion in water. In 1996, GABA-rich rice germ products were commercialized in Japan. Neurophysiology and neurological studies have shown that GABA is an important active substance in the human brain, although GABA can be from the brain glutamate in the specificity of glutamate decarboxylase under the action of transformation, but age and mental stress increase the difficulty of GABA accumulation, and through daily diet supplement can effectively improve the situation, thereby promoting human health [11].

Recently, Japanese scientists use rice germ and other raw materials developed and manufactured GABA-rich functional ingredients, has been widely used in beverages, jams, cakes, biscuits, seasonings and other products. It is also reported that high concentrations of GABA beverages made from biotechnology and GABA-rich food ingredients made from lactic acid bacteria can be used directly or as functional foods for anti-hypertension, brain function and liver function. China's GABA food research and development reports rarely, this research and development is still in its infancy.

1.2. Atmospheric pressure at room temperature plasma mutagenesis system

1.2.1 Basic principles of ARTP biological breeding

Atmospheric pressure low temperature plasma is a new type of plasma source which began to study in recent years. It has the advantages of low gas temperature, high active particle concentration, high energy and easy operation. Basic experimental studies have shown that high concentrations of active particles in atmospheric and low temperature plasmas can act on the genetic material of microorganisms and produce teratogenic mutations in a short time, which is a good source of mutagenesis [12]. Compared with traditional physical mutagenesis methods (such as ion beam implantation, radiation mutagenesis) and chemical mutagenesis methods, atmospheric and low temperature plasma mutagenesis methods are safe, simple, have no side effects on the human body, and no pollution to the environment.

1.2.2 ARTP development advantages

Conventional mutagenesis breeding methods have chemical mutation breeding and physical mutation breeding. Microbial mutagenesis breeding, mainly by means of artificial mutagenesis to induce microbial gene mutation, thereby changing the genetic structure and function, and then through the screening, from a lot of mutant bacteria in the selection of the required high yield, excellent traits of mutations And the optimum culture medium and the optimum culture conditions were found, so that it could synthesize the desired product under the optimum environmental conditions.

Tsinghua University, Department of Plasma Health Science and Technology Research Group (Department of Engineering Physics), Environmental Biotechnology Laboratory (Department of Chemical Engineering) Beijing Siqingyuan Biotechnology Co., Ltd., using the international advanced atmospheric pressure low temperature plasma generation technology, combined with recent years. The latest research results and automatic control methods, jointly developed a second generation ARTP biological breeding machine. The breeding machine has the advantages of stable operation, no preheating, simple operation, good human-computer interaction performance, and can produce

atmospheric temperature and low temperature plasma in the industrial environment to induce mutagenesis, accelerate the process of mutagenesis and improve the industrial production efficiency [13].
ARTP mutagenesis research can promote the integration and cross of plasma and biological science, on the one hand to promote multi-parameter, high-throughput, more controllable multi-channel atmospheric pressure low temperature plasma technology equipment development and innovation. On the one hand, through the study of different biomolecules and species regulation and action methods, the formation of plasma biotechnology (Plasma biotechnology) new research field - life sciences.

1.3. According to the meaning and meaning

γ-amino butyric acid (GABA) is a non-protein amino acid, which is an inhibitory transmitter in the mammalian center. GABA has the advantages of lowering blood pressure, enhancing memory, activating renal function, improving liver function, preventing and treating obesity, nutrition, nerve cells and other functions [14], through the microbial fermentation method to obtain GABA is of great significance.

With the GABA physiological function characteristics gradually recognized by human beings, the use of GABA enrichment technology to develop GABA-rich health food products will be a promising research field, chemical synthesis of GABA less safe, less GABA content in plant, the use of biosynthesis in the microbial (lactic acid bacteria) fermentation production GABA shows a broad development prospects [15]. However, the industrial production of GABA produced by lactic acid bacteria fermentation is not high.

In view of the fact that UV mutation breeding has the problems of heavy breeding process and poor mutation effect, chemical mutation breeding process is complex and has a large operating environment with great harm to human body.

In this study, the lactic acid bacteria were mutagenized by the new mutagenesis technique (atmospheric temperature chamber temperature mutagenesis breeding system), and the lactic acid bacteria strains with high yield GABA were obtained.

1.4. The main content of this study

(1) In this experiment, lactic acid bacteria producing γ-aminobutyric acid, which had been screened in the laboratory, were subjected to room temperature plasma mutagenesis at room temperature, and the mutants were screened by gradient plate.

(2) Fermentation of a large number of mutant strains in order to obtain lactic acid bacteria with high growth rate and high yield of γ-aminobutyric acid.

2. Experimental Materials

2.1. Source of bacteria

By the Anhui Wuhu Anhui University of Biotechnology Laboratory, No. HX-3-6.

2.2. Experimental drugs

Table 1. Experimental Drugs (Reagents)

Drug Name	Drug Type	Manufacturer
Agar	Biochemical Reagents	Sinopharm Chemical Reagent Co., Ltd
Glucose	Analysis Pure	Guodu Chemical Reagent Co., Ltd
Yeast Extracts	Biochemical Reagents	Sinopharm Chemical Reagent Co., Ltd
Tryptone	Biochemical Reagents	Pharmaceutical Group Chemical Reagent Co., Ltd
Fish meal peptone	Biochemical Reagents	Pharmaceutical Group Chemical Reagent Co., Ltd
Tween	Analysis Pure	Chemical Industry Group Chemical Reagent Co., Ltd
NaCl	Analysis Pure	Guodu Chemical Reagent Co., Ltd
$MgSO_4$	Biochemical Reagents	Shanghai reagent four plants
Manganese sulfate	Biochemical Reagents	Bengbu chemical reagents plant
95% Ethanol	Analysis Pure	Guodu Chemical Reagent Co., Ltd
Sodium acetate	Analysis Pure	Chemical Industry Group Chemical Reagent Plant
Disodium phosphate	Analysis Pure	Bengbu Chemical Reagent Factory
Sodium succinate	Analysis Pure	Biology Biological Shanghai Ltd
Citrate triamine	Analysis Pure	Chemical Industry Group Chemical Reagents Plant
Sodium glutamate	Analysis Pure	Nanjing Jirui Chemical Reagent Co., Ltd
Sodium hydroxide	Analysis Pure	Chemical Industry Group Chemical Reagents Plant

$GaCO_3$	Chemical Reagents	Bengbu Chemical Reagent Factory
Phenol	Analysis Pure	Chemical Industry Group Chemical Reagent Plant

2.3. Experimental apparatus

Table 2. Test Instruments

Experimental Equipment	Manufacturer
SPX-250BS-II Biochemical Incubator	Shanghai new seedlings of medical equipment Manufacturing Co., Ltd.
ARTP Mutation Breeding System	Beijing Siqingyuan Material Technology Co., Ltd
Automatic Microplate Reader Multiskan Fc	Thermo Fisher Scientific (Shanghai) Co., Ltd
Meiling BCD-181KC refrigerator	Meiling Electric Co., Ltd.
Automatic Autoclave HVE-50	Huaxia Line Instrument Co., Ltd
SW-CJ-CO-type purification table	Suzhou purification Equipment Co., Ltd.
DHG-9143BS-III electric thermostat blast drying oven	Shanghai new seedlings of medical equipment Manufacturing Co., Ltd.
LDZX-50KBS vertical electric pressure steam sterilization pot	Jintan Ronghua Instrument Manufacturing Co., Ltd.
Constant temperature water bath HH-2	Jintan Jerelli Electric Co., Ltd.
SK-1 Quick Mixer	Jintan Jie Ruier Electric Co., Ltd.
FC104 electronic balance	Shanghai Precision Science Instrument Co., Ltd.
PHSJ-3F laboratory pH meter	Changzhou Guohua Electric Co., Ltd.
100 μl Precise Micropipettor	GilsonSAS.France
1000 μl Precise Micropipettor	GilsonSAS.France
5000ul Precise Micropipettor	GilsonSAS.France
80-2 Centrifuge	Jiangsu Jin Tan Ronghua Instrument Manufacturing Co., Ltd.
15W UV Lamp	Suzhou Purification Equipment Co., Ltd.
Laboratory pH meter PHSJ-3F	Shanghai Precision Science Instrument Co., Ltd.
Electric furnance	Danyang City, flying and metal electrical plant
Visible Spectrophotometer 722	Shanghai Youke Instrument \u0026 Meter Co., Ltd
Magnetic stirrer SK-1	Jintan Jerelli Electric Co., Ltd.
The United States of the refrigerator BCD-195TC	Hefei Rong's refrigerator Co., Ltd.
High - Flux Thermostat Oscillator Canvic HTS - T008	Shanghai Shi Ping Experimental Equipment Co., Ltd
Stainless steel distilled water.	Shanghai Health Silver Medical Instrument Co., Ltd

This test also supplies: Eppendorf centrifuge tube, beaker, glass rods, tweezers, reagent bottles, measuring cylinders, bottles, asbestos, cones, petri dishes, scissors, white wok, alcohol lamp, tube rack, ice bag, reagent spoon , Coated rods, glycerin tubes and so on.

2.4. Preparation of culture medium and related reagents

(1) MRS liquid medium: fish meal peptone 10 g yeast extract 10 g glucose 5 g sodium acetate 5 g potassium dihydrogen phosphate 0.2 g citric acid triamine 0.2 g magnesium sulfate 0.2 g manganese sulfate 0.05 g Tween-80 0.1 mol / L water 1000 mL PH 6.5 at 115 °C for 25 minutes.

(2) MRS solid medium: fish meal peptone 10 g yeast extract 10 g glucose 5 g sodium acetate 5 g potassium dihydrogen phosphate 0.2 g citric acid triamine 0.2 g magnesium sulfate 0.2 g manganese sulfate 0.05 g agar 20 to 25 g water 1000 mL pH 6.5 115 °C for 25 minutes.

(3) GABA-containing MRS solid medium: fish meal peptone 10 g yeast extract 10 g glucose 5 g sodium acetate 5 g dipotassium hydrogen phosphate 0.2 g citric acid triamine 0.2 g magnesium sulfate 0.2 g manganese sulfate 0.05 g agar 20 to 25 g GABA 60 g water 1000 mL pH 6.5 at 115 °C for 25 min

(4) Fermentation medium: Yeast extract 5g Tryptone 5g Glucose 10g Sodium succinate 5g 1% Sodium glutamate Water 1000mL pH 6.5 115 °C Sterilization 25 minutes

(5) Physiological saline solution: 1.8 g of NaCl dissolved in 200 mL of distilled water. 115 ℃ sterilization 25min.

(6) Boric acid buffer 1.525 g borax and 0.247 g boric acid dissolved to 100 mL.

(7) Sodium glutamate solution: 0.1 g of sodium glutamate was added to 100 mL of sterile water to prepare a sodium glutamate solution for the control.

3. Experimental Methods

3.1. Breeding of lactic acid bacteria HX-3-6 by ARTP

3.1.1 Determination of Growth Curve of Lactobacillus HX-3-6

(1) Activation of the strain: After the standard stock was removed and returned to room temperature, 1 mL of lactic acid bacteria were inoculated with 10 mL (v / v) in 50 mL Erlenmeyer flask containing 9 mL of MRS liquid seed medium. Placed in 30 ℃ incubator for 24 hours.

(2) Lactobacillus growth curve was drawn: The activated suspension was removed and inoculated with 10% (v / v) inoculated with 1 mL of the bacterial suspension to a 50 mL Erlenmeyer flask containing 9 mL of MRS liquid medium, incubation in the incubator, every 3h with a spectrophotometer at 600nm conditions measured bacteria OD value, and the time for the abscissa, OD value for the vertical axis of the growth curve of lactic acid bacteria.

The pH of the lactic acid bacteria suspension was also measured with a pH meter.

3.1.2 ARTP mutagenesis

(1) Preparation of the suspension: After the standard stock was removed and returned to room temperature, 1 mL of the lactic acid bacteria was inoculated into a 50 mL Erlenmeyer flask containing 9 ml of MRS liquid seed medium at 10% (v / v) And then centrifuge for 5 minutes, centrifuge to remove the supernatant by adding 1mL of sterile water using the oscillator to shake (1) uniformly, made of bacteria suspension reserve.

(2) ARTP mutagenesis operation: 10 μL of the above prepared bacteria suspension was applied to sterilized small iron pieces (8 groups in total) using a 20 μL pipetting on a sterile console. Eight groups of bacteria were subjected to ARTP processing time is 0s, 30s, 60s, 90s, 120s, 150s, 180s, 210s and 240s.

Atmospheric pressure room temperature (ARTP) plasma mutation breeding device, in this experiment using the conditions described below; power supply: 220V, 50Hz, voltage fluctuation range ≤ 5%, gas flow selection 10SLM, temperature 20 - 40 ℃ below, power 200W.

Use the tweezers to place the sample to be placed in the fixed groove on the rotating positioning table, and then adjust the height of the rotary table so that the sample to be processed is 3 mm away from the plasma generator outlet and the sample is closed and closed operate room door, select the irradiation time.

3.1.3 Post-culture and plate count

(1) Post-culture: The mutated microfuel containing the bacterial solution was quickly placed in a centrifuge tube containing 1.5 mL of MRS liquid medium, and the mutated lactic acid bacteria were then placed in a thermostatic incubator at 30 ℃ 2h.

(2) Plate count: 2h after the removal of mutagenic bacteria using the oscillator to centrifuge tube bacteria in the mix, with 1000μL pipette to draw 0.5mL placed in 4.5mL saline with 7mL centrifuge tube, followed by dilution To 10-3,10-4,10-5,10-6, take the dilution fold 10-4,10-5 two gradient bacteria suspension (0s take the dilution factor of 10-5,10-6 two gradient bacteria coated plate), with 100 μL of the 200 μL pipettes, add MRS plates, apply evenly, and each dilution was done in three parallel lines. The coated plates were placed in a thermostatic incubator and incubated at 30 ℃ for 72 h.

(3) Calculation of ARTP lethality: After 72 hours of culture, the colonies on the plate were counted and compared with the corresponding concentration of the control group, according to the formula: lethality = (control group - mutagenic colonies)/control number of colonies, calculated lethality.

3.1.4 Screening of mutants

(1) Preparation of gradient plate: In the aseptic console, the plate tilted about 15 degrees, put about 10mL MRS agar medium, to be solidified and put back to the horizontal position, and then pour into the same volume of GABA concentration 60 g/L MRS medium, after solidification to form a gradient plate back.

(2) Screening of mutant strains: The mutant strains were diluted 10-4,10-5 with gradient plates, and the coated gradient plates were placed in a constant temperature incubator and incubated at 30 ℃ for 72 hours (0s no need to coat the gradient plate). The colony size was determined by GABA concentration and colony size after 72 hours of culture. The relationship between colony size and anti-GABA concentration and GABA production was verified.

(3) Mutation of the mutant strain: The mutant strain was re-screened with the fermentation medium, and the fermentation conditions were 50 mL of a 250 mL Erlenmeyer flask. And cultured at 30 ℃ for 72 h at room temperature. After 72 h, the yield of GABA was measured by measuring the mutant strain at 640 nm.

3.1.5 Determination of GABA content

Take 1ml fermentation broth in 1.5mL centrifuge tube 10000 r/min centrifugation 2min, draw 0.5mL supernatant diluted with sterile water 10 times standby.

Take 0.8 mL of the stock solution in a flat bottom tube. Then, 0.2 mL of 0.2 mol/L sodium carbonate solution, 0.2 mL of a borate buffer of 0.2 mol/L Ph10.0, and 2.5 mL of 6% re-distilled phenol were added, followed by addition of 2 mL of sodium hypochlorite (NaClO). Mix and place for 4 to 8 minutes, then boiling water bath for 10min, immediately ice bath for 20min, until the solution appears blue and green, add 4mL 60% ethanol solution, mix room temperature after 3 minutes 20 min, after the solution is made from 200 μL of each solution was added to the plate, and the content of GABA was determined by the microplate reader at 640 nm. The GABA standard was determined by a microplate reader.

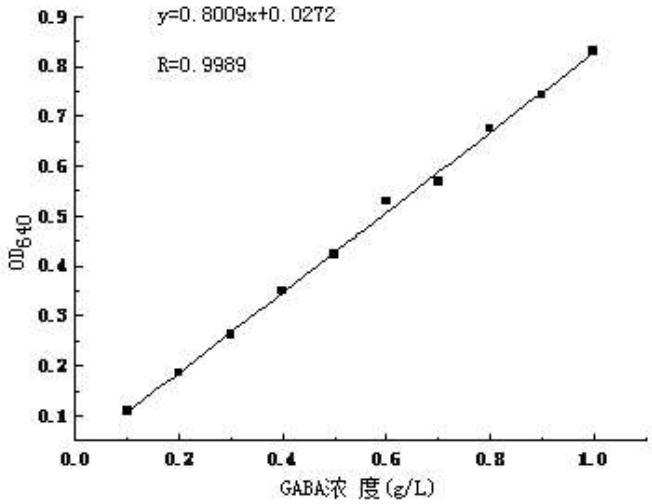

Figure 1. GABA microplate reader standard curve

3.1.6 Species preservation

The mutant strains of high yield GABA obtained by repeated screening and repeated passage were frozen and frozen at -70 ℃ with glycerol tubules.

3.2. Breeding of lactic acid bacteria L-120-1 by ARTP

3.2.1 Source of the strain

L-120-1 was the mutant strain of HX-3-6 strain that was mutated by ARTP and screened with hereditary and yield stable.

3.2.2 Basic operation of the experimental process

The activation of the strain → the growth curve of the lactic acid bacteria → the preparation of the bacterial suspension → the induction of ARTP → the post-culture → the plate count → the calculation of the lethal rate of the ARTP → the screening of the mutant strain → the determination of the GABA content → the preservation of the strain.

4. Results and Discussion

4.1. Lactic acid bacteria HX-3-6 ARTP mutagenesis results

4.1.1 Determination of HX-3-6 growth curve

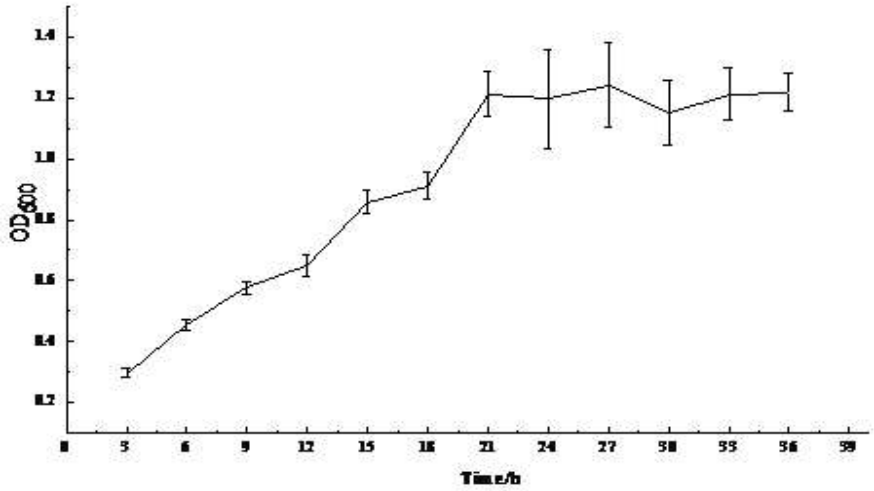

Figure 2. HX-3-6 growth curve

Chart analysis:

Lactic acid bacteria HX-3-6 lactic acid bacteria after activation treatment, the use of spectrophotometer in A600 under the conditions of HX-3-6 lactic acid bacteria growth curve shown in Figure 4-1, the figure can be seen, the bacteria after inoculation culture After a short period of adaptation into the logarithmic growth period, about 18h into the logarithmic period, during which the cell growth and metabolism of strong and stable is the best time to mutagenesis. At the same time we can see that the strain enters the stable phase after about 21 hours.

4.1.2 Determination of lethal rate of lactic acid bacteria HX-3-6

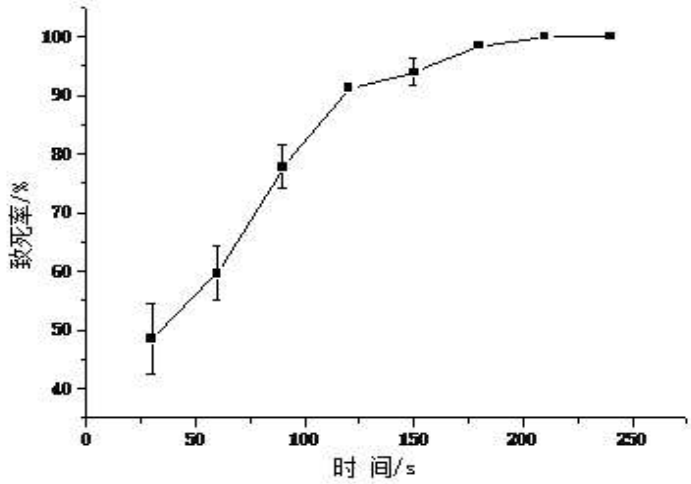

Figure 3. HX-3-6 mutagenic lethality curve

It can be seen from Figure 4-2: HX-3-6 lactic acid bacteria after ARTP mutagenesis treatment, lactic acid bacteria mutagenesis rate increases with the strain mutation time increases. 90s mortality rate of 77.81%, 120s lethal rate of 91.2%; experiment to select the lethality of 90% of the strains were screened in order to obtain high-yield strains.

4.1.3 Determination of GABA content after HX-3-6 mutagenesis

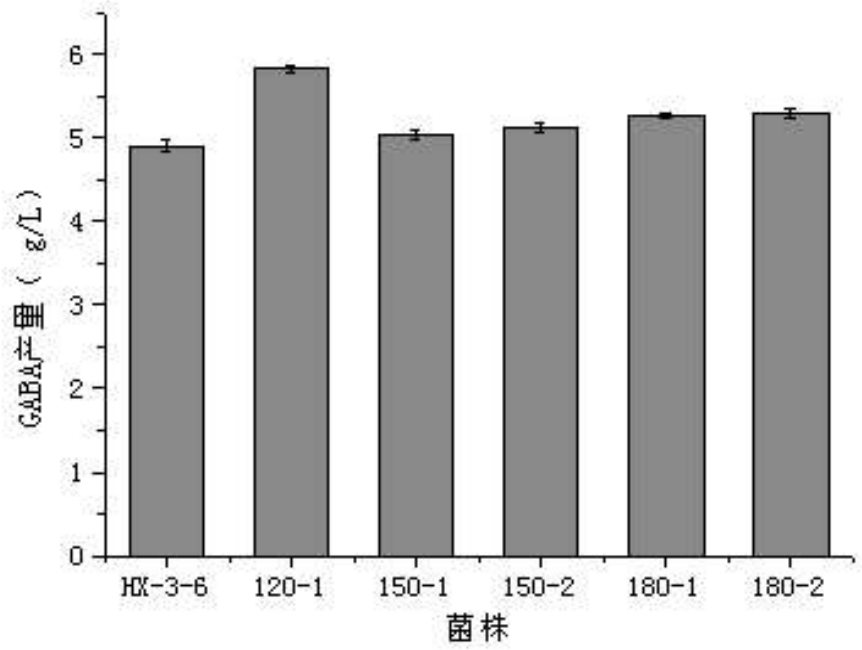

Figure 4. Yield of GABA after HX-3-6 mutagenesis

As can be seen from Figure 4-3, after the first mutagenesis, it was found that the yield of GABA was increased with most of the mutated strains compared with the control group; the yield of 120-1 GABA was 5.828 g / L; The highest yield of GABA mutant strain l-120-1 was 18.84% higher than that before mutagenesis. And after several generations of yield stability.

4.2. Lactic acid bacteria L-120-1 ARTP mutagenic results

4.2.1 L-120-1 growth curve drawing

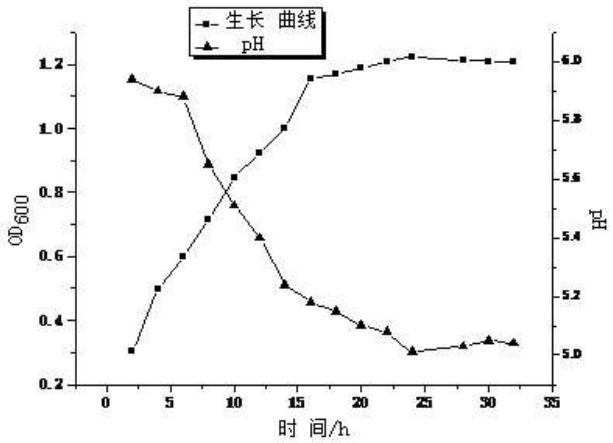

Figure 5. L-120-1 growth curve and pH curve

L-120-1 growth curve shown in Figure 4-4, it can be seen from the figure, the bacteria in the vaccination after a short period of adaptation into the logarithmic growth period, about 14h into the logarithmic period, during this period

Cell growth and metabolism is strong and stable is the best time to mutagenesis. At the same time we can see that the strain enters the stable phase after about 18h. L-120-1 L-120-1 Lactobacillus L-120-1 L-120-1 L-120-1 L-120-1 L-120-1 L-120-1 L-120-1 L-120-1 L-120-1 L-120-1 L-120-1 L-120-1 L-120-1 L-120-1 L-120-1 L-120-1 L-120-1 L- 1 Lactobacillus growth curve and HX-3-6 lactic acid bacteria growth curve is different. Lactic acid bacteria L-120-1 after the activation of the above steps, the use of pH meter to determine the lactic acid bacteria L-120-1 culture pH change shown in Figure 4-4, in the incubation time of 0 - 24h culture between the pH The value has been reduced from 6.5 to 5.0. The pH value of L-120-1 was significantly different from that of HX-3-6 culture, and the pH and growth curve of L-120-1 were correlated, probably due to genetic changes caused by the results.

4.2.2 L-120-1 lethality determination

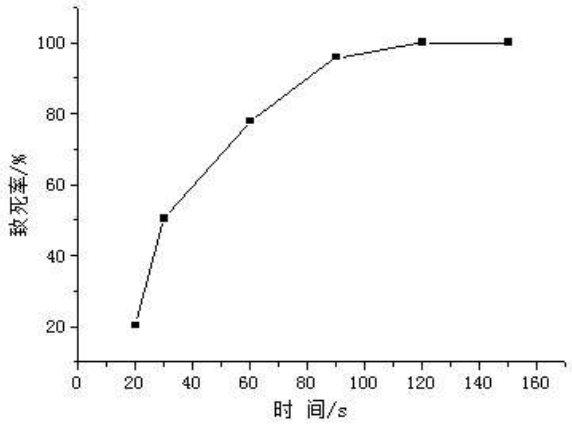

Figure 6. L-120-1 mutagenic lethality curve

It can be seen from Figure 4-5: L-120-1 lactic acid bacteria after ARTP mutagenesis treatment, lactic acid bacteria mutagenic mortality rate increases with the mutation time of the strain increased. The lethal rate of lactic acid bacteria strain was 95.8% at 90 s mutation time, and the mutant strain on 90s gradient plate was screened and screened.

4.2.3 Determination of GABA content at 90s after L-120-1 mutagenesis

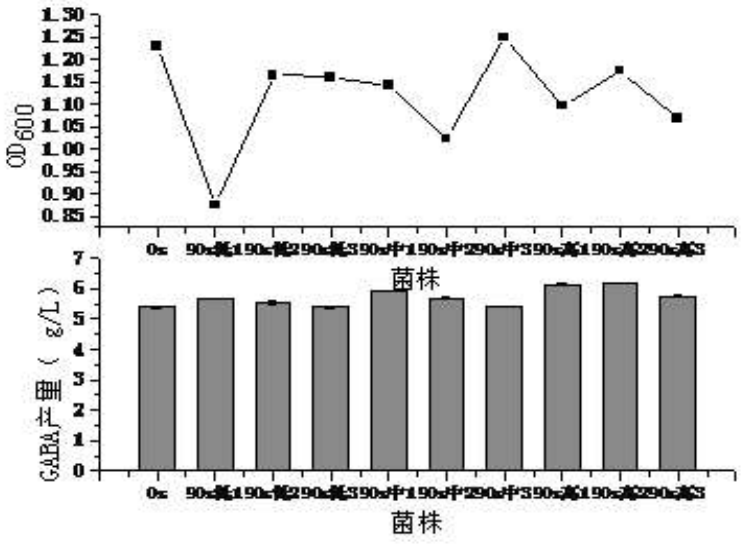

Figure 7. L-120-1 mutant strain GABA content and OD curve

In Figure 4-6, 90s-low 1,90s represents the time of mutation, low on the low concentration of GABA (medium to medium concentration, high for high concentration), 1 for larger colonies (2 for moderate colonies, 3 for smaller

colonies). From Fig. 4-6, it can be seen that the yield of GABA produced by 90s-high 1,90s-high 2 mutant was significantly higher than that of control group after L-120-1 lactic acid bacteria mutagenesis. The yield was 6.116g / L, 6.178g / L, respectively, and the yield of GABA was 14.98% and 16.15%, respectively.

In this study, the strain with high yield and high GABA yield was obtained, but the OD value of the mutant strain was not correlated with the yield of GABA, and the higher yield of GABA did not show a higher OD value. , pending further mutagenesis screening.

Conclusions

The purpose of microbial breeding is to guide the biosynthetic metabolic pathways in the direction that people want, or to optimize genetic traits after mutations in the cells, artificially accumulate some metabolites excessively and achieve the high yield, high quality and low consumption of bacteria. Gene mutations are one of the most important pathways and have been widely used [16]. At present, the domestic microbial breeding industry is mainly used in conventional physical, biological and chemical factors such as mutagenesis methods to reorganize. This experiment is through the new mutagenesis method - atmospheric pressure plasma temperature (ARTP) mutation breeding system.

Through this experiment, the following conclusions are drawn:

(1) From the growth curve of HX-3-6 lactic acid bacteria before the first mutation, the logarithmic phase of the strain was obtained at 3 - 21h and about 18 hours later, the best timing for mutation. The mutant L-120-1 yield was 5.828 g/L, which was 18.84% higher than that of the original strain.

(2) The growth curve of lactic acid bacteria HX-3-6 measured in this experiment is different from that of lactic acid bacteria L-120-1, which may be the change of gene after lactic acid bacteria mutagenesis.

(3) In this experiment, the mutant strain with the highest GABA yield was 25.98% higher than that of the original lactic acid bacteria strain HX-3-6, which was obtained by mutagenizing the Lactobacillus strain L-120-1.

(4) The Lactobacillus strain L-120-1 was mutagenized, and single colonies were selected from the three high, medium and low concentrations on the gradient plate. The yield of the 90s-high 2 mutant strain was the highest, and the yield of L-120-1 (5,319 g/L) was 16.15% higher than that before mutagenesis.

Due to lack of time, the quality of GABA lactic acid bacteria that produced in the end of experiment is not ideal enough, it is recommended to carry out further works for improvement:

(1) Mutant breeding of mutant strains by UV mutation breeding and chemical mutation breeding in order to obtain high yield GABA lactic acid bacteria mutant strains.

(2) Mutagenesis combined with high-throughput screening to improve the efficiency of screening mutants, to obtain high-yield strains.

(3) To study the mechanism and principle of high yield induced by mutagenesis in combination with genetic engineering.

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