

## Lactic Acid Bacteria Screening of High-yield LAB-EPS and its Application in Steamed Buns

Xiaoman Yao, Yuanyuan Zhang, Kexue Yu, Guifang Chang, He Zhu\*,

Zhaoxue Zhang, Yang Yu, Mengliu Zhu, Bo Li, Yanxia Xing

Shandong Agriculture and Engineering University, Jinan, 250100, China

\*251763119@qq.com

### Abstract

In this experiment, the lactic acid bacteria ZH-7 with the strongest ability to produce exopolysaccharides were isolated and screened from the sourdough, and after 16s RNA identification, it was determined to be a Class I lactic acid bacterium (ZH-7), and the yield of its exopolysaccharides was determined by phenol-sulfuric acid method. The effects of fermentation characteristics and steamed bun quality were explored by combining the fermentation characteristics of dough and the quality of steamed buns by combining different lactic acid bacteria (ZH-3), Class I lactic acid bacteria (ZH-7) and Class III lactic acid bacteria (ZH-2). Through single-factor test and orthogonal test, according to the sensory evaluation of steamed bun and the texture of steamed bun, the best time for fermentation of steamed bun and the best combination of culture compound were explored.

### Keywords

Lactic Acid Bacteria; Extracellular Polysaccharide; Screening; Optimization of Culture Conditions; Strains; Combination; Fermentation Characterization; Steamed Bread Quality.

## 1. Prolegomenon

### 1.1. Lactic Acid Bacteria Screening

Lactic acid bacteria are a generic term for a class of gram-positive bacteria that ferment carbohydrates to produce large amounts of lactic acid. According to the biochemical and morphological classification methods in the internationally accepted Bergey Systematic Bacteriology Handbook, lactic acid bacteria are currently divided into four categories: gram-positive bacillus non-bacillus, endophytic bacillus, gram-positive facultative anaerobic, irregular shape obligate anaerobic bacteria, a total of 23 genera. At present, it is known that lactic acid bacteria exert the mechanism of major physiological functional characteristics, in addition to colonization, main metabolites (such as lactic acid, etc.) to improve the intestinal environment, secondary metabolites of lactic acid bacteria such as bacteriocins, superoxidase dismutase (SOD), exopolysaccharide (EPS) and so on also play an important role.

Polysaccharides refer to carbohydrate compounds composed of more than 20 monosaccharides, which are divided into plant polysaccharides, animal polysaccharides and microbial polysaccharides according to different sources. According to the different composition of sugars, it can be divided into homopolysaccharides and heterotypic exopolysaccharides. Lactic acid bacteria exopolysaccharides (LAB-EPS) are polysaccharides synthesized and secreted outside the cell wall by lactic acid bacteria during growth and metabolism. As a food thickener and gelling agent, lactic acid bacteria exopolysaccharides have been widely used in food production. Lactic acid bacteria exopolysaccharides can be used as

antioxidants, prevent aging, maintain the body's youthful vitality, and can stimulate the increase in the number of immune cells, enhance the body's immune ability, etc., which can be used as antioxidants in medicine[1], so there is important theoretical and practical significance in the study of LAB EPS.

This experiment is dedicated to the enrichment and centralized culture of strains in sourdough. Then analysis and screening, after rescreening, the polysaccharide-producing lactic acid bacteria were found for morphological observation and cultured separately, and the content of exopolysaccharides was determined. After finding a suitable lactic acid bacteria, the ability of lactic acid bacteria to produce exopolysaccharides was tested, including the amount of addition, culture method, culture temperature, culture pH and culture time, so as to determine the most suitable ability of lactic acid bacteria to produce exopolysaccharides.

### 1.1.1. Introduction to Lactic Acid Bacteria

Lactic acid bacteria are a general term for a class of spore-free, gram-stain-positive bacteria whose main product of fermented sugars is lactic acid. All bacteria that can produce lactic acid from the fermentation process of glucose or lactose are collectively called lactic acid bacteria. Lactic acid bacteria were first isolated from milk, and then the presence of lactic acid bacteria was gradually found in some fermented products and foods. Lactic acid bacteria are widely distributed in nature, and the types of lactic acid bacteria are also very diverse, bacteria, cocci and bacilli account for the vast majority of the total number of lactic acid bacteria[2]. At present, there are at least 23 taxonomic genera of lactic acid bacteria found in nature, but the main ones used in food, medicine and other fields are *Lactobacillus*, *Lactococcus*, *enterococcus*, *Leuconostoc*, *Streptococcus*, and *Bifidobacterium*[3]. Its main physiological functions are to provide nutrition, enhance immune function, improve intestinal function, etc.[4]. Studies have shown that lactic acid bacteria can intervene in liver cholesterol accumulation and reduce serum cholesterol[5, 6]. In studies of natural antioxidants, strains with antioxidant function were mostly distributed in *Lactobacillus*[7]. At present, lactic acid bacteria are also widely used in human health food, including at least probiotic food (including beverages, solid drinks, health foods, etc.), dairy products, fruit and vegetable foods, wine, condiments, food preservation, etc. With the stable development of China's economy, people's demand for healthy food is becoming stronger and stronger, and the research and application of lactic acid bacteria has also become one of the hot spots[8].

### 1.1.2. Isolation, Screening and Identification of Lactic Acid Bacteria in Sourdough

China's traditional pasta starter culture is similar to the sourdough used in Western fermentation bread, which is generally called "old noodles", "fermented seeds", "noodle fat", etc. in China[9], and has a long history of use. The sour dough changes the texture under the influence of lactic acid bacteria, delays aging, and prolongs the shelf life of its noodle products[10], so the isolation, screening and identification of excellent lactic acid bacteria from the sourdough and their application play a crucial role in the process of fermenting noodle products.

Chen Junli[11] isolated 16 lactic acid bacteria from traditional starter cultures from different sources, added these 16 strains to the dough and applied them to steamed bun making, and conducted sensory evaluation, and screened out 2 strains with higher scores, namely DN812 and DM616, which were identified as Class I lactic acid bacteria (ZH-7) (*Lactobacillus planetarium*) and *Streptococcus thermophilus*. Li Zihong[12] isolated and screened 27 lactic acid bacteria from the traditional starter culture collected by the people, selected three lactic acid bacteria that had a greater impact on the quality of steamed buns through single-factor experiments, and compounded the amount of lactic acid bacteria isolated during the production of steamed buns, and concluded through orthogonal experiments that the steamed buns produced when the amount of *Lactobacoccus lactis* sub-added was 0.10g, the fermentation time

was 60min, and the fermentation temperature was 32°C. Ji Jinlong et al.[13]improved the acid production capacity of lactic acid bacteria by investigating influencing factors such as carbon source, nitrogen source, pH value, culture temperature, shaker speed, inoculation amount and so on. The results of molecular biology identification showed that the three strains of lactic acid bacteria screened were *Enterococcus faecalis* of the same genus as *Enterococcus*, and the optimal acid production conditions of the lactic acid bacteria were: glucose as the carbon source, the initial pH value of the medium was 6.8, the liquid filling capacity was 50mL, the culture temperature was 25 °C, the speed was 80 r/min, and the inoculation amount was 2.0%. The sensory evaluation results showed that the lactic acid bacteria had a significant effect on the color and epidermis condition of steamed buns.

### 1.1.3. Lactic Acid Bacteria Exopolysaccharides

#### 1.1.3.1 Overview

Lactic acid bacteria are long-established industrial production bacteria, widely used in the processing of dairy products, vegetables and meat products, especially in the production of fermented dairy products such as various yogurt and cheese, and play an extremely important role in their flavor, texture and nutritional health properties of products, mainly through the EPS substances formed during their metabolism[14, 15].

Lactic acid bacteria extracellular polysaccharides are a kind of carbohydrate compounds secreted by lactic acid bacteria into the cell during the anabolic process, often infiltrated into the culture medium, and some attach to the microbial cell wall to form a capsule and become a capsule polysaccharide; Some enter the medium to form mucus and become mucous polysaccharides[16]which are microbial metabolic subproducts, and the molecular weight is mostly between  $4.0 \times 10^4 \sim 6.0 \times 10^6$ . LAB EPS can impart special texture and flavor to fermented dairy products, play the role of food additives, and are widely used in thickening, stabilizing, emulsifying, gelling and holding water in various foods[17]; Exopolysaccharides also have biological activities such as immunoactivity, antitumor and antiulcer, and can be used in the field of medicine.

#### 1.1.3.2 Chemical Structure of Lactic Acid Bacteria Exopolysaccharides

Exopolysaccharides can be divided into two types: isotropic exopolysaccharides and heterotypic exopolysaccharides in terms of chemical structure. Homotypic exopolysaccharides consist of one type of monosaccharide, while heterotypic exopolysaccharides consist of repeating units of two or more different monosaccharides [18]

Isotropic polysaccharides are divided into four types:  $\alpha$ -D-glucan,  $\beta$ -D-glucan, fructan, and galactan [19]. Representative examples of  $\alpha$ -D-glucan include dextran and pullulan; Representative examples of  $\beta$ -D-glucan are curdlan and scleroglucan; Representative examples of fructans are fructans (Levan)[19-21]. These microbial polysaccharides have obvious differences in sugar bonding, branching type, polymer chain length, and high-level structure. Among the lactic acid bacteria isotropic polysaccharides, the more famous is dextran dextran, the main chain of dextran is connected by  $\alpha$ -D-Glcp (1→6) and  $\alpha$ -D-Glcp (1→3). This special chemical structure gives dextran unique physical properties, which can improve the rheological properties of products, increase the viscosity and stability of products, and play an important role in many fields such as food and medicine.

In general, the backbone of lactic acid bacteria heterosexual exopolysaccharides is composed of complex units composed of monosaccharides such as D-glucose, D-galactose, and L-rhamnose[22]. Since Doco et al.[23]reported the structure of *Streptococcus thermophilus* exopolysaccharides, more than 50 lactic acid bacteria exopolysaccharides with known chemical structures have been known, of which, There are 34 types of polysaccharides with unique structures. Compared with isotropic polysaccharides, the structure of the main chain and its branch chains of the heterotypic polysaccharide repeat unit is diverse, and a few heterotypic

exopolysaccharides repeat units contain N-acetylglucosamine (GlcNAc) and N-acetyl galactosamine (GalNAc). and substituents such as glucuronic acid, and sometimes exopolysaccharides containing non-sugar residues such as phosphate, acetyl and glycerol. Among the reported exopolysaccharides, N-acetyl groups are contained in repeat units of *S. thermophilus* Sc136 and *S. thermophilus* SFi20 heterotypic exopolysaccharides[24]. The repeating unit of *S. thermophilus* MR-1C contains L-fructose residues, the backbone of the polysaccharide is composed of galactose and rhamnose residues, on which two galactose residues and one L-fructose residue are linked, respectively, It is the polysaccharide with the largest number of repeat unit monosaccharides reported to date [24, 25].

There are many types of exopolysaccharides produced by lactic acid bacteria, and their chemical structures are also significantly different. In general,  $\beta$ -1,4-bond-linked exopolysaccharides are more viscous than  $\beta$ -1,3 or  $\beta$ -1,2-linked exopolysaccharides[26],  $\beta$  by  $\alpha$ -bond-linked exopolysaccharides Bond-linked exopolysaccharides have greater elasticity[25].

### 1.1.3.3 Biosynthesis Mechanism of Lactic Acid Bacteria Exopolysaccharides

The biosynthesis of microbial exopolysaccharides occurs at different stages of growth and environmental conditions depending on the species. According to the different synthetic sites and synthesis modes, the synthesis of microbial extracellular polysaccharides is divided into the synthesis of homopolysaccharides located outside the cell wall and the synthesis of heterotypic exopolysaccharides located on the cell membrane. The synthesis of homopolysaccharides does not depend on the synthetic mode of C55-lipid-PP, and the synthesis of heterotypic exopolysaccharides depends on the synthesis mode of C55-lipid-PP.

#### (1) Biosynthesis of lactic acid bacteria homotypic exopolysaccharides

Isocellular polysaccharides are polysaccharides synthesized extracellular under the catalysis of highly specific glycosyltransferases based on sucrose[26]. A representative example of isotropic polysaccharides is dextran, whose biosynthetic pathway has received widespread attention. The main production strains of dextrans are *Leuconostoc mesenteroides* subsp. *mesenteroides* or *Leuconostoc mesenteroides* subsp. *dextranicum*, which are synthesized on the cell surface or extracellular under the catalysis of glucansucrase, using sucrose as a matrix.

#### (2) Biosynthesis of lactic acid bacteria heterotypic polysaccharides

Polysaccharides are natural biological macromolecular substances that are present in almost all organisms. The biosynthesis process of lactic acid bacteria heterotypic polysaccharides is complex, similar to the synthesis process of peptidoglycan and lipopolysaccharides, which are components of the cell wall. These carbohydrates have certain similarities in terms of composition, synthesis mechanisms on the cell membrane, and delivery outside the cell membrane[23]. Take *Lc.lactis* NIZO B40 heterotypic exopolysaccharides is an example of the biosynthesis, synthesis of repeat units, polymerization and extracellular export of lactic acid bacteria heterotypic exopolysaccharides glycotides in detail.

### 1.1.4. Domestic and International Research

The accumulation of microbial secondary metabolites requires not only carbon sources, nitrogen sources, water, and a variety of inorganic salts, but also suitable values, incubation temperatures, and fermentation times[27]. When we need to obtain a more ideal number of metabolites, by optimizing and controlling the growth environment of microorganisms, microorganisms can grow in a suitable environment, so as to accumulate more metabolites and achieve the target number of products we need.

#### 1.1.4.1 Research Abroad

Optimizing the composition of the culture medium during microbial growth can increase the potential of the strain, thereby improving the production efficiency of the strain during the fermentation process. The optimization methods of media components mainly include single-

factor method, orthogonal design experimental method and response surface analysis method. Gancel et al.[28]sugar, lactose, glucose and fructose as carbon sources to optimize the fermentation conditions of EPS produced by *Streptococcus salivarius* thermophilic subspecies, and found that when lactose was used as carbon source, the amount of EPS synthesized by *Streptococcus salivarius* thermophilic subspecies was the largest. Cerning et al.[29]showed that when glucose was used as a carbon source, the amount of EPS synthesized by *Lactobacillus casei* LC2W was the largest. Liu Cuiping[30]LC2W synthesis of exopolysaccharides by response surface method (RSA), and finally concluded that the optimal culture conditions for the synthesis of exopolysaccharides by *Lactobacillus casei* LC2W were as follows: inoculation amount of 4%, fermentation temperature of 31.5°C, fermentation time of 32h, under which the yield of exopolysaccharides could reach 157.46 mg/L, which was 30.81% higher than the yield before optimization.

#### 1.1.4.2 Domestic Research

In 2008, Zhang Tianqi et al.[31]conducted an optimized study on the culture conditions of EPS produced by lactic acid bacteria, and the results showed that the yield of EPS of lactic acid bacteria could reach 155 mg/L by adding 1% tyrosine and 5% glucose to skimmed milk medium. In 2009, Zhang Xiuli et al.[32]studied the water retention of *Lactobacillus pentoseus*, and the results showed that when the culture medium was different, the yield of synthesized exopolysaccharides was also different. When *Lactobacillus pentosus* was cultured with synthetic and semi-synthetic medium, the yield was between 40-600 mg/L; When cultured with skim milk as medium, the yield is between 50-425 mg/L. Liu Jing et al.[33] production of *Paracasei* based on the isolation of one strain from the traditional Finnish mixed fermented dairy product Viili, and the results showed that the optimal nutritional conditions: fructose addition of 2%, soy peptone 2.5%, triammonium citrate 0.4%, the yield of exopolysaccharides under these conditions was 193.99 mg/L, which was 2.6 times higher than before optimization. Optimized culture conditions: 4% bacterial throughput, 28 h incubation time, temperature 32 °C, under these conditions, the sugar yield of exopolysaccharides reached 263.74 mg/L, which was 36% higher than before. Sun Junde et al.[34]screened a lactic acid bacteria L3 with high yield of exopolysaccharides from sauerkraut juice and raw milk, and changed the carbon source, nitrogen source, fermentation temperature, time, pH and other conditions of the basal medium, respectively, to explore its effect on the biosynthesis ability of lactic acid bacteria L3 exopolysaccharides. The results showed that the optimal biosynthesis conditions of exopolysaccharides in this strain were as follows: initial pH 6.5, fermentation temperature 37°C, fermentation time 24h, and the biosynthesis of exopolysaccharides under these conditions was 108.49 mg·L<sup>-1</sup>. In summary, by changing the growth environment of microorganisms, the amount of metabolites of microorganisms can be increased.

## 1.2. The Necessity and Implementability of Lactic Acid Bacteria in Steamed Buns

China has a vast land area and a variety of climate types. Due to the difference in climate types, the cultivation of crops in China also has obvious differences in the north and south, the south is mainly ricing cultivation, while wheat in the north is the main food crop. Due to the different cultivation of major food crops, there are also great differences between the north and the south in terms of staple foods, and most of the staple foods of southerners are mainly rice and rice flour; In the north of China, most of the noodle products are the mainstay, and steamed buns, flower rolls and other noodle products are indispensable staple foods in the homes of northerners. Since ancient times, northerners have mastered the production process of steamed buns very thoroughly, and the fermentation process of steamed buns can be traced back thousands of years. Ancient social development is backward, yeast industrial production is not popular enough, most northerners will make their own fermented head, which is what

we usually call fermentation, old noodles, also known as acid dough in the West[35]. With the development of industrialization and the commercialization of yeast starter cultures, in order to save fermentation time and make the production fast and effective when making steamed buns, more and more people choose to use yeast to ferment steamed buns. However, compared with steamed buns fermented by a single strain of yeast, the single-strain fermented steamed buns are inferior in taste and flavor, and the quality of the steamed buns is average. With the improvement of people's living standards, more and more people began to have higher requirements for the flavor and taste of steamed buns, and the use of yeast single culture fermentation steamed buns obviously cannot meet the needs of more people, and old noodle steamed buns can not only meet the needs of more people in flavor and taste, but also have higher nutritional value. Old noodle steamed bun is made of acid dough, old noodle steamed bun belongs to multi-species mixed fermentation, steamed out of the flavor of the steamed bun taste is better, but the acid dough is easily contaminated by bacteria in the air for a long time[36]these adverse effects, it is difficult to ensure the safety of steamed bun production.

According to He Xiaoyun [37] type of lactic acid bacteria on the flavor characteristics of old noodle steamed buns, it is mentioned that the fermentation system composed of yeast and lactic acid bacteria in the acidic dough is mainly used, and the multi-species compound fermentation gives the steamed buns a special flavor. In the process of using acidic dough, yeast fermentation plays an important role in the fluffiness of steamed buns, and lactic acid bacteria play a vital role in the flavor of steamed buns. Yeast in the fermentation process uses the decomposition of sugar substances in flour to generate carbon dioxide and alcohol, the alcohol in the steamed bun is volatilized by high temperature steaming, and carbon dioxide gas is left inside the steamed bun, so that the volume of the steamed bun increases, and the steamed bun is soft and palatable. In the fermentation process, lactic acid bacteria system uses the sugars in flour to produce lactic acid and a small amount of alcohols, ketones, esters and other flavor substances [38] , the right amount of lactic acid makes the steamed bun have a different flavor, and the taste is better when chewed. Generally, the acid dough culture system used in steaming old noodle steamed buns is complex, and the bacterial content of yeast and lactic acid bacteria in the dough cannot be determined, so the conditions for fermenting steamed buns with acid dough are difficult to control. The fermentation time is too short, the carbon dioxide gas produced by the yeast in the steamed bun is insufficient, and the steamed bun is smaller in size, harder to chew, and the quality of the steamed bun is poor; The long fermentation time will cause the lactic acid bacteria in the dough to ferment for too long, resulting in excessive lactic acid content, steamed buns with sour, and the taste and flavor will also deteriorate.

### 1.3. Background and Significance of the Topic

LAB EPS is a carbohydrate compound that is often infiltrated into the medium by fermentation of lactic acid bacteria during growth metabolism and secreted extracellular mucus or capsular polysaccharides[39, 40]. Lactic acid bacteria EPS can improve the tissue state of fermented milk and the adsorption of intestinal mucosa by strains. RAWSON et al. have concluded that the production of fermented milk from EPS-producing lactophilic acid bacteria can improve the tissue state of stirred yogurt and prevent whey separation without the need to add stabilizers. In addition, since GABRIETA reported that lactic acid bacteria EPS has anti-tumor effects in 1998, it has attracted the attention of many scholars[41]. In addition to the glucan produced by the fermentation of *Leuconostoc*, which has been widely used in petroleum, chemical, medical and other industries, no other lactic acid bacteria EPS have been seen application reports, this is mainly due to the high production cost of these products, which do not have application value in ordinary products. Therefore, it is necessary to further improve the research on biosynthesis, such as the screening of high-yield strains, the optimization of fermentation process, and the research of new fermentation methods, so as to have practical application and development

value. In this project, the lactic acid bacteria with the strongest ability to produce exopolysaccharides were isolated from sourdough, and the exopolysaccharides were extracted, and the optimized EPS synthesis conditions of lactic acid bacteria were obtained by changing the culture conditions such as addition amount, culture method, culture temperature, incubation time and pH. This optimal synthesis condition lays the foundation for further separation and purification of exopolysaccharides and structural analysis in the future.

According to the research progress on the application of sourdough in steamed bun fermentation by Wang Rong[42] et al., it is mentioned that the dominant bacteria in acidic dough are mainly yeast and lactic acid bacteria, among which more than 20 kinds of yeast in acidic dough were found; There are as many as 60 kinds of lactic acid bacteria in acidic dough, the most important of which are Class I lactic acid bacteria (ZH-7), Class II lactic acid bacteria (ZH-3), Class III lactic acid bacteria (ZH-2) and so on. Therefore, this paper intends to use a variety of single lactic acid bacteria for strain compounding, explore the effects of fermentation characteristics and steamed bun quality of different cultures through single factor test and orthogonal test, and find the best compound combination similar to the flavor of old noodle steamed bun to meet the requirements of more people for the flavor and taste of steamed buns.

## 2. Materials & Instruments

### 2.1. Main Reagents

Glucose, sodium hydroxide, agar, distilled water, peptone, bromocresol green indicator, Tween, K<sub>2</sub>HPO<sub>4</sub>, diammonium citrate, sodium acetate, MgSO<sub>4</sub>·7H<sub>2</sub>O, MnSO<sub>4</sub>, calcium carbonate, phenol, sulfuric acid, potassium sodium tartrate and other reagents are domestic analytical pure reagents, flour, Hebei Jinshahe Noodle Industry Co., Ltd.; Yeast, Angel Yeast Inc.

### 2.2. Main

**Table 1.** Experimental Instruments

Instrument name	Model	Origin
Biological microscopy	BM-1000 type	Nanjing Jiangnan Yongxin Optical Co., Ltd
Electronic balances	BS224S	Doris Scientific Instruments (Beijing) Co., Ltd
Electronic analytical balances	LE204E/02 type	METTLER TOLEDO Instruments Inc
Water bath thermostatic oscillator	SHA-C type	Changzhou Aohua Instrument Co., Ltd
Biosafety cabinets	AC2-4S1	ESCO
UV spectrophotometer	722s type	Changzhou Aohua Instrument Co., Ltd
Vertical automatic pressure steam sterilizer	GI54DWS	Zhiwei (Xiamen) Instrument Co., Ltd
Biochemical incubator	MIR-150A type	Shanghai Santeng Instrument Co., Ltd
Wake-up box	BDF-18PS	Guangzhou Boda Machinery Equipment Co., Ltd
Precision pH meter	S210-K	METTLER TOLEDO Instruments (Shanghai) Co., Ltd
Texture analyzer	TA.XT PlusC	Stable Micro Systems, UK
Induction		Zhongshan Haiyu Life Electrical Appliance Co., Ltd
Refrigerator	218WDPD	Qingdao Haier Co., Ltd

## 2.3. Culture Medium

MRS liquid medium: peptone 10g, beef paste 10g,  $K_2HPO_4$  2g, diammonium citrate 2g, sodium acetate 5g, glucose 20g, Tween-80 1mL,  $MgSO_4 \cdot 7H_2O$  0.5g,  $MnSO_4$  0.25g, distilled water 1000mL, pH 6.2-6.4, sterilization at 121 °C for 20min. Solid medium is added with 2% agar on top of the liquid medium.

MRS-bromocresol green (MRS modified medium) medium: beef paste 10g, peptone 10g, tomato juice 200g, glucose 10g, Tween 0.5ml, calcium carbonate 20g, bromocresol green 0.1g, agar 20g, distilled water 1000 mL, sterilization at 12 °C for 20min.

## 3. Experimental Methods

### 3.1. Isolation, Screening and Identification of Lactic Acid Bacteria

Initial screening: accurately weigh 10g of the activated old dough sample, put it into a triangle bottle containing 90mL of sterile water that has been sterilized, shake and shake well, so that the old dough and water are fully mixed. Under sterile conditions, pipette 100  $\mu$ L of enrichment solution into a tube containing 4.9 mL of sterile water, perform gradient dilution, dilute to  $10^{-1}$  to  $10^{-6}$ , and take  $10^{-3}$  to  $10^{-5}$ . The 200 $\mu$ L dilution was coated on MRS-bromocresol green medium, and MRS-bromocresol green medium was placed in a constant temperature incubator at 37°C and incubated for 48h to observe its color change. It is theoretically surrounded by yellow circles, record colony characteristics and morphology, and perform multiple scribing separations on plates of MRS solid medium, repeat 3-4 times, and finally obtain pure single colonies. The purified single colonies were added to MRS liquid medium, cultured overnight, and preserved with 30% glycerol in a -80°C freezer.

Rescreening: The purebred strains screened by the initial screening were inoculated in MRS liquid medium, fermented at 30°C for 24h, the content of exopolysaccharides in the fermentation broth was determined, and the strains with the highest content of exopolysaccharides were selected for ITS identification.

### 3.2. Morphological Observations

The purebred strains after initial screening were inoculated on MRS solid medium, cultured in a constant temperature incubator at 37°C for 48h, a bacterial ring was picked, appropriately diluted in sterile water, and the bacterial suspension was made into a bacterial suspension, and the bacterial suspension was pipetted on a glass slide with a pipette gun in a biological safety cabinet, covered with a coverslip and hydraulically prepared the bacterial suspension evenly and bubble-free. Observe under a microscope after 1 min.

### 3.3. Determination of Exopolysaccharides

#### 3.3.1. Lactic Acid Bacteria Activation

The low-temperature stored lactic acid bacteria were taken out, placed in a 37 °C water bath for 3 hours, the liquid lactic acid bacteria were connected to the solid medium by plate coating, and cultured for 3 days by a 37 °C biochemical incubator, and then the lactic acid bacteria were cultured in MRS solid medium by the plate scribing method for 24 hours, repeated 3 times, and the fully activated lactic acid bacteria were obtained for backup.

#### 3.3.2. Extraction of Exopolysaccharides

The screened strains were inoculated in the screening MRS liquid medium, fermented at 30 °C for 24h, 2mL of fermentation broth was taken, boiled in boiling water for 10 min, cooled to room temperature, and then centrifuged (4 °C 8000r/min) in a centrifuge for 20 min, the precipitate was washed once with distilled water, centrifuged again, and the supernatant was combined twice. Add 8% trichloroacetic acid (TCA) to the supernatant, precipitate the protein overnight at 4 °C, centrifuge (4 °C 12000r/min) for 15 min to remove the precipitate, add triple

volume of 95% absolute ethanol to the supernatant, precipitate overnight at 4 °C, centrifuge (4 °C 10000r/min) for 20 min to obtain polysaccharide precipitation.

### 3.3.3. Sulfuric Acid-phenol Method to Determine the Content of Lactic Acid Bacteria Exopolysaccharides

The determination of the content of extracellular polysaccharides in lactic acid bacteria was determined by phenol-sulfuric acid method, and glucose was used as the standard curve. The polysaccharide precipitation obtained in 3.3.2 was re-dissolved in water to make a polysaccharide solution, and the absorbance value was measured at a wavelength of 490nm by phenol-sulfuric acid method, and the measured absorbance value was substituted into the glucose standard curve to find its corresponding sugar content, that is, the yield of its extracellular polysaccharide was obtained, and the blank medium was used as a control to deduct background interference.

## 3.4. Identification of Lactic Acid Bacteria and Phylogenetic Tree Analysis

### 3.4.1. Identification of Lactic Acid Bacteria

(1) PCR sequence amplification of lactic acid bacteria

According to the conserved region of the 16S rDNA gene sequence of lactic acid bacteria, the amplification primers (synthesized by Shengong Bioengineering (Shanghai) Co., Ltd.) were amplified in this study using bacterial universal primers 27F:5'-AGTTTGATCMTGGCTCAG-3' and 1492R:5'-GGTACCTTGTTACGACTT-3'. First, centrifuge the primers at 10000rpm for 3min, add double distilled water to configure their desired concentration, and store them in the -20 °C refrigerator for later use.

**Table 2.** PCR amplification system

Reagent	Dosage
5X Buffer	20uL
Primers1	2uL
Primers2	2uL
template	2uL
dNTP	10uL
ddH <sub>2</sub> O	63uL
Primstar(High fidelity)	1uL
Total volume	100uL

**Table 3.** PCR amplification conditions

Temperature	Time	Note
94°C	5min	30piece cycle
94°C	30s	
55°C	30s	
72°C	1min 45s	
72°C	10min	
4°C	10h	

Refer to Carle P, Saillard C, Bové J M. DNA EXTRACTION AND PURIFICATION[M]// Methods in Mycoplasmaology. 1983. Genomic DNA from each strain was extracted and modified. Since the bacteria have no cell wall and do not need to be treated with wall breaking, a small amount of single colony weight was picked by a sterile toothpick and resuspended in sterile double

distilled water (ddH<sub>2</sub>O), and the strain template was obtained by shaking and mixing, and the negative control was amplified by strain's PCR sequence under the same conditions using ddH<sub>2</sub>O as the template. The PCR amplification system amplified by lactic acid bacteria is shown in Table 2, with a total volume of 100 $\mu$ L, and the entire system construction process should be operated on an ice box, and the PCR amplification conditions are shown in Table 3.

## (2) Gel electrophoresis

After the end of PCR amplification, when the bromophenol blue and the sample are mixed for spotting, a maker is used as an indicator standard to do a blank control experiment at the same time, and the PCR product is jointly detected by 0.8% agarose gel electrophoresis, and the GelStain staining solution is stained and scanned in an ultraviolet gel scanner, and the image is saved after photography. The 16S rRNA gene product of the amplified bacteria was sent to Shenggong Bioengineering (Shanghai) Co., Ltd. for sequence sequencing, using universal primers.

### 3.4.2. Strains Develop Tree Drawing

The measured 16S rRNA sequence is about 1500bp long and the known species sequence in the NCBI database is compared with blast homology, and the known sequence with the highest homology to the sequence of the strain to be tested is selected, and the species of the lactic acid strain to be tested are determined according to the comparison results. Using MEGA6.0 and other software, the Neighbour-Joining method was used to construct a phylogenetic tree of strains to study the kinship between strains.

## 3.5. Analysis of Exopolysaccharide Production Conditions by Strains

### 3.5.1. Effect of Lactic Acid Bacteria Addition on Exopolysaccharide Production by Lactic Acid Bacteria

After the screened lactic acid bacteria strains were activated, the culture mother liquor (OD<sub>600</sub>=1.680) was inoculated into MRS liquid medium according to the weight of 0, 0.4%, 0.8%, 1.2%, 1.6% and 2%, and it was placed in a constant temperature incubator at 37°C and incubated for 24h to measure the content of extracellular polysaccharides.

### 3.5.2. Effect of Culture Method on Exopolysaccharide Production by Lactic Acid Bacteria

After the screened lactic acid bacteria strains were activated, the culture mother liquor (OD<sub>600</sub>=1.680) was inoculated into the MRS liquid culture set at 1.2% weight, and they were placed in a constant temperature incubator at 37°C, 100r/min, 150r/min, 200r/min and liquid paraffin liquid sealed oxygen-free medium and incubated for 24h, and the content of extracellular polysaccharides was measured.

### 3.5.3. Effect of Culture Temperature on Exopolysaccharide Production by Lactic Acid Bacteria

After the screened lactic acid bacteria strains were activated, the seed mother liquor (OD<sub>600</sub>=1.680) was inoculated into MRS liquid medium at 1.2% weight, and they were placed in 20, 25, 30, 37 and 42°C constant temperature incubators for 24h, respectively, and the content of extracellular polysaccharides was measured.

### 3.5.4. Effect of Culture Time on Exopolysaccharide Production by Lactic Acid Bacteria

The screened lactic acid bacteria strains were activated, and the seed mother liquor (OD<sub>600</sub>=1.680) was inoculated into MRS liquid medium at 1.2% weight and cultured in a constant temperature incubator at 37°C for 0, 6h, 12h, 24h, 36h, 42h, and 48h, and the yield of extracellular polysaccharides was determined with uninoculated liquid MRS medium as a blank control.

### 3.5.5. Effect of pH on Exopolysaccharide Production by Lactic Acid Bacteria

The screened lactic acid bacteria strains were activated, and the seed mother liquor (OD<sub>600</sub>=1.680) was inoculated into MRS liquid medium with pH 3, 3.5, 4, 4, 4.5, 5, 5.5, 6, 6.5, 7 according to the 1.2% weight of the receiving weight, and incubated inverted in a constant temperature incubator at 37°C for 24h to measure the content of extracellular polysaccharides.

### 3.6. The Process of Making Steamed Buns

Through pre-experiments and referring to the steamed bun production process of Ding Zhili [43] et al., the steamed bun production method was determined. Weigh 120g of water, add 1g of yeast powder, and add lactic acid bacteria ZH-7, ZH-3, ZH-2 from 1g to 5g Single-factor experiment, after obtaining the experimental results, the proportion of three lactic acid bacteria changed, a total of 7g for compound experiments, so that yeast and lactic acid bacteria powder were completely dissolved and mixed in water, 250g of flour was added, first stirred the flour into a dough flocculent with chopsticks and then kneaded by hand for about 10min, kneaded until the surface of the dough was smooth, put into the proofing box for proofing, awakened until the dough was twice as large, the springing time was 90min, the temperature of the fermentation oven was set to 40 degrees Celsius, and the humidity was 60%-85%.

The awakened dough is exhaust shaped, each dough is divided into 100g and kneaded and rounded, the raw dough is kneaded into a smooth round shape, put it into the steamer, the water starts to time after boiling, the steaming time is 25min, after turning off the heat for 5min, the steamed bun is taken out, and the sensory evaluation and related indicators are measured after cooling.

#### 3.6.1. Univariate Testing

##### 3.6.1.1 Determination of Fermentation Time

In this experiment, a total of 5 fermentation times were designed, which were set at 30min, 60min, 90min, 120min, and 150min, respectively, to explore the effect of fermentation time on the sensory quality of steamed buns.

##### 3.6.1.2 Determination of the Amount of Different Lactic Acid Bacteria Added

In this experiment, three different lactic acid bacteria strains, namely class I lactic acid bacteria (ZH-7), class II lactic acid bacteria (ZH-3), and class III lactic acid bacteria (ZH-2), a total of 5 additions were designed for the three lactic acid bacteria strains, respectively 1g, 2g, 3g, 4g, 5g, to explore the effect of the addition of different lactic acid bacteria on the sensory quality of steamed buns.

#### 3.6.2. The Method of Sensory Assessment of Steamed Buns

**Table 4.** Steamed buns sensory evaluation criteria

Project	Out	Scoring criteria
Bun appearance	15points	The appearance is smooth and glossy 11-15 points, the appearance is relatively smooth and slightly shriveled 6-10 points, and the appearance is shriveled and not round 1-5 points.
Epidermal color	15points	Bright white or milky white 11-15 points, white or slightly yellow 6-10 points, yellow or dark yellow 1-5 points.
Organizational structure	15points	The internal pores of the steamed bun are uniform 11-15 points, the internal pores of the steamed bun are basically uniform 6-10 points, and the internal pores of the steamed bun are uneven 1-5 points.
Bun elasticity	15points	Good compression recovery is 11-15 points, compression recovery is generally 6-10 points, and compression recovery is poor 1-5 points.

Bun flavor	20points	There is the fragrance of fermented noodle products, with aromatic taste 16-20 points, fermented noodle products with general fragrance, aromatic taste is generally 9-15 points, fermented noodle products have poor fragrance, poor aromatic taste, sour taste 1-8 points.
Bun taste	20points	Moderate chewing, softer, strong taste, non-sticky teeth 16-20 points, followed by 9-15 points, poor chewing, hard, average taste, slightly sticky teeth 1-8 points.

Referring to the sensory evaluation analysis table of steamed buns by Ai Zhilu [44] et.al. this experiment was formulated, and 7 students were selected to conduct sensory analysis of the quality of steamed buns in turn, and the corresponding indicators are seen.

### 3.6.3. Orthogonal Test

Through the univariate test, the optimal fermentation time, the amount of class I lactic acid bacteria (ZH-7), the amount of class II lactic acid bacteria (ZH-3), and the amount of class III lactic acid bacteria (ZH-2) were determined by univariate test. The three levels that are better are selected for the four factors, and the orthogonal test table is designed and the orthogonal test is carried out through SPSS software.

### 3.6.4. Determination of Dough pH

According to the different culture compounding schemes, the pH of the fermented dough was determined, and the pH determination method in Xing Xiaolong's [45] paper was modified and measured every 30 minutes until the dough fermentation was completed. Take 5g of the fermented dough, dissolve it in 45ml of distilled water, stir it with a glass rod, so that the dough is completely dissolved in distilled water, without large granules. The stirred sample is measured with a pH meter, which is the pH of the dough.

### 3.6.5. Determination of the Fermentation Power of Dough

The fermentation power of dough was measured by the graduated cylinder method [46]. Take 30g of kneaded smooth dough, put the dough into a 250ml measuring cylinder, so that the dough fills the entire lower part of the measuring cylinder without gaps, so that the surface of the dough is smooth, the initial volume  $V_{\text{initial}}$  is recorded, and the fermentation completion volume is  $V_{\text{end}}$ . The dough is placed in the fermentation box for fermentation, and the dough mass volume is read and recorded every 30 min, the dough mass volume changes throughout the fermentation process, and the volume of the dough after fermentation is completed.

### 3.6.6. Determination of the Specific Volume of Steamed Buns

The volume of steamed buns was determined by millet substitution [47]. After cooling the steamed buns at room temperature for 1.5h, the volume of steamed buns was determined, and the measured volume steamed buns were weighed, the results were recorded, and the analysis was made. The ratio of the volume of the steamed bun to the quality of the steamed bun is the specific volume of the steamed bun.

### 3.6.7. Determination of the Texture of Steamed Buns

After cooling the steamed buns at room temperature for 2h, slice them on a clean board, measure the uniform part in the middle of the steamed buns by 20mm with calipers, and cut them into slices parallel up and down with a knife [48]. The parameters of the texture analyzer are set, and the TPA mode is used for measurement, and the P100 round probe is used, the pre-measurement speed is set to 3.5mm/s, the test speed is 1.5mm/s, the post-measurement speed is 10mm/s, the contact force is 5g, and the return distance is 20mm.

### 3.6.8. Data Analysis

The experimental data in this test are all three or more test results and are analyzed by SPSS software system.

## 4. Results & Analysis

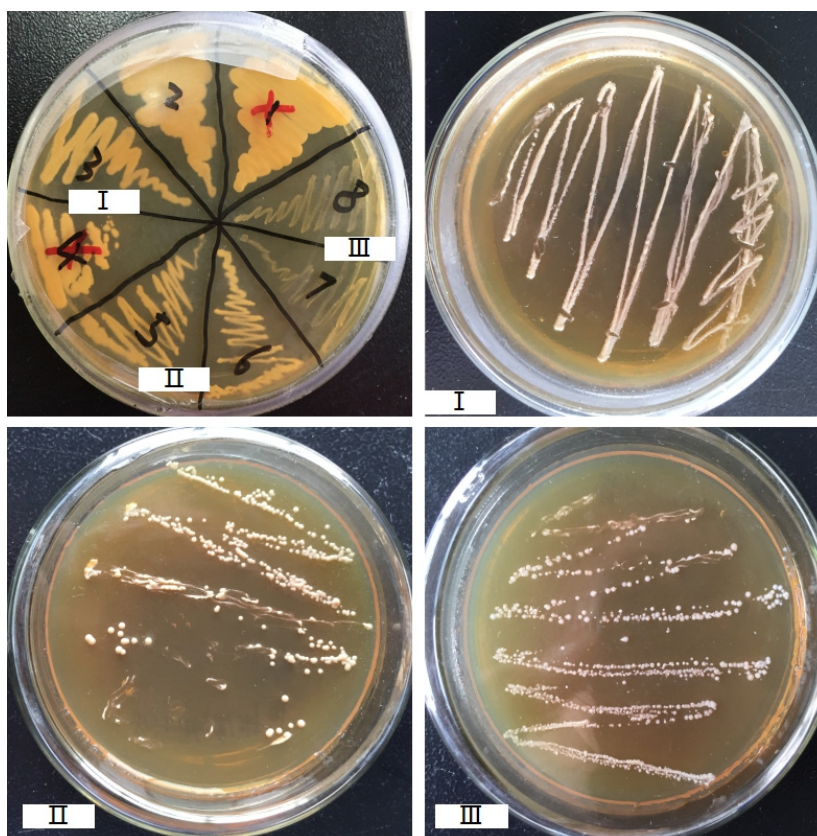
### 4.1. Isolation, Screening and Identification of Lactic Acid Bacteria

#### 4.1.1. Isolation and Screening of Lactic Acid Bacteria

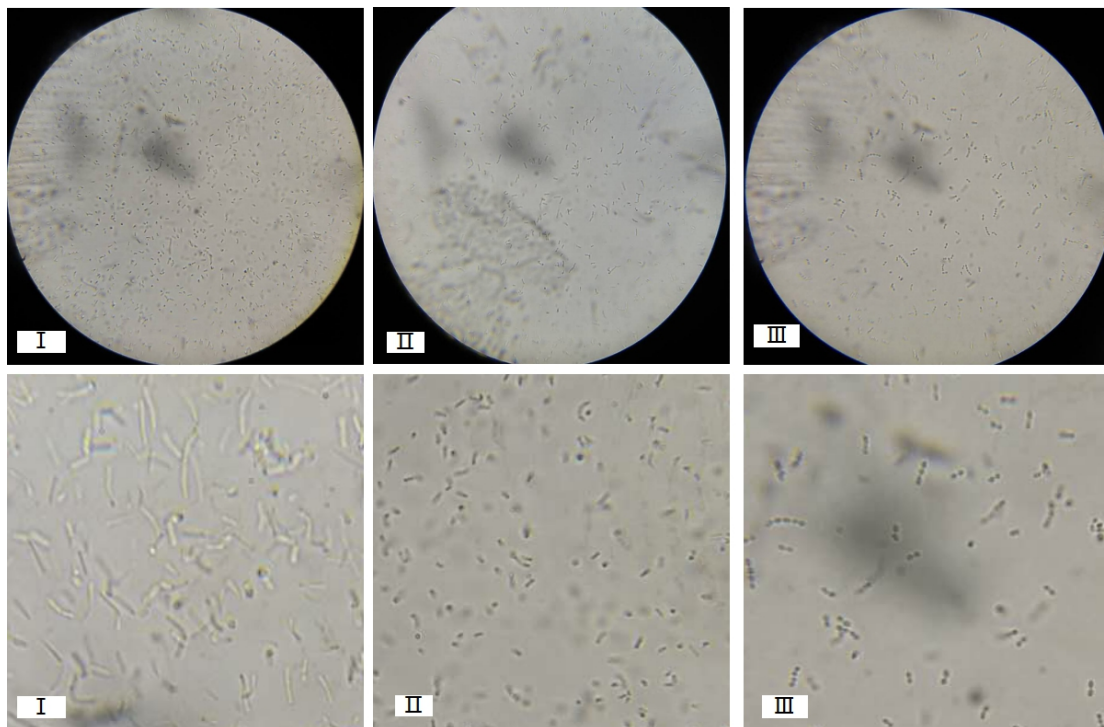
According to the morphological characteristics of lactic acid bacteria colonies and the characteristics of forming yellow circles on MRS-bromocresol green medium, 10 suspected strains of lactic acid bacteria were obtained by scribing and purification, and the colony numbers were ZH1-10. The isolated strains can be divided into three categories by observing their colony morphology and cell morphology, and the colony morphology and cell morphology are shown in Table 5. Class I lactic acid bacteria (colony numbers ZH-1, ZH-4, ZH-7, ZH-10), class II lactic acid bacteria (colony numbers ZH-3, ZH-8), class III lactic acid bacteria (colony numbers ZH-2, ZH-5, ZH-6, ZH-9), their colony morphology is shown in Fig 1, microscopic observation is shown in Fig 2.

**Table 5.** Colony Morphology and Cell Morphology of Lactic Acid bacteria

Fungus	Color	Shape	Center	Edge	Diameter size	Surface	Transparency	Cell morphology
I	white	round	convex	neat	3mm	smooth	opaque	Rod
II	gray-white	round	convex	neat	2mm	Moist smooth	opaque	Short rod or short chain
III	white	oval	convex	neat	1mm	moist	opaque	spherical or chain



**Fig 1.** Lactic acid bacteria colony diagram

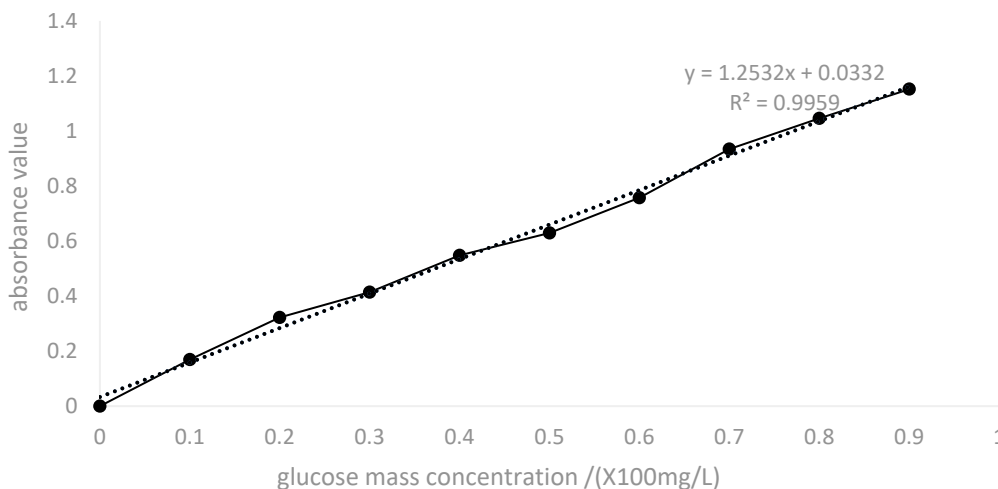


**Fig 2.** Microscopic observation of lactic acid bacteria

Shown in Table 5, Fig 1, Fig 2, It can be seen that the three types of lactic acid bacteria have different colors, the color of Class II lactic acid bacteria is gray-white, and the color of Class I and III are white. Class I and II lactic acid bacteria are round in shape, and Class III lactic acid bacteria are oval. The center of the three types of lactic acid bacteria is convex, and the edges are neat. Class I lactic acid bacteria have a diameter size of 3mm and a moist and smooth surface, Class II lactic acid bacteria have a diameter size of 2mm and a smooth surface, and Class III lactic acid bacteria have a diameter size of 1mm and a wet surface. From the perspective of transparency, the three types of lactic acid bacteria are opaque. Class I lactic acid bacteria cells are rod-shaped, Class II lactic acid bacteria cells are short rod-shaped or short-chain-shaped, and Class III. lactic acid bacteria cells are spherical or chain-shaped. It can be seen that these three types of lactic acid bacteria belong to different species.

**4.1.2. Rescreening of Lactic Acid Bacteria**

**4.1.2.1 Drawing of Standard Curves**

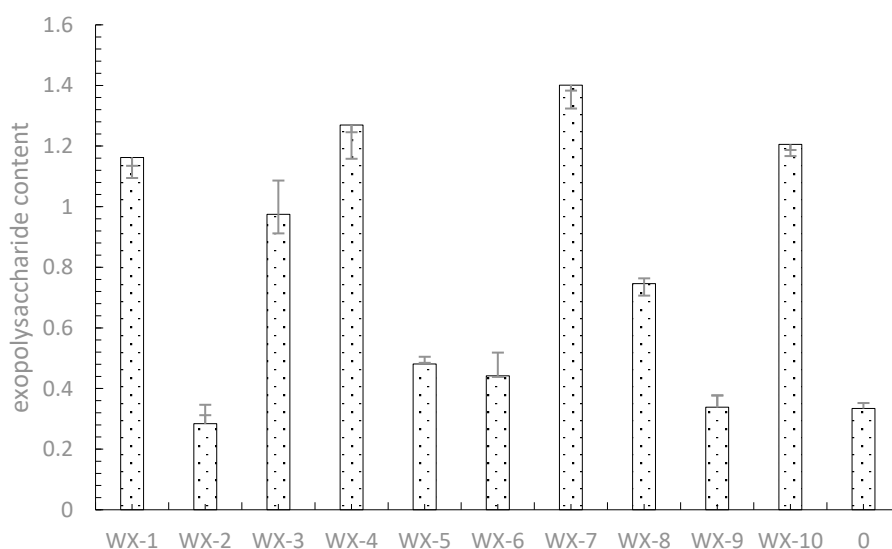


**Fig 3.** Glucose standard curve

Take the mass concentration of the glucose diluent as the abscissa and the absorbance value as the ordinate to plot the glucose standard curve (shown in Fig 3). The standard curve is  $y=0.6266x+0.0332$ ,  $R^2=0.9959$ , which indicates that the glucose mass concentration is in the range of 0.2-1.8 (x100mg/L), and the linear relationship between glucose mass concentration and absorbance value is good.

#### 4.1.2.2 Determination of Exopolysaccharide Content

The isolated lactic acid bacteria were inoculated in a liquid MRS culture concentration according to the inoculation amount of 1%, placed in a biochemical constant temperature incubator at 37 °C for 24h, and the sugar yield of the fermentation broth was measured by phenol-sulfuric acid method as shown in the Fig 4.

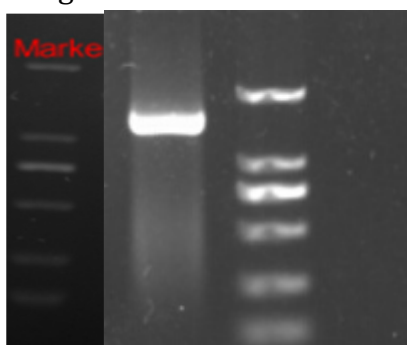


**Fig 4.** Content of extracellular polysaccharide produced by different lactic acid bacteria strains

As can be seen from Fig 4 under the same culture conditions, the strains ZH-1, ZH-3, ZH-4, ZH-7, The content of exopolysaccharides produced by ZH-8 and ZH-10 was higher than that contained in the medium, among which the lactic acid bacteria strain ZH-7 had the highest ability to produce exopolysaccharides, reaching 0.14g/l, and the fermentation performance was stable. It is an ideal producer of exopolysaccharide lactic acid bacteria.

#### 4.1.3. Identification of Lactic Acid Bacteria

The DNA of the strain, ZH-7, screened in the sour dough, was PCR amplified, and the concentration of amplification product was detected by 0.8% agarose gel electrophoresis, and the detection results were shown in Fig 5.



**Fig 5.** ZH-7 16SrDNA amplification results

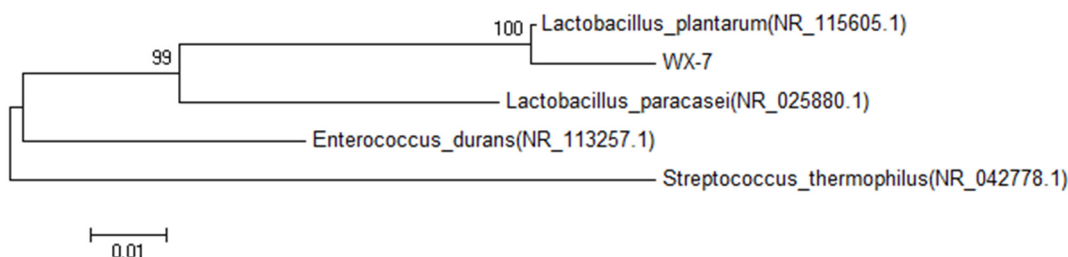
It can be seen from Fig 5 that compared with the results of Marker electrophoresis, a clear bright band appeared near 1500 bp of the strains, no specific amplification, and no diffusion and tailing of the bright band, which met the sequencing requirements.

The sequencing results were analyzed by NCBI's BLAST comparison, and the results were shown in Table 6. (the homology of the 16S rDNA sequence of the lactic acid bacteria strain and the model strain was not less than 99%, which could be directly identified to the species), and the isolated exopolysaccharide-producing lactic acid bacteria were determined to be Class I lactic acid bacteria (ZH-7). The E value indicates the probability of random matching, and the larger the E value, the greater the probability of random matching. When the E value is close to zero or zero, it is an exact match.

**Table 6.** Identification of ZH-7strain ITS

Description	Max score	Total score	Query cover	E value	Ident	Accession
Lactobacillus plantarum strain JCM 1149 16S ribosomal RNA gene, partial sequence	2590	2590	96%	0.0	99%	NR_115605.1

The phylogenetic tree of the 16sRNA sequence of this strain was constructed by MEGA6 software, and the results are shown in Fig 6. It can be seen from Fig 6 that the lactic acid bacteria and its corresponding model strain on the phylogenetic tree belong to the same branch, indicating that there is a close relationship between them, and the comparison results of Class I lactic acid bacteria (ZH-7) and BLAST are consistent.



**Fig 6.** Construction of phylogenetic tree of lab based on 16SrDNA sequence

#### 4.1.4. Optimization of Conditions for Exopolysaccharide Production by Lactic Acid Bacteria

##### 4.1.4.1 Effect of Added Amount on the Ability of Lactic Acid Bacteria to Produce Exopolysaccharides

The sugar content begins to decline. Therefore, the inoculum dose of 0.8% is most suitable for the strain to produce exopolysaccharides. In MRS liquid medium, the inoculation culture stock liquor (OD<sub>600</sub>=1.680) was fermented for 24h at 0, 0.4%, 0.8%, 1.2%, 1.6% and 2%, and the content of exopolysaccharides in the fermentation broth was shown in Fig 7.

It can be seen from Fig 7 that the amount of addition has an effect on the content of exopolysaccharides produced by Lactobacillus implanted. (Under the premise of fixing other indicators, based on the description and empirical analysis in other papers, passed t Inspection, the amount of exopolysaccharide production when vaccination is in 0.8% is significantly higher than other treatment groups ( $p < 0.05$ ). As the amount of strain inoculated increases, The strain produces more extracellular.

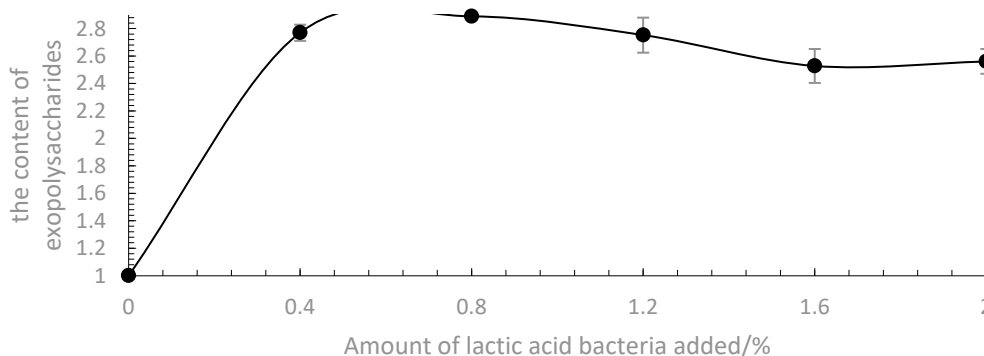


Fig 7. Construction of phylogenetic tree of lab based on 16SrDNA sequence

#### 4.1.4.2 Effect of Culture Method on the Ability of Lactic Acid Bacteria to Produce Exopolysaccharides

In MRS liquid medium, inoculate 1.2% culture mother liquor (OD600=1.680), the culture methods are standing, anaerobic and rotational speed are 100, 150 and 200, respectively, and incubated in a 37°C incubator for 24h, the content of exopolysaccharides in the fermentation broth, as shown in Fig 8.

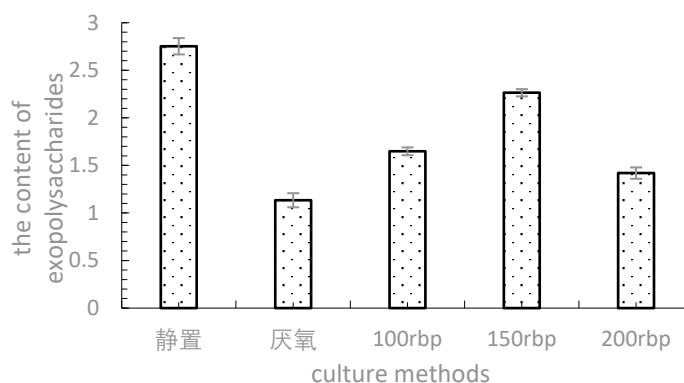


Fig 8. The production of ester producing under different culture methods

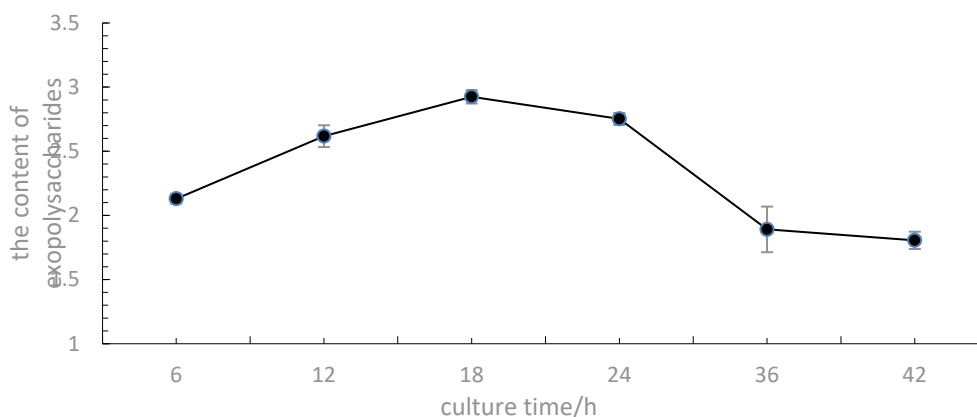
It can be seen from Fig 8 that Class I lactic acid bacteria (ZH-7) are cultured anaerobic and at speeds of 100 and 200, and the content of exopolysaccharides produced by Class I lactic acid bacteria (ZH-7) is basically the same, and when the speed is 150, the content of exopolysaccharides increases, but its content is slightly lower than that of standing culture, and in standing culture, the content of extracellular polysaccharides produced by Class I lactic acid bacteria (ZH-7) reaches 0.275g/L. Therefore, standing culture is most suitable for strains to produce exopolysaccharides.

#### 4.1.4.3 Effect of Fermentation Time on the Ability of Lactic Acid Bacteria to Produce Exopolysaccharides

In MRS liquid medium, inoculate with 1.2% culture stock liquor (OD600=1.680) and culture in a 37°C incubator for 6, 12, 18, 24, 36, and 42 h, respectively, with the content of exopolysaccharides in the fermentation broth, as shown in Fig 9.

As can be seen from Fig 9, the culture time has an effect on the yield of exopolysaccharides produced by Class I lactic acid bacteria (ZH-7). In a certain time, range, with the extension of fermentation time, the yield of exopolysaccharides produced by Class I lactic acid bacteria (ZH-7) increased, and when the culture time was 18h, the yield of exopolysaccharides reached a maximum, that is 0.292g/L. Beyond this culture time range, fermentation culture continued,

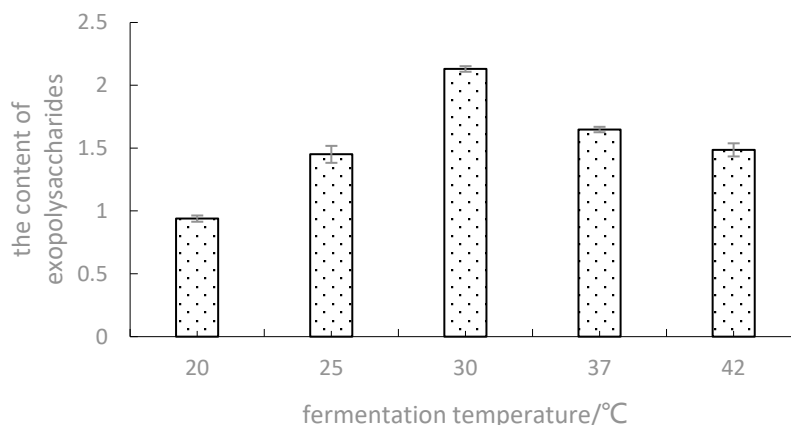
and exopolysaccharide production decreased. Therefore, fermentation and culture for 18h is most suitable for strains to produce exopolysaccharides.



**Fig 9.** Effect of culture time on Production of EPS

#### 4.1.4.4 Effect of Fermentation Temperature on the Ability of Lactic Acid Bacteria to Produce Exopolysaccharides

In MRS liquid medium, inoculate 1.2% seed mother liquor ( $OD_{600}=1.680$ ) and incubate for 24h in 20, 25, 30, 37 and 42 °C incubators, respectively, with the content of exopolysaccharides in the fermentation broth, as shown in Fig 10.



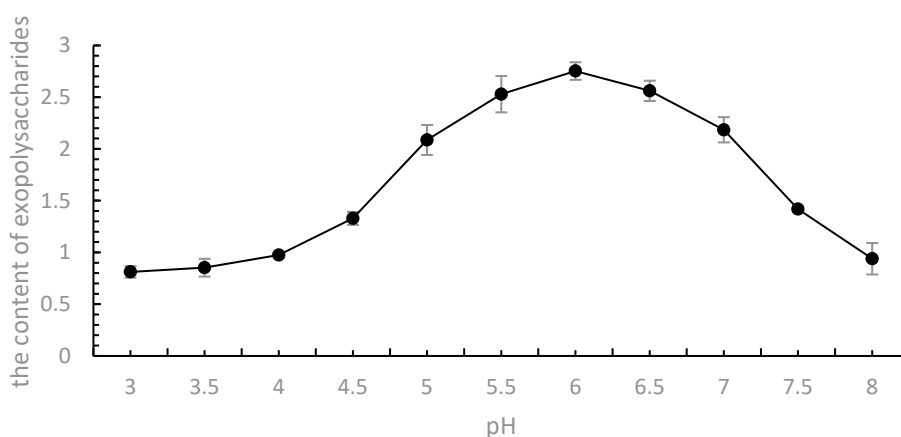
**Fig 10.** Effect of temperature on growth and yield of ester producing

It can be seen from Fig 10 that the production of exopolysaccharides of Class I lactic acid bacteria (ZH-7) is significantly different at different fermentation temperatures. At a fermentation temperature of 30 °C, exopolysaccharide yields are the highest. Although 37 °C is the most suitable temperature for bacterial growth for Class I lactic acid bacteria (ZH-7), exopolysaccharides are secondary metabolites, and as the temperature rises, the stability period of the strain will arrive earlier, and the yield of polysaccharides will be significantly reduced. Therefore, the fermentation temperature of 30 °C is most suitable for the production of exopolysaccharides by strains.

#### 4.1.4.5 Effect of pH on the Ability of Lactic Acid Bacteria to Produce Exopolysaccharides

In MRS liquid medium, inoculate 1.2% culture mother liquor ( $OD_{600}=1.680$ ), the initial pH values of fermentation are 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, and after incubation in a 37°C

incubator for 24 h, and the content of exopolysaccharides in the fermentation broth is shown in Fig 11.



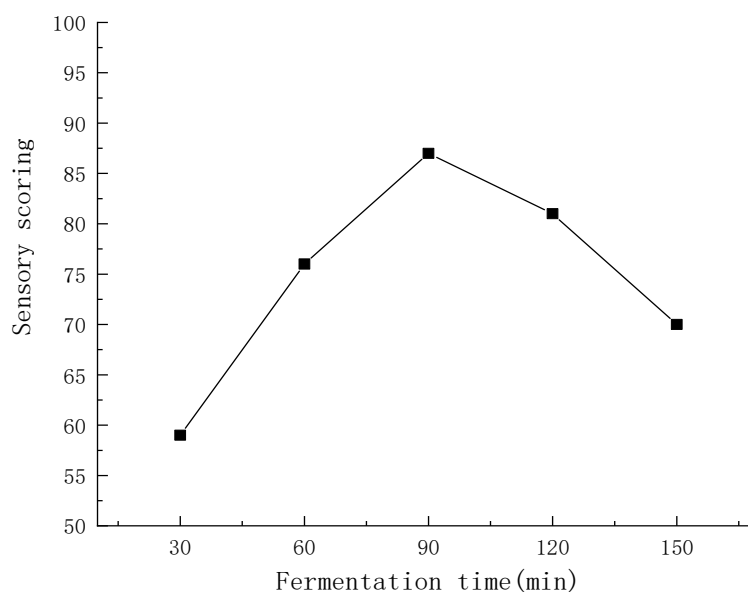
**Fig 11.** Effect of pH on growth and yield of ester producing

It can be seen from Fig 11 that with the increase of pH, the production of exopolysaccharides first increases and then decreases. Too high or too low pH is not conducive to the production of exopolysaccharides by Class I lactic acid bacteria (ZH-7). This is because below pH 5.0, because of the inhibition of acid, lactic acid bacteria are not suitable for growth, the production of exopolysaccharides is less, and when the pH is above 7.0, it exceeds its optimal growth pH range, and the bacteria are not suitable for growth, so the production of exopolysaccharides is less. Therefore, when the pH value ranges from 5.0 to 7.0, the maximum number of exopolysaccharides can be produced.

## 4.2. Application in Steamed Buns

### 4.2.1. Univariate Testing

#### 4.2.1.1 Determination of Fermentation Time



**Fig 12.** Effect of different fermentation time on sensory score of steamed buns

The effect of different fermentation times on the sensory score of steamed buns is shown in Fig 12. It can be seen from Fig 12 that the fermentation time has a greater impact on the sensory score of steamed buns, and with the increase of fermentation time, the sensory score of steamed buns first increases and then decreases, which is the same as the research trend of Guo Wuqiong [49] et al., which is the result of the interaction between yeast and lactic acid bacteria with fermentation time. Among them, the sensory score of steamed buns when the fermentation time is 30min is the lowest, which is due to the shorter fermentation time, the content of yeast and lactic acid bacteria in the dough in the early stage of fermentation is less, the aromatic smell such as CO<sub>2</sub> gas and lactic acid produced in the steamed buns is less, the steamed buns are smaller in volume and hard in texture, and the appearance, color and taste of the steamed buns are poor. With the increase of fermentation time, yeast and lactic acid bacteria begin to multiply in large quantities, yeast growth and reproduction period produces a large amount of CO<sub>2</sub> gas, lactic acid bacteria growth and reproduction produce lactic acid [50], yeast and lactic acid bacteria affect each other during the fermentation process, and when the fermentation time of the steamed buns is 90min, the steamed buns have the best color, flavor and taste, and the sensory score of the steamed buns is the highest. As the fermentation time continues to increase, the sensory score of the steamed buns decreases, which is caused by excessive propagation of lactic acid bacteria in the dough, the steamed buns begin to appear sour, and the sensory quality of the steamed buns begins to decline. Therefore, the fermentation time is too short, less CO<sub>2</sub> gas is produced in yeast, and the steamed buns are harder and have poor color and taste; if the fermentation time is too long, the large number of lactic acid bacteria will make the lactic acid in the steamed bun too much, resulting in sour steamed buns, seriously affecting the flavor and taste of the steamed buns, and making the quality of the steamed buns worse. According to the influence of fermentation time on the sensory score of steamed buns in a single factor, it can be seen that when the fermentation time is 90min, the steamed buns have the highest sensory score.

#### 4.2.1.2 Determination of the Amount of Different Lactic Acid Bacteria Added

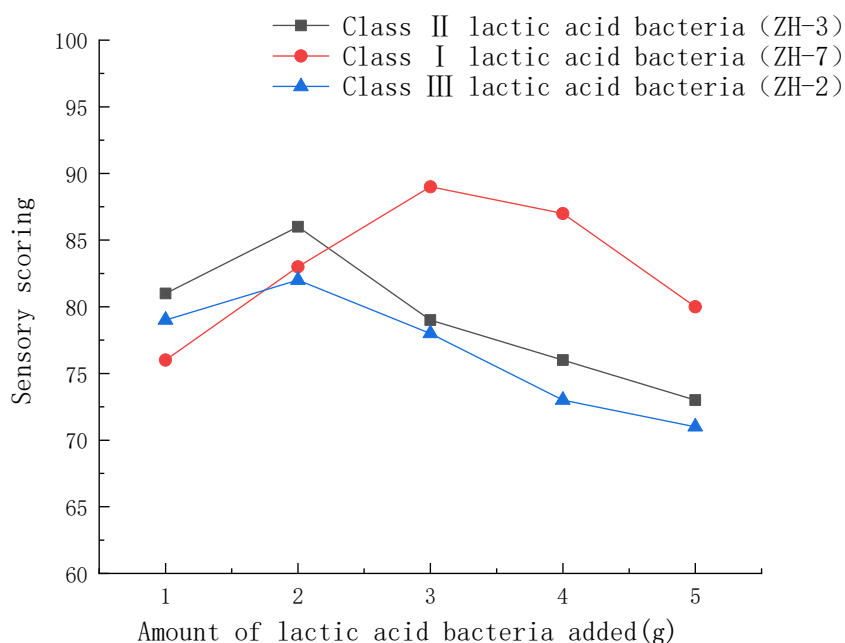


Fig 13. Effects of different lactic acid bacteria added on sensory scores of steamed buns

The effect of different lactic acid bacteria added on the sensory score of steamed buns is shown in Fig 13. The amount of lactic acid bacteria added was different from that of lactic acid bacteria, which had a certain impact on the sensory score of steamed buns. With the increase of lactic acid bacteria added, the sensory score of steamed buns first increased and then decreased. This is due to the increase in the amount of lactic acid bacteria added in the dough, the amount of acid produced by lactic acid bacteria is different during the same fermentation time, and the aromatic substances generated are different; The more lactic acid bacteria are added, the more acid is produced, and the flavor and texture of steamed buns are also very different. It can be seen from Fig 13 that in the univariate experiment, the effects of Class II lactic acid bacteria (ZH-3), Class I lactic acid bacteria (ZH-7), and Class III lactic acid bacteria (ZH-2) on the sensory score of steamed buns are different. With Class I lactic acid bacteria (ZH-7) are added, the sensory score of steamed buns had the greatest impact, and the sensory score was generally high, indicating that Class I lactic acid bacteria (ZH-7) had a good effect on the flavor and texture of steamed buns; However, the amount of Class II lactic acid bacteria (ZH-3) and Class III lactic acid bacteria (ZH-2) had little effect on the sensory score of steamed buns. Through the sensory score of steamed buns by different lactic acid bacteria added in single-factor experiments, it can be seen that when the amount of Class II lactic acid bacteria (ZH-3) is 2g, when the amount of Class I lactic acid bacteria (ZH-7) is 3g, and when the amount of Class III lactic acid bacteria (ZH-2) is 2g, the sensory score of steamed buns is the highest.

#### 4.2.2. Orthogonal Test

Through the results of univariate test, three optimal levels were selected among the fermentation time, the amount of Class II lactic acid bacteria (ZH-3), the amount of Class I lactic acid bacteria (ZH-7), and the amount of Class III lactic acid bacteria (ZH-2), and the orthogonal test of four factors and three levels was carried out. The orthogonal test of L<sub>9</sub> (3<sup>4</sup>) is shown in Table 7, and the influence of different factors on the sensory quality of steamed buns is investigated, and the orthogonal test results of steamed buns are obtained through orthogonal tests as shown in Table 8.

**Table 7.** Steamed buns orthogonal test factors and levels

Level	A	B	C	D
	Fermentation time /min	Class II lactic acid bacteria (ZH-3)/g	Class I lactic acid bacteria (ZH-7)/g	Class III lactic acid bacteria (ZH-2)/g
1	60	1	2	1
2	90	2	3	2
3	120	3	4	3

According to the analysis of the orthogonal test results in Table 8, it can be seen that the sensory scores of steamed buns under different factors and levels vary greatly. It can be seen from Table 8 that the sensory scores of 1#, 2# and 3# steamed buns are relatively low, indicating that fermentation time has a greater influence on the quality of steamed buns. Through range analysis, it can be seen that the primary and secondary factors affecting the quality of steamed buns in the orthogonal test are A>C>B=D, that is, fermentation time> Class I lactic acid bacteria (ZH-7) >Class II lactic acid bacteria (ZH-3) = Class III lactic acid bacteria (ZH-2), and the optimal level is A<sub>2</sub>B<sub>1</sub>C<sub>2</sub>D<sub>3</sub> from the orthogonal test results, and the optimal group contract 4# steamed bun fermentation conditions are the same, that is, the fermentation temperature is 90min, Class II lactic acid bacteria (ZH-3) 1g, Class I lactic acid bacteria (ZH-7) 3g, Class III lactic acid bacteria (ZH-2) 3g.

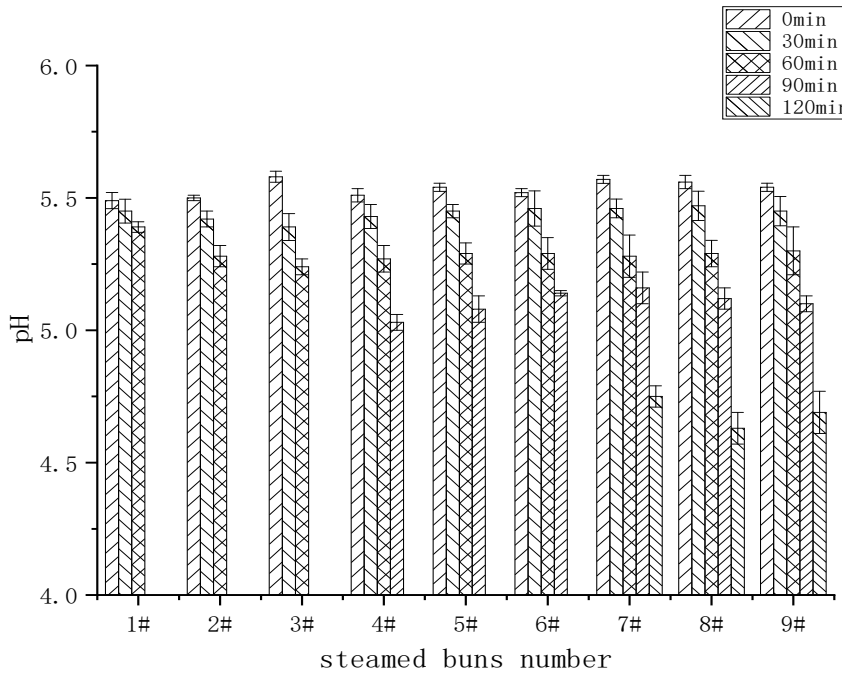
**Table 8.** Orthogonal test results of steamed buns

Steamed buns number	A	B	C	D	Sensory scores
	Fermentati on time	Class II lactic acid bacteria(ZH-3)	Class I lactic acid bacteria (ZH-7)	ClassIII lactic acid bacteria (ZH-2)	
1#	1	1	1	1	76.3
2#	1	2	2	2	80.6
3#	1	3	3	3	78.1
4#	2	1	2	3	91.0
5#	2	2	3	1	82.4
6#	2	3	1	2	79.7
7#	3	1	3	2	83.4
8#	3	2	1	3	82.6
9#	3	3	2	1	81.1
K1	235	250.7	238.6	239.8	
K2	253.1	245.6	252.7	243.7	
K3	247.1	238.9	243.9	251.7	
k1	78.3	83.6	79.5	79.9	
k2	84.4	81.9	84.2	81.2	
k3	82.4	79.6	81.3	83.9	
R	6.1	4	4.7	4	
Primary and secondary factors	A>C>B=D				
Optimization level	A <sub>2</sub>	B <sub>1</sub>	C <sub>2</sub>	D <sub>3</sub>	
Optimal combination	A <sub>2</sub> B <sub>1</sub> C <sub>2</sub> D <sub>3</sub>				

#### 4.2.3. Effect of Dough pH in Complex Pairing of Different Cultures

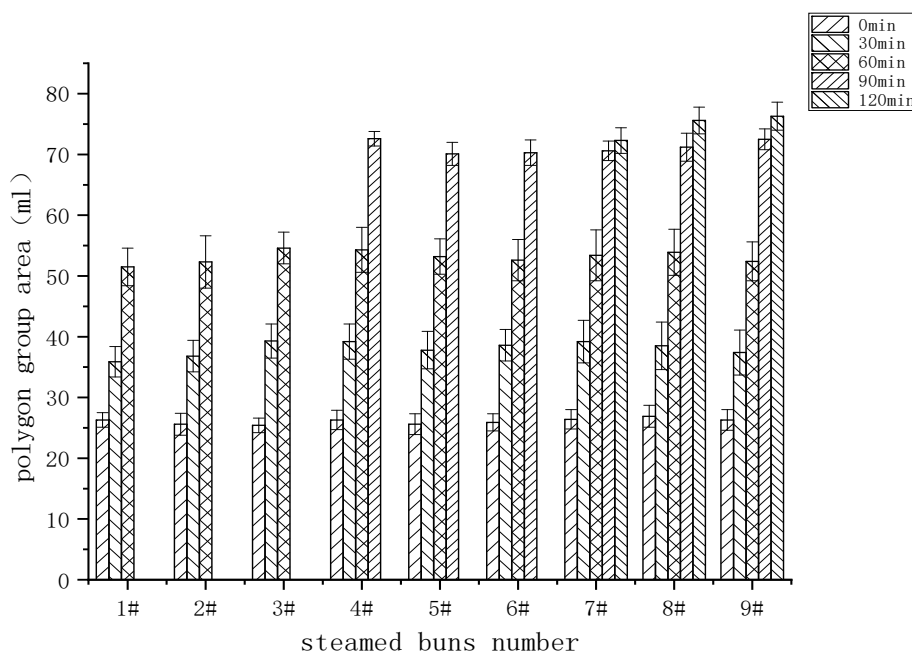
The effect of the pH of the dough of different cultures is shown in Fig 14. It can be seen from Fig 14 that the initial pH of each dough is between 5.49 and 5.58, the pH of the dough after 30min fermentation is maintained between 5.39~5.47, the pH of the dough after 60min fermentation is maintained between 5.24 and 5.39, the pH of the dough after 90min is maintained between 5.03 and 5.16, and the pH of the dough after 120min fermentation is maintained between 4.69 and 4.75. It can be seen from Fig 14 that the pH drop in the early stage of dough fermentation is slow, which is due to the fact that the yeast in the dough first undergoes short aerobic respiration, the yeast ferments faster, and the lactic acid bacteria ferment slowly. With the increase of fermentation time, yeast and lactic acid bacteria grow and multiply in large quantities in an oxygen-free environment, the dough volume becomes larger, and the pH in the dough continues to decrease. The total amount of Class II lactic acid bacteria (ZH-3), Class I lactic acid bacteria (ZH-7), and Class III lactic acid bacteria (ZH-2) in the dough is different in 1#, 2#, 3#, with the increase of the total amount of lactic acid bacteria added, the pH under the same fermentation time has a certain change, and the more lactic acid bacteria are added, the lower the pH when the dough fermentation is completed[36]. 2#, 4#, 5#, 6#, 7#, 8#, 9# steamed buns can be seen that under the condition that the total amount of lactic acid bacteria added remains unchanged, the pH decreases with the increase of fermentation time, which is caused by the growth and reproduction of lactic acid bacteria in the dough; Over time, lactic acid

bacteria continue to use the sugars in the dough to produce more lactic acid, so the pH in the dough continues to decrease. It can be seen from Fig 14 that the pH changes of different lactic acid bacteria are different, indicating that Class II lactic acid bacteria (ZH-3), Class I lactic acid bacteria (ZH-7) and Class III lactic acid bacteria (ZH-2) have different acid production performance during fermentation, resulting in different pH changes in the dough.



**Fig 14.** Effect of dough pH in complex pairing of different cultures

**4.2.4. Effect of Fermentation Power of Dough in Combination with Different Cultures**

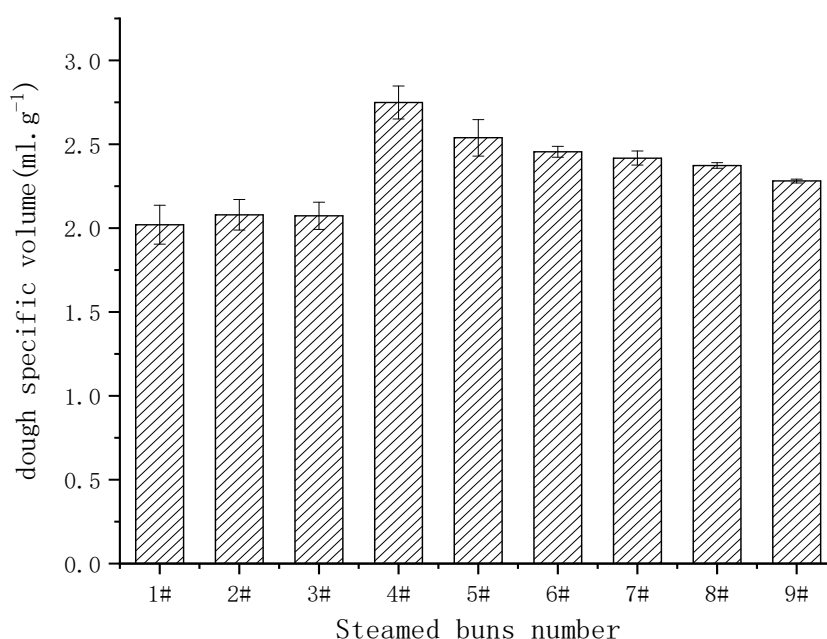


**Fig 15.** Effect of fermentation volume of dough in combination with different cultures

The effect of the fermentation volume of different cultures on the dough is shown in Fig 15. The initial volume of dough in 1#~9# is between 25 and 27ml, the group volume after 30min

fermentation is between 35 and 40ml, the group volume after fermentation is between 50 and 55ml, the group volume after fermentation is between 70 and 75ml, and the group volume after fermentation is between 73 and 75ml after 120min, and the volume of dough continues to increase with the growth of fermentation time. From the growth trend of Fig 4, it can be seen that the fermentation rate of dough in the early stage of fermentation is faster and the volume change is large, and with the increase of fermentation time, the fermentation rate of dough slows down and the volume change decreases. When the dough ferments for 90min and 120min, the dough fermentation rate slows down, and the volume change of the dough is small, which is due to the large number of yeasts and lactic acid bacteria in the dough with the increase of fermentation time, the number of lactic acid bacteria multiplies, and the lactic acid content in the dough gradually increases[51]; The pH in the dough drops, causing changes in the growing environment of yeast and lactic acid bacteria. As the fermentation time increases, the volume of the dough continues to increase and the network structure in the dough changes. In the later stage of dough fermentation, the dough will produce air leakage, and the volume change of the dough will slow down.

#### 4.2.5. Effects of Different Strains on the Specific Volume of Steamed Buns



**Fig 16.** Effects of different strains on the specific volume of steamed buns

The effect of the complex pairing of different cultures on the specific volume of steamed buns is shown in Fig 16. It can be seen from Fig 16 that the specific volume of 1#, 2#, and 3# steamed buns is the smallest, the specific volume of 5#, 6#, 7#, 8#, and 9# steamed buns is not much different, and the specific volume of 4# steamed buns is the largest. Among them, the amount of lactic acid bacteria added to 1#, 2#, and 3# steamed buns increased sequentially, and the specific volume of steamed buns increased relatively, and it can be seen that the difference in the total amount of lactic acid bacteria added will have a certain impact on the specific volume of steamed buns. The total amount of lactic acid bacteria added to 4#, 5#, 6#, 7#, 8#, 9# steamed buns is the same, and the fermentation time is different, and the specific volume of steamed buns varies greatly. It can be seen that the different amount of lactic acid bacteria added in steamed buns and different fermentation times will have a certain impact on the specific volume of steamed buns, the amount of lactic acid bacteria added has little effect on the specific

volume of steamed buns, and the fermentation time has a greater impact on the specific volume of steamed buns.

#### 4.2.6. Effect of Texture of Steamed Buns with Different Strains

**Table 9.** Effect of texture of steamed buns with different strains

Steamed buns number	hardness	recovery	cohesion	viscosity	chewiness	elasticity
1#	14536.302	0.923	0.725	9663.693	9663.693	0.290
2#	13124.299	0.911	0.757	9905.315	9070.477	0.417
3#	13627.862	0.839	0.799	10799.566	9076.663	0.407
4#	11676.947	0.896	0.772	9011.943	8095.648	0.455
5#	12967.225	0.912	0.778	10060.631	9177.643	0.450
6#	12741.669	0.921	0.783	9973.504	9178.337	0.461
7#	11943.949	0.915	0.750	8954.529	8185.207	0.408
8#	12617.174	0.890	0.772	9712.724	8644.019	0.441
9#	12646.427	0.907	0.778	9841.329	8923.213	0.447

\*The data in the table are averages.

The effects of different cultures on the texture of steamed buns are shown in Table 9. The sensory score of steamed buns has a certain correlation with the texture of steamed buns, which is the same as that of Zhao Zheng[52]. Through the analysis of the texture of steamed buns, it can be concluded that the greater the hardness of steamed buns, the greater the chewiness and less elasticity of steamed buns[53]. The recovery, cohesion and viscosity of steamed buns have a certain impact on the change of hardness of steamed buns, but the difference is small. 1#, 2#, 3# steamed buns can be seen that the hardness of these three steamed buns is large, and the sensory score of the steamed buns is low. This is due to the influence of fermentation time on the sensory quality of steamed buns, the shorter the fermentation time of steamed buns, the less yeast and lactic acid bacteria in the steamed buns grow and reproduce, the relatively less CO<sub>2</sub> produced by dough fermentation, and less different flavor substances are generated in the dough; During the steaming process, the steamed bun is not easy to expand, resulting in greater hardness, poor taste and flavor, relatively low sensory quality and low sensory score of steamed buns. The hardness of 4#, 5#, 6#, 7#, 8#, 9# steamed buns is not much different, indicating that there is enough gas produced in the dough during fermentation of 90min, compared with 120min fermentation, there is not much difference, and the difference in texture of steamed buns is small. In the fermentation process, the combination of different strains will have a certain impact on the texture of steamed buns, and the different types and proportions of lactic acid bacteria will also affect the texture of steamed buns, and the texture of steamed buns with the same fermentation time also has certain differences. As can be seen from Table 9, 4# steamed buns have the smallest hardness, the least chewiness, greater elasticity, relatively good taste, and relatively high sensory scores.

Through the analysis of the results of this experiment, the conclusions drawn are:

1. ZH-7, the lactic acid bacterium with the strongest extracellular polysaccharide production ability isolated and screened from the sourdough, was identified as a Class I lactic acid bacterium (ZH-7) after 16s RNA identification.
2. By optimizing the conditions for exopolysaccharide production of strain ZH-7, it was finally determined that it had the strongest ability to produce exopolysaccharides when the pH of the

medium was between 5 and 7 and the addition amount was 0.8%, and it was incubated in a constant temperature incubator at 30°C for 18h.

3. The best scheme for fermentation time and strain combination in the production of steamed buns was obtained, among which the optimal combination was 90min fermentation time of steamed buns, 1g of Class II lactic acid bacteria (ZH-3) added, 3g of Class I lactic acid bacteria (ZH-7), and 3g of Class III lactic acid bacteria (ZH-2), the sensory quality of steamed buns was the best.

4. Through physical and chemical indicators and sensory evaluation, the pH value of 4# steamed bun after fermentation is 5.03, the volume of dough is 72.6, the specific volume of steamed bun is 2.749, the hardness is 11676.947, and the elasticity is 0.455, under this condition, the steamed bun is better in appearance, smell, hardness, elasticity, chewiness and other aspects.

## References

- [1] TANG X. Study on Screening for Lactic Acid Bacterium with High EPS-producing Capacity and Optimization of Culture Condition from Koumiss in Xin Jiang [D]; Xinjiang Agricultural University, 2012.
- [2] WANG S. Talking about the biological basis and application of lactic acid bacteria [J]. Learning Weekly, 2018, (06): 169-70.
- [3] WANG J. Study on Isolation & Identification and Fermentation Properties of Microorganisms from Sourdough [D]; Inner Mongolia Agricultural University, 2015.
- [4] DAI YONGGANG T Z, NAN XIPING. Progress of the Lactobacillus and Its Physiological Function [J]. Academic Periodical of Farm Products Processing, 2009: 24-6+9.
- [5] TIAN JIANJUN Z K, LI QUANWEI, ZHAO YANHONG, JIN YE. Recent Progress in Understanding the Substance Basis for the Regulation of Cholesterol Metabolism by Lactic Acid Bacteria [J]. Food Science, 2019, (19): 334-9.
- [6] GUO JINGJING Z P, CAO CHENGXU, ZOU TINGTING, WU RINA. Cholesterol-lowering lactic acid bacteria isolated from the naturally fermented soybean sauces and their effects on serum cholesterol levels in rats [J]. Food and Fermentation Industries, 2019, (02): 15-24.
- [7] SHI-JIE Z T Z Y-K X Y-P Z D W Y-X Z H W. Research progress on antioxidation and regulation mechanism of lactic acid bacteria [J]. China Food Additives, 2018, (09): 202-9.
- [8] GUANZHONG Z X L F L Y X Y L. Progress of the application of lactic acid-producing bacteria in healthy foods [J]. Shanghai Medical & Pharmaceutical Journal, 2018, (15): 25-8.
- [9] ZHANG GUOHUA H G. Traditional Starter Cultures of Chinese Steamed Bread--a Review [J]. Journal of Chinese Institute of Food Science and Technology, 2012, 12(11): 115-20.
- [10] LIU CHANG-JIAN J B, CUI YU-BO, LIU QIU, QI XIAO-HUI. Advancement of Lacto-Bacteriocin as Bio-Preservative [J]. First Journal of Microbiology, 2010, (03): 87-90.
- [11] CHEN J. Effects of Lactic Acid Bacteria on Sourdough Fermentation and Chinese Steamed Bread [D]; Henan University of Technology, 2012.
- [12] LI Z. Microbial Screening and Identification of Traditional Starter and the Impact on the Quality of Mantou [D]; Henan University of Technology, 2011.
- [13] XIUTING L J S Y T C Z Y L. Improving Acidic Ability of Lactic Acid Bacteria from Sourdough and Its Effect on Steamed Bread Quality [J]. Journal of Chinese Institute of Food Science and Technology, 2018, (05): 106-14.
- [14] PATRICIA RUAS-MADIEDO J H, PIETERNELA ZON. An overview of the functionality of exopolysaccharides produced by lactic acid bacteria [J]. International Dairy Journal, 2002.
- [15] PHILIPPE DUBOC B M. Applications of exopolysaccharides in the dairy industry [J]. International Dairy Journal, 2001.

- [16] ZHAO YUCHEN W Y, WANG ZHONGFENG. Lactic acid bacteria exopolysaccharides [J]. China Dairy, 2003, (04): 32-5.
- [17] MOUMING Z Y P Z Z. The Studies on Exopolysaccharides Produced by Lactic Acid Bacteria [J]. China Dairy Industry, 1999, (04): 7-10.
- [18] N. H. A. ADSA Foundation Scholar Award: possibilities and challenges of exopolysaccharide-producing lactic cultures in dairy foods [J]. J Dairy Sci, 2008: 1282-98.
- [19] MALIK A R M, KRALJ S, ET AL. Screening of lactic acid bacteria from Indonesia reveals glucansucrase and fructansucrase genes in two different *Weissella confusa* strains from soya [J]. FEMS Microbiol Lett, 2009: 131-8.
- [20] BADEL S B T, MICHAUD P. New perspectives for lactobacilli exopolysaccharides [J]. Biotechnol Adv, 2011: 54-66.
- [21] VAN HIJUM S A K S, OZIMEK L K, ET AL. Structure-function relationships of glucansucrase and fructansucrase enzymes from lactic acid bacteria [J]. Microbiol Mol Biol Rev, 2006: 157-76.
- [22] DE VUYST L D B. Heteropolysaccharides from lactic acid bacteria [J]. FEMS Microbiol Rev, 1999: 153-77.
- [23] DOCO T W J M, FOURNET B, ET AL. Structure of an exocellular polysaccharide produced by *Streptococcus thermophilus* [J]. Carbohydr Res, 1990: 313-21.
- [24] LOW D A J A, HORNE D, ET AL. Role of *Streptococcus thermophilus* MR-1C capsular exopolysaccharide in cheese moisture retention [J]. Appl Environ Microbiol, 1998: 2147-51.
- [25] KLEEREBEZEM M V K R. Exopolysaccharides produced by *Lactococcus lactis*: from genetic engineering to improved rheological properties? [J]. Antonie van Leeuwenhoek, 1999: 357-65.
- [26] SCHWAB C W J, TANNOCK G W, ET AL. Sucrose utilization and impact of sucrose on glycosyltransferase expression in *Lactobacillus reuteri* [J]. Syst Appl Microbiol, 2007: 433-43.
- [27] P. RUAS-MADIEDO C G D L R-G. Invited Review: Methods for the Screening, Isolation, and Characterization of Exopolysaccharides Produced by Lactic Acid Bacteria [J]. Journal of Dairy Science, 2005.
- [28] F GANCEL G N. Exopolysaccharide production by *Streptococcus salivarius* ssp. *thermophilus* cultures. 1. Conditions of production. [J]. Journal of Dairy Science, 1994.
- [29] CERNING R, THIBAUT, ET AL. Carbon source requirements for EPS Produced by *Lactobacillus* CGII and Partial structure analysis of the Polymer [J]. applied and enviroMicro 1994.
- [30] LIU CUIPING W Z, WANG YINYU, GUO BENHENG. Optimization of Culture Conditions for the Production of Exopolysaccharides by *Lactobacillus casei* LC2W Using Response-surface Methodology [J]. Food and Fermentation Industries, 2008, (12): 85-9.
- [31] ZHANG TIANQI Z X, KONG BAO-HUA, HUTTUNEN EINE, YANG ZHEN-NAI. Isolation and screening of exopolysaccharide-producing lactobacilli from northeast sauerkraut and their applications in yoghurt [J]. China Dairy Industry, 2009, (07): 8-12.
- [32] ZHANG X. Optimized Production and Application of Exopolysaccharides Produced by *Lactobacillus Pentosus* in Fermented Milks [D]; Beijing Forestry University, 2009.
- [33] YAXIN L J Y S C Y L Y L X S. Optimized Condition and Antioxidant Properties of Exopoly Saccharides by *Lactobacillus paracasei* VL8 [J]. Journal of Chinese Institute of Food Science and Technology, 2017: 82-9.
- [34] SUN JUN-DE L X. Screening for A Lactic Acid Bacteria with High EPS-producing Capacity and Optimization of Culture Conditions [J]. Journal of Shenyang Agricultural University, 2010, 41(01): 90-3.
- [35] QING-PING Z H H. Exploration the primary source of strain using in natural fermentation of sour dough [J]. Science and Technology of Food Industry, 2013, 34(17): 185-8.
- [36] REN-HE Z H Y X-L L. Effect of fermentation agent on the quality of dough and steamed bread [J]. Cereals & Oils, 2018, 31(04): 72-5.
- [37] HE X. Effects of Lactic Acid Bacteria Fermentation Types on the Flavor Characteristics of Sourdough Steamed Bread [D]; Jiangnan University, 2013.

- [38] SANAE OKADA M I, IWAO YOSHIDA. Identification and Characteristics of Lactic Acid Bacteria Isolated from Sour Dough Sponges [J]. Taylor & Francis, 2014: 572-5.
- [39] [THAN] E J WANDAM THAN S D BEY D A S B. Polysaccharide I—prokaryotic polysaccharides [M]. Beijing: Chemical Industry Press, 2004.
- [40] I W S. Structure-function relationships in microbial exopolysaccharides [J]. Biotech Adv, 1994, 12(2): 393-4.
- [41] WENSHUI L Q X. The Researching Aspect of Lactic Acid Bacteria Exopolysaccharide [J]. Food and Fermentation Industries, 2002, 29(05): 86-90.
- [42] XIUHONG W R Z T Z S Q B S L Z. Research Progress on the Application of Sourdough in Steamed Bread Fermentation [J]. Science and Technology of Food Industry, 2021, 42(15): 389-95.
- [43] CHANGHONG D Z L. The Effect of Different Steaming Conditions on the Quality of Steamed Bread [J]. Grain Processing, 2019, 44(02): 1-5.
- [44] BIAO A Z-L N W-J X X-L L Z P Z-L H Z-M S. Effects of Chinese traditional starters from different regions on fermentation characteristics of dough and quality of steamed bread [J]. Food and Fermentation Industries, 2018, 44(10): 71-8.
- [45] XING X. Microbial Communities in Chinese Traditional Fermented Dough from Henan Province and Compound Properties of Predominant Microorganism [D]; Henan Agricultural University, 2020.
- [46] ZHI-LU F H-P L R Z X-L A. Effects on fermentation characteristics of dough and quality of steamed bread of yeast on the market [J]. Cereals & Oils, 2016, 29(06): 9-13.
- [47] ZHIJIAN L C S X Z H W Y L. Biological Activity of Different Mixtures of the Old Dough and Yeast and Their Effects on Steamed Bread Quality [J]. The Food Industry, 2018, 39(10): 31-5.
- [48] BIAO A Z-L N W-J L Z W N H Z-M S. Effects of traditional starter culture from different regions of China on texture and sensory quality of steamed bread [J]. Effects of traditional starter culture from different regions of China on texture and sensory quality of steamed bread, 2018, 9(06): 1427-33.
- [49] BU-JIANG G W-Q C H Z Y-F Y Y N Q X Y-Y W. Effect of Compound Strains Fermentation on the Quality of Steamed Bread [J]. Food Research and Development, 2017, 38(05): 134-7.
- [50] DILEK BİRCAN C T G, KAMIL BOSTAN. Studies on Sour Dough Bread Making by Different Methods [J]. Aydın Gastronomy, 2017: 1-8.
- [51] LI ZHI-JIAN Y X-L, LIU CHANG-HONG, CHEN JUN-LI. Effects of lactic acid bacteria on sourdough fermentation of steamed bread [J]. Food Science and Technology, 2013, 38(03): 139-41.
- [52] ZHAO Z. Effects of Different Sourdoughs on the Quality of Potato Steamed Bread and the Preparation of Composite Starter [D]; Chinese Academy of Agricultural Sciences, 2019.
- [53] Sun X. Different yeast studies that affect the fermentation characteristics of dough and the quality of steamed buns [J]. Technological Pioneers, 2013, (19): 230.