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Article

# Finding and classifying a microfunctional bacterial strain in fermented sourdoughs

**Chuanfeng Li*, Yuanyuan Zhai, Gailing Wang**

School of Biological and Food Processing Engineering, Huanghuai University, Zhumadian 463000, China
*** Corresponding author:** Chuanfeng Li, lichuanfeng0921@126.com

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**Abstract:** The aim of this study was to examine the variety of Lactic acid bacteria and yeasts found in traditionally fermented sourdough, as well as their functional characteristics. Bengal red agar medium, Potato-based liquid media (PDA) medium, and DeMan, Rogosa and Sharpe (MRS) medium were used in that order to isolate, purify, and preserve *Saccharomyces* cerevisiae and *Lactobacillus* lactis. In order to analyze single strand breaks, the isolates underwent DNA extraction, Polymerase Chain Reaction (PCR) amplification, and sequencing techniques. The main lactic acid bacteria in the yeast dough were identified by the experimental results as *Enterococcus* faecalis, *Enterococcus* pseudomallei, *Enterococcus* faecalis, *Lactobacillus* plantarum, and *Lactobacillus* casei. Moreover, *Lactobacillus* plantarum and *Enterococcus* faecalis were found. Tests for resistance to bile salt, acid, and salt were also conducted. The current study offers a theoretical framework for additional investigation and real-world application of this strain, and it provides an initial understanding of the strain composition of *Saccharomyces* cerevisiae and *Lactobacillus* found in traditional fermented meals and doughs.

**Keywords:** traditional fermented sourdough; lactic acid bacteria; MRS medium; PCR amplification; molecular characterization

## 1. Introduction

Traditional fermented sourdough has a long history, and as early as the Zhou Dynasty, people consciously added yeast-containing wine mother to flour for fermentation. By the end of the Western Han Dynasty, its fermentation technology had matured [1,2]. In the thirteenth century, naturally fermented dough began to be used as a leavening agent for steamed buns, which are made by co-fermenting grains or other substrates with water and microorganisms such as yeasts and lactic acid bacteria in the environment, and can be used as a leavening agent as well as an acidifying agent and a flavor-carrying agent [3]. In addition, sourdough fermented with cornmeal is a traditional specialty food commonly eaten by the Manchu people in summer, and is mainly popular in the eastern Liaoning, southeastern Jilin, and Heilongjiang regions of northeastern China. This type of sourdough is made by mixing grains and water, and then fermented by yeast and lactic acid bacteria, with the goal of enhancing the dough's gluten structure, nutritional value, and textural qualities, and thus prolong the shelf life [4,5].

Up until now, the conventional method of fermenting dough has been employed. The use of conventional fermentation techniques has been somewhat restricted since the advent and widespread use of yeast in pasta products in the 1980s [6,7]. However, traditional sourdough significantly improves the flavour of steamed

bread when compared to industrial products made with a single strain of fermenting bacteria. This is because it produces a variety of flavour substances, such as alcohols, phenols, aldehydes, and esters, through the saccharification, fermentation, and esterification of the substrate under the action of various microorganisms [8]. As a result, the buns made with sourdough have a distinct flavour and improved texture that set them apart from commercial items that use quick active dry yeast as a leavening agent. Sourdough is still widely used by small workshops and many families to manufacture noodles, despite the maturity of industrial yeast production. Thus, it's critical to identify the optimum fermenting bacteria and research the microbial community structure of traditional sourdough [9,10].

The primary method used in traditional fermented doughs is continuous fermentation, which is defined by the microorganisms' ability to sustain high fermentation and metabolic capacity while being physiologically active throughout the dough's fermentation phase. The pH is kept at roughly 4.0 and the temperature is kept at room temperature, or roughly 25 °C, during the fermentation process. Traditional fermented sourdough is mostly leavened by old noodles and leavened seeds [11,12].

Made from maize flour, wheat flour, etc., leavened seeds are created by drying, crushing, and other procedures after being injected with bacterial strains (small or big kinds) during several fermentations. Leavened seeds are manufactured from a blend of different strains of bacteria, which gives the dough produced during fermentation a distinct flavour and a wide range of flavour components. A popular traditional technique for producing steamed bread is old noodle fermentation, sometimes referred to as noodle fertiliser fermentation or starting fermentation [13]. To make traditional fermented steamed buns, part of the fermented dough is set aside as a leavening agent, flour and water are added, and the dough ferments overnight. The next day, the proper amount of flour and additives are added, and the buns are shaped, woken, and steamed. The product of the ancient noodle fermentation has a distinct flavour and is much sought after. It also involves a blend of different microorganisms.

A multi-species complicated fermentation including yeast, lactic acid bacteria, and other microflora in the leavening agent system produces traditional fermented sourdough. Numerous microorganisms collaborate during fermentation to give the dough a distinct flavour and texture. Yeast gives dough its structure and volume by the creation of gas [14]. Sourdough gets its distinct aroma and texture from a variety of microorganisms that are involved in starch saccharification, proteolysis, cross-linking, esterification, and gas production. The metabolism also yields soluble sugars, volatile acids, hydrocarbons, alcohols, esters, and aldehydes. The metabolites' acids contribute to the dough's acidification while also enhancing its texture and quality. Traditional fermented dough products have a longer shelf life because to the exopoly saccharides (EPS) released by *Saccharomyces* cerevisiae, which also have an inhibitory impact on dangerous microbes and slow down the ageing process of the dough [15,16].

## 2. Materials and procedures

### 2.1. Test sample

Six regions of Henan Province, namely Zhengzhou, Zhumadian, Shangqiu, Luohe, Luoyang, and Langfang, provided a total of 24 finished samples of naturally fermented sourdough at varying fermentation times (12 h, 2 d, 4 d, and 6 d). 50 g of the same dough samples were then combined. Simultaneously, 50 g of dough samples were mixed at the same time, and the combined samples were assigned the numbers D1, D2, D3, and D4. The gathered samples were kept at 80 °C, and **Table 1** displays the samples' comprehensive data.

**Table 1.** Collection information of acid dough samples.

| Number | Sample number | Producer | Specification (g) |
|---|---|---|---|
| 1 | ZZ | Zhengzhou | 4 × 150 |
| 2 | ZMD | Zhumadian | 4 × 150 |
| 3 | SQ | Shangqiu | 4 × 150 |
| 4 | LH | Luohe | 4 × 150 |
| 5 | LY | Luoyang | 4 × 150 |
| 6 | LF | Langfang | 4 × 150 |

#### 2.1.1. Main reagents

Main reagents as shown in **Table 2**.

**Table 2.** Main reagents.

| Name | Manufacturer |
|---|---|
| DNA extraction kit | OMEGA corporation in the United States |
| Axy prepdna gel recovery kit | AXYGEN, USA |
| Fastpfu DNA Polymerase | Beijing Transgen Company |
| Agarose | Wabison Spain |
| Proteinase k | Beijing Dingguo Changsheng Co., Ltd |
| PCR primers and amplification system | Beijing Dingguo Changsheng Co., Ltd |
| Illumina Miseq platform | Illumina, USA |
| Hydrochloric acid | China National Pharmaceutical Group Chemical Reagent Co., Ltd. Announcement |
| Sodium chloride | China National Pharmaceutical Group Chemical Reagent Co., Ltd. Announcement |

#### 2.1.2. Media configuration

In the test, yeast was isolated using Bengal red agar medium, Potato-based liquid media (PDA) medium was used for culture and preservation, and DeMan, Rogosa and Sharpe (MRS) medium was used for the isolation, purification, and preservation of lactic acid bacteria. All of the reagents were obtained from Shenyang Dingguo Biotechnology Co. and were analytically pure:

MRS medium: two grammes of sodium citrate, two grammes of $K_2HPO_4$, 0.58

g of $MgSO_4$-$7H_2O$, 0.25 g of $MnSO_4$-$4H_2O$, 10 g of peptone, 8 g of beef paste, 4 g of yeast extract, 20 g of glucose, 5 g of sodium acetate $3H_2O$, 1 g of Tween 80, Distilled water was added as an addition, and agar was added in accordance with the requirements (0.75% for semi-solid medium, 2% for solid medium). Following the addition of 1000 mL of distilled water, the sample was autoclaved at 121 °C for 15 min.

Bengal red agar medium: readily accessible goods; add distilled water in accordance with directions; sterilise at 121 °C for 20 min. Potato-based liquid media (PDA): distilled water, 1000 mL, 20 g of dextrose, 20 g of agar, and 200 g of peeled potatoes. Dice the potatoes and put them in a beaker with some water. Boil for 30 min or until the potato is mushy. After that, strain the mixture through eight layers of gauze, add dextrose, and stir thoroughly. Let the mixture cool slightly to reclaim 1000 mL of distilled water. Sterilise the mixture for 20 min at 121 °C, remove, and store for later use. To make semi-solid culture medium, mix the aforementioned solution with 0.75% agar.

## 2.2. Testing instruments

**Table 3** displays specifics of the primary testing equipment utilised in this investigation.

**Table 3.** Main instrument and equipment list.

| Instrument name | Manufacturer |
|---|---|
| Biochemical incubator SHP-250 | Jinghong Experimental Equipment Co., Ltd. in Shanghai |
| Clean bench | Antai Air Technology Co., Ltd. Suzhou |
| Vertical pressure of LDZX Pot for steam sterilisation | Shanghai Shen'an Medical Device Factory |
| Electronic balance | Shanghai Yueping Scientific Instrument Co., Ltd |
| Thermostat water bath | Jiangsu Jintan Ronghua Instrument Manufacturing Co., Ltd |
| VS-1 Vortex Instrument | Beijing Dinghao Raw Material Technology Co., Ltd |
| PCR instrument | Applied Biosystems |
| DGW-12 electrophoresis instrument | Beijing 61 Biological Instrument Co., Ltd |
| UVP GDS-8000 gel imager | Shanghai Precision Science Co., Ltd |
| UV-4802 UV spectrophotometer | Shanghai Lichi Measuring Instrument Co., Ltd |
| High speed desktop freezer centrifuge | Eppendorf Company |
| Ultra trace spectrophotometer | Therno Fisher Scientific |
| MISEQ sequencer | Illumina, USA |

## 2.3. Test method

### 2.3.1. Extracting and amplifying DNA

Following the directions provided by the manufacturer when using a DNA extraction kit, microbial DNA was isolated from sourdough samples [17]. The PCR reaction was conducted in accordance with the following programme: Phase II involves cycling 29 times, Phase I involves preheating at 95 °C for 3 min; Phase III:

storage at 10 °C after a 10-minute extension at 72 °C. For every sample, three replicate experiments were carried out. The PCR products of the same samples were found using 2% agarose gel electrophoresis for the amplification results. The PCR amplification products were recovered by cutting the gel using the DNA gel recovery kit, eluting it with Tris-HCl, and detecting it using 2% agarose electrophoresis.

### 2.3.2. Illumina MiSeq sequencing

On the Illumina MiSeq platform, double-end sequencing (2 × 300) was carried out in accordance with Majorbio Bio-Pharm Technology Co. Ltd.'s standard operating procedure [18]. The basic idea of operation is as follows: PCR amplification creates DNA clusters, which are then linearized to form a single strand. After that, one end of the DNA fragment is fixed on the chip by complementary to the primer base, while the other end is fixed to form a structure resembling a "bridge" by randomly complementary to the primer nearby. Only one base is synthesised per cycle using DNA polymerase and dNTP with four fluorescent markers; a laser scans the reaction plate's surface to determine the type of nucleotide added in the first round of reaction for each template sequence; chemical cleavage removes the "fluorescent group" and "termination group" and restores the 3s. To restore the stickiness of the 3s, remove the "termination group" and "fluorescent group" by chemical cleavage and proceed with the synthesis of the second nucleotide; After tallying the fluorescent signals gathered in every iteration, examine the sequence of the designated section of the template DNA.

### 2.3.3. Sequencing of pairs of data

Data were inspected for operational taxonomic units (OTUs) with 97% similarity using UPARSE (version 7.1, http://drive5.com/uparse/), and chimeric sequences were found and removed using UCHIME. To classify species, taxonomic classifications were applied to all OTUs using the Ribosomal Database Programme (RDP) classifier, with a confidence level of 0.8 [19,20]. The microbiological diversity of the samples was evaluated using alpha diversity [21]. To examine the differences between each sample, beta diversity analysis using unifrac was carried out [22]. PICRUSt predicted the function of bacterial communities.

### 2.3.4. Initial lactic acid bacteria isolation and purification from sourdoughs

Two or three suitable gradient dilutions were chosen from the collected sourdough samples, and two parallel samples were set up for each gradient. The samples were then inoculated into MRS solid medium containing 2% calcium carbonate and cultured partially anaerobically for 24-36 h at 20 °C and 37 °C, respectively. Following colony formation, the distinctive *Lactobacillus* colonies containing calcium rings were selected, inoculated into MRS liquid medium for a duration of 24 h, and subsequently purified using MRS solid medium for approximately three times to yield single colonies, thereby obtaining the putative *Lactobacillus* strain.

### 2.3.5. Initial finding of microorganisms that produce lactic acid

The Manual of Systematic Identification of Common Bacteria and *Lactobacilli* Bacterial Classification Identification and Experimental Methods were used in order to conduct a preliminary physiological and biochemical identification of the isolated

*Lactobacilli*.

#### 2.3.6. Preservation of lactic acid bacteria during freeze-drying

The first-identified pure lactic acid bacteria were seeded in MRS liquid medium, cultivated for 24 h at a steady temperature of 37 °C, pierced, and then stored at 4 °C in a refrigerator. After being punctured and preserved, the strains were reactivated for two to three generations. The tubes were then washed with sterile saline after being centrifuged for five minutes at 4000 rpm and 4 °C. The supernatant was then poured off. This process was repeated several times. Next, 1 mL of 15% skim milk was added, mixed with a pipette gun, and injected into three to four 1.5 mL freezing tubes. The tubes were then stored at 80 °C in an ultra-low temperature freezer. A vacuum freeze-dryer was used to complete the freeze-drying process, and the finished product was kept in an ultra-low temperature refrigerator at −80 °C [23].

#### 2.3.7. Isolation and purification of yeast

The sourdough samples that were collected underwent a 10-fold gradient dilution procedure to dilute them with saline. After selecting two to three optimal gradient dilutions, two parallel samples were set up for each gradient, inoculated onto Bengal red agar medium, and incubated for 48 h at 28 °C. A microscope was used to examine the cell morphology of the single colonies that were obtained after the colonies with smooth, wet, sticky surfaces, uniform textures, and ease of picking up were chosen and placed in PDA liquid medium for 48 h. The single colonies were then purified using PDA solid medium two to three times.

#### 2.3.8. Preservation of *Saccharomyces* cerevisiae with freeze-drying

After being grown for 24 h at a consistent temperature of 37 °C, the pure strain of *Saccharomyces* cerevisiae that was believed to have been taken from the initial isolation, which was punctured and stored at 4 °C in a refrigerator. After reactivating for two to three generations, for ten minutes, the puncture-preserved strain was centrifuged at 4000 rpm and 4 °C. This process was repeated multiple times. Next, 1 mL of 15% skim milk was added, mixed with a pipette gun, and injected into three to four 1.5 mL freezing tubes. Finally, the tubes were preserved at minus 80 °C in an ultralow-temperature refrigerator. Finally, it was vacuum freeze-dried to lyophilize it, then kept at −80 °C in an ultra-low temperature refrigerator.

### 2.4. Strain identification

#### 2.4.1. DNA extraction and detection

To determine the quantity and quality of the extracted DNA from the isolated strains, a small UV spectrophotometer was utilised, which was extracted using a kit procedure. As long as the observed DNA concentration is more than 50 ng/μL, the PCR amplification system's criterion for DNA concentration of 50 ng/μL is satisfied. The OD260/OD280 ratio, which is typically between 1.6 and 1.8 and indicates the best purity, was used to assess the DNA's purity. 1.8 is the optimal purity value. The protein content is excessive if the OD260/OD280 value is less than 1.6; the RNA content is excessive if the value is more than 1.8.

#### 2.4.2. PCR amplification

Universal primers were used to amplify the *Lactobacillus* DNA that had been

extracted. The forward primer was designated as 27f, which stands for 5′-AGAGTTTGATCCTGGCTCAG-3′, and the reverse primer was designated as 1495r, which stands for 5′-CTACGGCTACCTTGTTACGA-3′. *Saccharomyces* cerevisiae was amplified using the forward primer NL-1: 5′-GCATATCAATAAGCGGAGGAAAAG-3′ and the reverse primer NL-4: 5′-GGTCCGTTTCAAGACGG-3′. The sample of DNA was used as a template.

The amplification system had a volume of 25 μL, and the following ingredients were used: 0.5 μL of forward and reverse primers (25 pmol/μL), 1 μL (50 ng/μL) of genomic DNA template, 2.5 μL of 10 × PCR buffer, 2 μL of dNTP MIX, 0.2 μL of TaKaRa ExTaq enzyme, and 18.3 μL of dd$H_2O$ were used. **Table 4** lists the conditions for the PCR process.

**Table 4.** PCR reaction conditions.

| Serial number | Step | Temperature | Time |
|---|---|---|---|
| 1 | Pre denaturation | 94 °C | 5 min |
| 2 | Denaturation | 93 °C | 1 min |
| 3 | Anneal | 93 °C | 1 min |
| 4 | Extend | 75 °C | 1 min 20 s |
| 5 | Circulate | | 35 times |
| 6 | Extend | 70 °C | 10 min |
| 7 | Heat preservation | 2 °C | |

### 2.4.3. Strain identification

Initially, a 1.0% agarose gel was set up. Following the PCR reaction, 1 μL of 6 × sampling buffer was combined with 5 μL of the reaction product, resulting in an even spike of the sample. The electrophoresis settings included a voltage of 5 V/cm, a 0.5 × TAE electrophoresis solution, and an approximate 30-minute electrophoresis period. Following the completion of the electrophoresis, the sample was imaged under UV light and stained with Ethidium Bromide (EB) for approximately 30 min. Upon the appearance of distinct, brilliant bands at 1500 bp for *Lactobacillus* and 600 bp for *Saccharomyces* cerevisiae, it was deemed that the amplification was prosperous and could proceed with sequencing. Shanghai Sunny Biotechnology Co. carried out the sequencing.

### 2.4.4. Homology comparison analysis

To complete the molecular identification of the strains, the sequencing results from the isolation were compared using BLAST to the sequences of standard strains in GenBank and other databases for homology. This allowed us to determine which standard strains had the highest homology to the measured gene sequences.

### 2.4.5. Examining yeasts and *Lactobacilli* for probiotic and functional properties

After first activating thirty-nine strains of lactic acid bacteria, the bacteria were injected at a 2% (v/v) concentration into MRS liquid medium with pH. The inoculation was then left for twenty-four hours at 37 °C. First, the acid-tolerant lactic acid bacteria were screened, and the bacterial growth was gauged by measuring the Optical Density (OD) value with a UV spectrophotometer. The acid-tolerant lactic

acid bacteria that had been first screened were then further injected into MRS liquid medium at pH 1.5, 2.5, and 3.0 (5% inoculum, respectively), and left it to incubate at 37 °C for three hours. The survival rate was calculated and the quantity of viable bacteria was quantified using the decantation method.

#### 2.4.6. Yeast acid resistance test

After being cultivated for 24 h at 28 °C in a constant temperature incubator, the activated yeasts were inoculated into PDA liquid medium with pH. Their OD values were then determined to first screen out the acid-resistant yeasts. The amount of live bacteria was ascertained using a hemocyte counting plate after the initially screened acid-resistant yeasts were inoculated into PDA liquid medium was incubated for 24 hours at 28 °C with pH values of 1.5, 2.5, and 3.0, respectively.

#### 2.4.7. *Lactobacillus* salt tolerance test

First, thirty-nine strains of lactic acid bacteria were activated. Then, the bacteria in the logarithmic growth stage were injected into MRS liquid medium at concentrations of 4%, 6%, 8%, and 10% of sodium chloride, respectively, and incubated for twenty-four hours at 37 °C. The UV spectrophotometer was used to carry out the inoculation. The OD at 600 nm in the blank control group—which included a liquid medium free of any strain inoculation—was measured using a UV spectrophotometer. The salt-tolerant lactic acid bacteria that had been initially screened were then injected into MRS liquid medium with 10%, 15%, and 20% salt concentration at 5% inoculum, respectively, and left it to incubate at 37 °C for three hours. Next, the survival rate was computed using the decantation method to ascertain the quantity of viable bacteria.

### 2.5. Yeast salt tolerance test

After being inoculated into PDA liquid medium with sodium chloride concentrations of 4%, 6%, 8%, and 10%, and being cultured for 24 h at 28 °C, the activated yeasts in the logarithmic growth phase were first screened out of the salt-tolerant yeasts using their OD values. After the salt-tolerant yeasts were first screened, they were inoculated into PDA liquid medium containing 10%, 15%, and 20% salt concentrations. After a 24-hour incubation period at 28 °C, the mixture was counted for live bacteria using a hemocyte counting plate.

### 2.6. *Lactobacillus* bile salt resistance test

Following adequate mixing and a 24-hour incubation period at 37 °C constant temperature, the samples were taken. Two generations of the frozen lactic acid bacteria were activated by inoculating them into MRS liquid media, Moreover, a 2% inoculum of the second generation of bacterial suspension was added to PDA liquid medium at bile salt concentrations of 0.5%, 0.1%, 0.15%, and 0.2%. Using a UV spectrophotometer, the OD value at 600 nm was measured in the blank control group, which consisted of the liquid medium devoid of inoculation microorganisms. After a preliminary screening, 0.2%, 0.25%, and 0.3% of bovine bile salt were used to inject 5% of the lactic acid bacteria resistant to bile salt into the MRS liquid medium. After that, the cultures were incubated at 37 °C for 4 h. Following the decantation method's determination of the number of viable bacteria, the viability

rate was calculated.

### 2.7. Yeast bile salt resistance test

In order to initially screen out the bile salt-resistant yeasts, the activated yeasts with logarithmic growth period were seeded into PDA liquid medium with 0.5%, 0.1%, 0.2%, and 0.15% of bile salt, in that order. The yeasts were then grown at 28 °C for 24 h, and their OD values were determined. The first-screened bile salt-resistant yeasts were seeded into PDA liquid medium containing 0.2%, 0.25%, and 0.3% of salt, respectively. The cultures were then allowed to grow for 24 h at 28 °C, after which the quantity of live bacteria was counted using a hemocyte counting plate.

## 3. Isolation and characterisation of lactic acid bacteria in sourdough

### 3.1. Isolation of lactic acid bacteria from sourdough

Based on the results of high-throughput sequencing, lactic acid bacteria were recovered from a total of 18 samples of sourdough from the last three fermentation periods by using the selective impact of MRS medium. Initially, 42 putative lactic acid bacteria were identified according to their morphological traits, including colour, transparency, and colony shape.

Based on their morphological traits, including colony structure, colour, and transparency, First found were 42 strains of potential lactic acid bacteria. Tests including the Gramme stain test and other physiological and biochemical procedures were performed on 42 strains of probable lactic acid bacteria, the catalase test, and the growth test. Of these, 39 strains were confirmed to be lactic acid bacteria due to positive Gramme stain reactions and negative catalase tests. **Figure 1** displays the Gramme staining results of a few *Lactobacillus* strains, and **Table 5** displays the outcomes of physiological and biochemical tests.

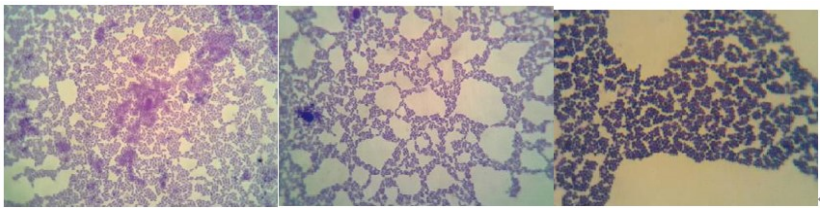

**Figure 1.** Gram staining images of some lactic acid bacteria strains.

**Table 5.** Physiological and biochemical test results of suspected strains of lactic acid bacteria.

| Bacterial strain | 37 °C Growth Test | Growth test with a pH value of 3 | Growth test with a pH value of 5 | Growth test with a pH value of 7 | Catalase test | Gram staining test |
|---|---|---|---|---|---|---|
| BX2-1 | + | + | + | + | − | + |
| BX2-2 | + | − | + | + | − | + |
| BX2-3 | + | + | + | + | − | + |
| BX3-1 | + | + | + | + | − | + |
| BX3-2 | + | + | + | + | − | + |

**Table 5.** (*Continued*).

| Bacterial strain | 37 °C Growth Test | Growth test with a pH value of 3 | Growth test with a pH value of 5 | Growth test with a pH value of 7 | Catalase test | Gram staining test |
|---|---|---|---|---|---|---|
| BX4-1 | + | − | + | + | − | + |
| BX4-2 | + | + | + | + | − | + |
| AS2-1 | + | + | + | + | − | + |
| AS2-2 | + | + | + | + | − | + |
| AS3-1 | + | − | + | + | − | + |
| AS3-2 | + | + | + | + | − | + |
| TL2-1 | + | + | + | + | − | + |
| TL2-2 | + | + | + | + | − | + |
| TL3-1 | + | + | + | + | − | + |
| TL3-2 | + | + | + | + | − | + |
| HR3-2 | + | + | + | + | − | + |
| HR3-3 | + | + | + | + | − | + |
| SY2-1 | + | + | + | + | − | + |
| SY2-2 | + | − | + | + | − | + |
| SY2-3 | + | + | + | + | − | + |
| DD2-1 | + | + | + | + | − | + |
| DD2-2 | + | − | + | + | − | + |

### 3.2. Genomic DNA extraction from lactic acid bacteria

A kit was used to extract the genomic DNA of *Lactobacillus* species, and all of the isolated species' genomic DNA was also extracted. Was chosen so that the micro-ultraviolet spectrophotometer could determine its concentration and purity. The findings demonstrated that all 39 *Lactobacillus* strains had DNA concentrations more than 50 ng/μL, meeting the specifications of the *Lactobacillus* PCR amplification technique. Furthermore, all *Lactobacillus* DNAs had OD260/OD280 values about between 1.6 and 1.8, which meant that the following experimental study could proceed.

### 3.3. PCR products amplification

*Lactobacillus* DNA that had been successfully extracted was utilised as a template for PCR amplification, and the PCR findings were examined using 1.0% agarose gel electrophoresis. According to the results, the PCR was successful and the 39 Lactobacillus strains' PCR products had distinct brilliant bands at 1500 bp, indicating that they could be sent off for sequencing. **Figure 2** displays the PCR results of a few typical strains of *Lactobacillus*.

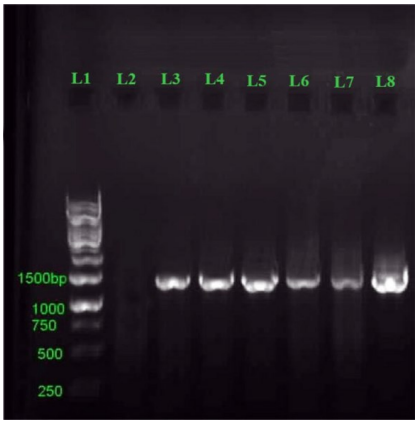


**Figure 2.** Partial electrophoretic diagram of lactic acid bacteria PCR product detection.

### 3.4. *Lactobacillus* species' 16S r DNA sequence homology analysis

The homology between the genomes of the sequenced *Lactobacillus* spp. and the standard strains in the National Center for Biotechnology Information (NCBI) Data GenBank in the United States of America was examined. Refer to **Table 6** for specific results.

**Table 6.** Comparison of homology of 16s RNA sequences of *Lactic Acid Bacteria*.

| Strain number | Appraisal results | Homology | Serial number | Strain number | Appraisal results | Homology | Serial number |
|---|---|---|---|---|---|---|---|
| BX2-1 | L. plantarum | 98% | GU290217.1 | | L. plantarum | 98% | FJ844944.1 |
| BX2-2 | L. plantarum | 97% | KP345891.1 | | L. plantarum | 98% | KJ |
| BX2-1-3 | L. plantarum | 75% | NR_104573.1 | | E. faecalis | 100% | KP137385.1 |
| AS2-1 | L. plantarum | 96% | KC416993.1 | SY2-1 | L. plantarum | 98% | KF673529.1 |
| AS2-2 | L. plantarum | 96% | KC422325.1 | SY2-2 | L. plantarum | 92% | NR_104573.1 |
| TL2-1 | L. plantarum | 98% | NR_115605.1 | SY3-3 | L. casei | 99% | KP165839.1 |
| TL2-2 | E. faecalis | 99% | JF903802.1 | SY4-1 | E. faecium | 97% | NR_114742.1 |

According to **Table 6**, a 16S rDNA sequence homology analysis was used to identify 39 lactic acid bacteria that were recovered from 8 sourdough samples as *Lactobacillus*. Of these, 32 strains of *Lactobacillus* showed more than 98% identity with the standard strains, 25 strains showed more than 99% homology, and 6 strains showed 100% homology with the standard strains. Specify the ingredients and their concentrations (e.g., peptone, beef extract, glucose, and agar). we Provide temperature (typically 37 °C), pH (around 6.5–7.0), and incubation time (e.g., 24–48 h).

### 3.5. Lactic acid bacteria population structure in sourdough

To gain a basic understanding of the lactic acid bacteria's community structure and makeup in sourdough, **Table 6**'s molecularly identified lactic acid bacteria were categorised. Refer to **Table 7** for specific results.

**Table 7.** Population structure of lactic acid bacteria in sour dough.

| Strain name | Quantity/plant |
|---|---|
| L. plantarum | 16 |
| L. casei | 5 |
| E. faecalis | 14 |
| E. pseudoavium | 2 |
| E. faecalis | 2 |
| Total | 39 |

Based on the information presented in **Tables 3–5**, two genera and five species may be distinguished among the 39 lactic acid bacteria strains. Of these, 16 strains of *Enterococcus* faecium accounted for 41.03% of all isolated lactobacilli; 1 strain of *Enterococcus* pseudoavium accounted for 3.00% of all strains; 1 strain of *Enterococcus* faecalis accounted for 3.00%; 18 strains of *Lactobacillus* plantarum (*Lactobacillus* plantarum) accounted for 46.15% of all strains; and 3 strains of *Lactobacillus* casei (*Lactobacillus* casei) accounted for 7.69% of all strains. This leads to the preliminary conclusion that *Enterococcus* spp. and Lactobacillus spp. made up the majority of the lactobacilli community composition in this collection of traditionally fermented sourdough. One of these, *Enterococcus* spp., was found in significant amounts in the home-made laboratory samples, suggesting that this species is the primary functional group of bacteria involved in the sourdough's natural fermentation process. *Enterococcus* faecalis and *Lactobacillus* plantarum were the two strains that were most prevalent in terms of isolates. These two bacteria are thought to be the predominant ones in traditionally fermented sourdough and may be essential to the fermentation process. To substantiate the descriptions of traditional fermented sourdough and its microbial communities with specific experimental data and research findings, perform DNA extraction from sourdough samples followed by sequencing of the 16S rRNA gene to identify bacterial species present. For fungal species identification, use Internal Transcribed Spacer (ITS) sequencing of the fungal community. Monitor pH changes, gas production ($CO_2$), and organic acid profiles during fermentation to correlate with microbial activities. Include control experiments where sourdough is prepared under sterile conditions or with specific microbial inhibitors to validate microbial contributions.

## 4. Related work

The production process of fermented noodles varies in different regions. For example, the existing methods of making yeast in different regions of China are as follows: in the southern region, Daqu is used as the bacterial strain, cornmeal is used as the raw material, and it is fermented by adding flour and keeping warm multiple times; In northern regions, wheat koji, yellow wine, and aged yeast are mainly used as bacterial strains, mixed with wheat flour and water to form uniform flour flocs, and then fermented at high temperature; Taking the commercialized Nanyang yeast as the research object, this study analyzed the changes in physical and chemical indicators such as moisture, value, and enzymes during the production process of yeast. It was found that the internal moisture content of yeast during the fermentation

stage showed an upward trend, while the value showed a downward trend. The starch fermentation and protein fermentation activity showed an upward trend. Studied the changes in microbial count during the production process of yeast; The total number of yeast cells is generally on the rise, while the trend of mold changes is irregular, providing theoretical reference for the research of yeast production technology [13]. Selecting different temperature levels to store yeast, the fermentation power and texture and sensory of Mantou were evaluated. It was found that the low-temperature conditions were more conducive to the storage of yeast. At the same time, they also studied the fermentation power of yeast packaged in vacuum and self-sealing bags, and the texture and sensory differences of Mantou. Hadef et al. [14] suggested that yeast products should be industrialized and vacuum packaging should be used to better preserve the quality of yeast, laying a theoretical foundation for the traditional industrial production of yeast [15]. Attention has been paid to the research, but it mainly focuses on the yeast preparation process and the quality of Mantou. Systematic research on the micro ecosystem in yeast has not been reported yet. As Brahimi et al. [16] studied the growth and metabolic characteristics of microorganisms in yeast complex lactic acid bacteria or single fermentation processes of two types of bacteria, it was found that different strains have different utilization of raw materials, and the changes in acid and soluble sugar content during their metabolic processes are also different. Banik et al. [17] take traditional yeast from Henan region as the research object, the influence of Kongju bacteria on the quality of traditional yeast was studied using the fermentation and acetylation abilities of the strains as indicators. Pourjafar et al. [18] found that the addition concentration gradient was lactic acid bacteria. In the process of fermentation of Mantou with active dry yeast, Angel's yeast was added to study the effect of rhizopus on the sensory quality of Mantou. It was found that Mantou fermented with yeast and rhizopus had the highest score, while Mantou fermented with yeast and lactic acid bacteria had the lowest score. It can be seen that Rhizopus has a certain impact on the quality of Mantou in China's traditional yeast [24].

## 5. Discussion

In this study, we conducted a series of carefully designed experiments aimed at revealing the mechanisms behind the microbial community classification phenomenon in fermentation surfaces. The first step of the experiment is to select suitable samples and use the microbial community classification method in the fermentation surface for pretreatment. The preprocessed samples are analyzed using microbial community classification techniques in the fermentation surface to ensure the accuracy and reliability of the data. Subsequently, we used the microbial community classification method in the fermentation surface for data collection and processing in order to obtain detailed experimental results [25,26].

Through experiments, we have discovered several important characteristics of microbial community classification in fermentation surfaces. Firstly, under the classification conditions of microbial communities in the fermentation surface, the sample exhibited significant characteristics of microbial community classification in the fermentation surface. This result is consistent with previous studies, but differs in

specific parameters. Through comparison, we speculate that this difference may be due to differences in sample selection and minor variations in experimental conditions [27,28].

In addition, the experiment also revealed that under the classification conditions of microbial communities in the fermentation surface, the intensity of microbial community classification phenomenon in the fermentation surface significantly increased. This indicates that the classification factors of microbial communities in fermentation surfaces play a key role in influencing the phenomenon of microbial community classification in fermentation surfaces. Specifically, when the classification parameters of microbial communities in the fermentation surface change, the microbial community classification in the fermentation surface of the sample shows a linear growth trend. This discovery provides a new perspective for understanding the classification mechanism of microbial communities in fermentation surfaces and points the way for future research [29,30].

Our research results were compared in detail with existing literature (e.g., Huang et al. [12], Pourjafar et al. [15], Banik et al. [17]). Although the overall trend is consistent, there are some differences in specific details. Huang et al. [12] reported that under the classification conditions of microbial communities in the fermentation surface, the classification phenomenon of microbial communities in the fermentation surface showed nonlinear changes, while our experiment showed a linear relationship. Possible reasons include differences in experimental equipment, variations in data processing methods, and the influence of environmental factors.

Through further analysis, we believe that the classification parameters of microbial communities in the fermentation surface during the experiment are the key factors leading to differences in results. We used the microbial community classification technique in the fermentation surface, while Pourjafar et al. [15] used the microbial community classification method in the fermentation surface. Although the two are similar in principle, there are significant differences in sensitivity and accuracy. Therefore, future research can further validate and expand our findings by unifying experimental conditions and methods [27–29].

This study not only enriches the understanding of microbial community classification phenomena in fermentation surfaces in theory, but also has important practical application value. Firstly, the classification of microbial communities in fermentation surfaces can be applied to the field of microbial community classification in fermentation surfaces, such as optimizing performance in microbial community classification techniques in fermentation surfaces. In addition, the methods and results of this study provide reference for other studies. For example, in the field of microbial community classification in fermentation surfaces, our method can be applied to the study of microbial community classification in fermentation surfaces to improve the accuracy and reliability of research [30].

Although this study has achieved significant results, there are still some limitations. Firstly, a small sample size may affect the generalizability of the results. Secondly, the experimental conditions are relatively ideal, and in practical applications, there may be more complex factors to consider. Therefore, future research should expand the sample size and repeat experiments under more diverse conditions to validate our results [19].

Future research should further explore more details about the classification mechanism of microbial communities in fermented noodles. For example, further research on the influence of microbial community classification parameters in fermentation surfaces on microbial community classification phenomena in fermentation surfaces, and how different environmental factors regulate microbial community classification phenomena in fermentation surfaces. This will help to comprehensively understand the classification mechanism of microbial communities in fermentation surfaces and provide a more scientific basis for practical applications.

This study revealed several new characteristics of microbial community classification in fermentation surfaces through systematic experiments and detailed analysis. These findings not only provide new perspectives for theoretical research, but also lay the foundation for practical applications. Despite some limitations, our research provides valuable references and guidance for future in-depth research and practical applications.

## 6. Conclusion

By carefully isolating, describing, and assessing the functional traits of yeasts and lactic acid bacteria in traditional fermented sourdough, a number of strains with significant application value were obtained in this work. The particular findings are as follows:

1) Strain isolation and identification

From traditionally fermented sourdough, 39 strains of lactic acid bacteria were recovered; these strains mostly belonged to two genera and five species, *Enterococcus* and *Lactobacillus* faecium. With 41.03% and 46.15% of all identified *Lactobacillus* strains, respectively, *Enterococcus* faecium and *Lactobacillus* plantarum were the predominant strains. The presence of a diverse yeast population in the sourdough was also revealed by the isolation and characterization of *Saccharomyces* cerevisiae.

2) Functional features filtering

Strong tolerance to acid, salt, and bile salts was demonstrated by *Lactobacillus* and *Saccharomyces* cerevisiae. Among them, *Lactobacillus* plantarum and *Enterococcus* faecium demonstrated a good acid tolerance by being able to maintain high activity in a low pH environment.

According to the salt tolerance test, several strains of *Lactobacillus* and *Saccharomyces* cerevisiae were still able to grow well in an environment with high salt concentrations. Notably, *Lactobacillus* plantarum and certain strains of Saccharomyces cerevisiae had a high survival rate at a concentration of 10% NaCl. Certain *Lactobacil*lus and *Saccharomyces* cerevisiae had higher survival rates in media containing varying concentrations of bile salts during the bile salt tolerance test, suggesting that they possess superior bile salt tolerance traits.

3) Checking for strains that are functionally significant

The present investigation involved the screening of strains of *Lactobacillus* and *Saccharomyces* cerevisiae that exhibited notable resistance against acid, salt, and bile salt. These strains hold promise for a multitude of future uses, particularly in the

domains of food fermentation and probiotic product development.

Since *Lactobacillus* plantarum and *Enterococcus* faecalis are the predominant strains in traditional fermented sourdough, it is important to continue studying and using these two bacteria since they may be important players in the fermentation process.

Propose future research directions focusing on leveraging advanced technologies to overcome current limitations and further enhance the scientific basis and application of traditional fermentation techniques. Emphasize the potential of these integrated approaches to not only preserve but also innovate upon traditional food processing practices in a sustainable and scientifically rigorous manner.

In summary, this study added to our knowledge of the microbial variety found in traditional fermented sourdough while also offering a technological and scientific foundation for its use in probiotic goods and food fermentation. Further research on these useful strains will contribute to the development of high-value probiotic products and other fermented meals in the future.

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