Scifood
vol. 20, 2026, p. 244-264
https://doi.org/10.5219/scifood.90
ISSN: 2989-4034 online
https://scifood.eu

Received: 15.1.2026
Revised: 23.3.2026
Accepted: 31.3.2026
Published: 2.4.2026

Screening and identification of lactic acid bacteria and yeast strains from raw materials with potential for application in sourdough technology

Lazat Umiraliyeva, Elvira Ismailova, Ivan Filatov, Asan Ospanov

ABSTRACT

This study focuses on the screening and identification of lactic acid bacteria (LAB) and yeast strains isolated from Kazakh raw materials, specifically wheat flour and kumis, to evaluate their potential for sourdough technology. The research was designed as a controlled experiment assessing the biotechnological properties of eight specific microbial isolates. These experimental units were allocated to standardized treatments for genetic identification via 16S rRNA and ITS sequencing, morphological characterization, and functional screening for acid production and antagonistic activity. The results identified the most active isolates as *Lacticaseibacillus paracasei* (strain B-319), *Lacticaseibacillus rhamnosus* (B-449), and *Lacticaseibacillus paracasei* (B-27). Morphological analysis confirmed the presence of stable, genus-specific traits, including cell morphology and the absence of sporulation. In the acidity determination assays, strain B-319 exhibited the highest acidifying capacity, reducing the medium pH to 4.55. The antagonistic screening against key bread spoilage agents—*Bacillus subtilis*, *Bacillus mesentericus*, and *Penicillium notatum*—revealed that strains B-449 and B-27 possess superior inhibitory potential. The findings demonstrate that these indigenous strains provide a robust biological barrier against microbial spoilage. These isolates represent high-value candidates for the development of specialized starter cultures to improve the safety, shelf life, and quality of bakery products.

Keywords: lactic acid bacteria, yeast, antagonism, microbial morphology, 16S rRNA, ITS-region

INTRODUCTION

The history of sourdough bread dates back to ancient civilizations, where it was made by fermenting naturally occurring wild yeasts and bacteria in flour and water. This natural fermentation process gives sourdough its distinctive tangy flavor and chewy texture. In contrast, traditional yeast-based bread is made using commercial yeast, which ferments the dough much faster but does not provide the same health benefits **[1]**.

In recent decades, there has been a resurgence of interest in natural breadmaking and sourdough. People are increasingly returning to traditional methods, shifting from commercial yeast to sourdough fermentation and cultivating their own sourdough starters. This shift is driven not only by a desire for a flavorful, wholesome product but also by a growing awareness of the importance of natural ingredients **[2]**.

A characteristic feature of most bakery products is the presence of a well-aerated crumb structure (in fact, the physical-chemical indicator “crumb porosity” is included in normative and technical documentation for bread, buns, and pastries). The leavening effect in dough refers to the saturation of a uniform, elastic dough mass with gas bubbles, resulting in a well-developed, spongy gluten framework. The resulting crumb structure is achieved through chemical, mechanical, or biological treatment of the dough. Chemical leavening is often used in the production of flour-based confectionery. However, some bread recipes also use chemical leaveners—for example, traditional Irish and Scottish soda bread, as well as grain- and gluten-free breads **[3]**, **[4]**, and **[5]**.

To implement mechanical leavening in industrial production, systems are being developed that aerate dough using compressed air **[6]**. The resulting dough is a foam-like mass with stable physical and chemical properties, and the final bread exhibits a well-developed, elastic crumb structure **[7]**, and **[8]**. However, to achieve the flavor and aroma profiles characteristic of traditional bread products, additional ingredients are often required into the recipe, such as organic acids, fruit juices, whey, or pre-ferments containing lactic acid bacteria and their metabolites **[6]**, and **[9]**.

The most common method of dough leavening in bread production is the biological method, which involves the use of special ingredients (baker's yeast and brewer's yeast *Saccharomyces cerevisiae*) or pre-ferments (sourdoughs and liquid yeasts) that serve as sources of technological microbiota (yeast cells and lactic acid bacteria) and are prepared on-site at the bakery **[10]**, **[11]**, **[12]**, and **[13]**. The leavening effect is achieved by the carbon dioxide produced during the metabolic activity of microorganisms (yeasts and heterofermentative lactic acid bacteria). In addition to the characteristic crumb texture, porosity structure, and loaf volume, products made with biological leavening also exhibit unique and complex flavors and aromas **[10]**, **[14]**, **[15]**, **[16]**, and **[17]**.

In industrial baking, commercial baker's yeast (dried, instant, and compressed forms, as well as yeast milk) is widely used. These products are made from various strains of *S. cerevisiae*, as well as liquid yeasts and sourdoughs produced using pure microbial cultures—yeasts and lactic acid bacteria **[10]**, **[11]**, **[18]**, **[19]**. The combination of yeast cells and lactic acid bacteria in sourdoughs enables the production of baked goods with a broad range of flavor and aroma compounds, which are highly valued by consumers **[15]**, **[16]**, **[20]**, and **[21]**.

Other key advantages of sourdough-based bakery products include extended shelf life (due to increased acidity and microbial stability, as well as slower staling); the ability to eliminate excessive crumbliness of the crumb by improving its elasticity; reduced glycemic index; enhanced bioavailability of mineral nutrients; and lower gluten content **[22]**, **[23]**, **[24]**, **[25]**, and **[26]**.

Depending on the source of technological microbiota, all sourdoughs can be divided into two major groups. The first group is initiated using preparations of pure microbial cultures during the initial propagation stage **[18]**. The second group—spontaneous (natural) sourdoughs—is developed using the "beneficial" microbiota naturally present in flour and additional raw materials **[19]**, and **[24]**. The main advantage of sourdoughs based on pure cultures is the ability to produce large volumes of baked goods with stable quality parameters, including consistent organoleptic properties, over extended periods.

Bread production involves numerous interrelated technological processes that create a favorable environment for the active functioning of microorganisms such as lactic acid bacteria and yeasts, which are the core of sourdough starters **[15]**, and **[16]**.

Each of these microorganisms plays a specific role:

- Lactic acid bacteria (*Lactobacillales* including genera *Leuconostoc*, *Pediococcus*) synthesize a complex of organic acids (lactic, acetic, etc.) from sugars, contributing to a decrease in pH and forming an acidic environment that inhibits pathogenic microorganisms **[27]**. They also release various enzymes such as proteases and lipases, which break down proteins and fats, enhancing the product's flavor.
- Yeasts (*Saccharomycetales*, *Candida*) participate in alcoholic fermentation, converting sugars into ethanol and carbon dioxide, thus improving the aroma and taste of the product.
- Molds (*Penicillium*, *Aspergillus*) are less commonly used in breadmaking and more frequently applied in the production of cheeses and soy sauces.

Sourdoughs consist of strains of bacteria and yeasts that interact with each other and with the substrate to produce specific enzymes and metabolites that promote fermentation. Alongside improving the nutritional value of bread, sourdough fermentation reduces phytic acid levels, releases prebiotic polysaccharides, and promotes protein hydrolysis **[17]**. Phytic acid interferes with the absorption of minerals such as K, P, Ca, Mn, and Mg found in cereal grains **[18]**.

The yeasts and bacteria in sourdough produce phytase, which catalyzes the hydrolysis of phytic acid into inositol and phosphoric acid **[12]**. The reduction in acidity to pH 5.5 due to lactic acid fermentation further promotes the hydrolysis of phytic acid.

Currently, the bakery industry employs various types of sourdoughs to meet production needs, including:

- Concentrated lactic acid sourdough **(CLAS)** – a flour-based pre-ferment fermented with selected pure strains of lactic acid bacteria (*Lactiplantibacillus plantarum*, *Levilactobacillus brevis*, *Limosilactobacillus fermentum, Lacticaseibacillus casei*), prepared either in liquid form or as dry lactobacterin. CLAS is produced in two stages: propagation and production. In the first stage, liquid cultures of each lactic acid bacterial species are accumulated in malt wort, then transferred to a water-flour mixture or saccharified mash. The second stage is carried out under production conditions by adding a flour and water mixture to the ready starter and incubating it at 32–38°C until the acidity reaches 14–

18 degrees. Lactic acid bacteria promote rapid acid accumulation and contribute to dough leavening, while yeasts further enhance this effect.

- Vitamin sourdough contains carotene-synthesizing yeast (*Bullera armeniaca*) and *Saccharomyces cerevisiae*, as well as *Lactobacillus acidophilus*, and is used to improve the quality of baked goods made from flour with weak gluten properties.
- Complex sourdough is a mixture of yeast strains, lactic acid, and propionic acid bacteria (*Lacticaseibacillus casei*, *Levilactobacillus brevis*, *Limosilactobacillus fermentum*), along with *Saccharomyces cerevisiae*, selected in specific proportions. It is used to enhance the flavor and aroma of bread and to increase resistance to "rope spoilage" caused by *Bacillus* spp. and mold contamination.
- Acidophilic sourdough contains *L. acidophilus* and *S. cerevisiae*, improving the taste and aroma of bakery products. It also prevents "potato disease" (rope spoilage) and is used in accelerated dough preparation methods and for improving the quality of bread from strong gluten flours.
- Propionic acid sourdough contains *Propionibacterium freundenreichii* spp. *Shermanii*, known for its strong bactericidal properties and vitamin B_{12} synthesis. It is used in baking to inhibit the development of rope spoilage and mold fungi **[10]**, **[12]**, and **[13]**.

Bakery products are susceptible to physical, chemical, and microbiological spoilage. While physical and chemical spoilage limits the shelf life of products with low to medium moisture content, microbiological spoilage caused by bacteria, yeasts, and molds is a major concern in high-moisture baked goods **[10]**, **and [14]**.

One effective strategy to prevent microbial spoilage and enhance the quality and safety of flour and bakery products is the use of biopreparations based on lactic acid bacteria and yeasts with antibacterial properties.

Scientific Hypothesis

In the modern baking industry, where high production rates and long-term product storage are integral requirements, the search for and implementation of biological products with antagonistic properties are particularly relevant. Undesirable microbiota such as spore-forming bacteria (*Bacillus spp.*) and molds (*Penicillium spp.*), often causes significant economic losses and also reduces the quality and safety of bakery products. The use of biological products based on lactic acid bacteria and yeast may effectively reduce microbial spoilage, while simultaneously contributing beneficial microbiota and improving its organoleptic characteristics.

Modern practice among small and emerging bakeries often relies on "spontaneous" fermentation starters. However, such starters are characterized by high inter- and intra-production variability of microbiota composition and acid- and gas-formation kinetics; therefore, the technological outcomes (dough rise, proofing time, aromatic profile) are difficult to predict and reproduce without long-term stabilization of the culture. Moreover, the absence of an identified microbial composition, combined with warm and humid storage conditions, increases the risk of microbiological spoilage—including rope spoilage ("potato disease") and mold growth—which leads to economic losses and reduced product quality and safety.

The central hypothesis of this study is that wild-type strains of LAB and yeast isolated from specific Kazakhstan raw materials (kumis and kazakh wheat flour species) exhibit superior functional properties compared to standard expectations for these species. Specifically, we hypothesized that these isolates would demonstrate a measurable ability to:

- reduce the medium pH to 4.4 - 4.8, optimal for sourdough stability;
- exhibit strong antagonistic activity (quantified by inhibition zones) against *Bacillus spp.* and *Penicillium notatum*.

These measurable parameters served as the criteria for selecting the most promising strains for further technological application.

Objectives

Primary objective: Identification and study of morphological and cultural properties of new strains of lactic acid bacteria and yeast, promising for use in the bakery industry.

MATERIAL AND METHODS

Samples

Samples description: Six isolates of lactic acid bacteria isolated from wheat flour and kumiss, and two yeast strains deposited at the Kazakh Research Institute of Processing and Food Industries (Table 1).

Table 1 Investigated strains of microorganisms and their natural sources of origin.

Isolate/strain name	Source of origin
Lactic acid bacteria	
B-27	Wheat flour (highest grade)
B-449	Kumys
B-302	Wheat flour (1st grade)
B-157	Wheat flour (1st grade)
B-319	Wheat flour (1st grade)
B-329	Wheat flour (highest grade)
Yeast	
LVg	Flour sourdough
A-28g	Flour sourdough

Samples collection: Samples of wheat flour (highest and first grades) were collected during 2023–2024 from milling enterprises in the Almaty region of Kazakhstan: JSC AsiaAgroFood, Almaty Flour Mill LLP, MukaMoll LLP. Samples of kumis were purchased from local farmers' shops selling products from farms in the Almaty region, during the summer production season. Flour samples (300 g each) were taken from freshly milled batches, placed in sterile containers, and transported to the laboratory under refrigerated conditions (+4 °C) within 24 h. Kumis samples (150 mL each) were aseptically collected into sterile tubes and transported at +4 °C within 6 h.

Samples preparation: In the laboratory, 10 g of flour samples were suspended in sterile saline solution (0.85% NaCl), serially diluted, and plated on MRS agar for lactic acid bacteria isolation. Kumis samples were directly diluted and plated in the same way. Yeast strains were isolated from spontaneously fermented flour sourdoughs prepared in the laboratory by mixing wheat flour and water (1:1) and incubating at 28 °C for 5 days, with daily refreshments. All isolates were purified by repeated streaking on selective media before deposition in the microbial collection of the Kazakh Research Institute of Processing and Food Industries. The cultures has been stored in sealed glass ampoules in a lyophilized state under laboratory refrigeration conditions at 0°C.

Number of samples analysed: Six samples of lactic acid bacteria and two samples of yeast were analysed.

Chemicals

Media: MRS Broth (TM Media, Titan Biotech Ltd.), MRS Agar (TM Media, Titan Biotech Ltd.), Sabouraud dextrose agar (TM Media, Titan Biotech Ltd.).

Primers: Universal 16S rRNA primers, reverse – 5′ ACG GCT ACC TTG TTA CGA CTT 3′ and forward – 5′ AGA GTT TGA TCC TGG CTC AG 3′ (Eurogentech), for ITS - ITS1 – 5′ GGAAGTAAAAGTCGTAACAAGG 3′ and ITS4 – 5′ TCCTCCGTTATTGATGC 3′ (Eurogentech). DNA ladder "Step 100 S-8100" (Biolabmix).

Chemicals: 10x reaction buffer (Fermentas), Taq DNA Polymerase (Fermentas), Taq Polymerase (Syntol), 1× PCR buffer (ThermoFisher), $MgCl_2$ (Sigma-Aldrich), agarose (TopVision, ThermoFisher) shrimp alkaline phosphatase (SAP) (ThermoFisher), exonuclease I (ThermoFisher), BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), phenolphthalein solution (Sigma-Aldrich), NaOH (Sigma-Aldrich).

All the reagents and auxiliary materials used in the experiments were of analytical grade and suitable for molecular genetic research.

Animals, Plants and Biological Materials

Wheat flour produced in Kazakhstan.

Kumis produced in Kazakhstan.

Isolates of lactic acid bacteria and yeast.

Test-cultures: *Bacillus mesentericus* - the type strain, deposited in the collection of the Scientific and Production Center of Microbiology and Virology (Almaty, Kazakhstan), inventory number - FS-15.

Penicillium notatum (*Penicillium sp.*) - the type strain, deposited in the collection of the Scientific and Production Center of Microbiology and Virology (Almaty, Kazakhstan), inventory number - 79.

Bacillus subtilis – the strain compared with type strain ATCC 6051, deposited in the collection of the Kazakh research Institute of Processing and Food Industries (Almaty, Kazakhstan), inventory number – B-18.

Instruments

Devices: Thermal Cycler VeritiPro (Applied Biosystems), spectrophotometer NanoDrop 1000 (ThermoFisher), horizontal electrophoresis chamber SubCell GT (Bio-Rad), PowerPac Basic electrophoresis power supply (Bio-Rad), 3730xl DNA Analyzer (Applied Biosystems), vortex (IKA), TS-1/80 SPU dry air thermostat (Smolensk SKTB SPU), Benchtop pH/ORP/Ion Meter PH-920 (BIOBASE).

Software: SeqMan (DNASTAR, version 11.2), MEGA (version 12.0.11), Excel (Microsoft Inc., 2025), G*Power (version 3.1.9.7).

Laboratory Methods

Genomic DNA from lactic acid bacteria was extracted using the method described by K. Wilson **[28]**, while for yeasts, DNA extraction was performed according to the protocol by R. Rapley **[29]**.

Identification of lactic acid bacteria as members of the *Lactobacillus* genus based on morphological characteristics was performed according to GOST 10444.11-89 "Food Products. Methods for the Detection of Lactic Acid Microorganisms." The criteria included Gram staining, motility, endospore presence, and catalase activity **[30]**.

The titratable acidity of the culture liquid was determined by the Turner titration method, following the principle described in GOST 3624-92 «Milk and milk products. Titrimetric methods of acidity determination», which was applied to the microbial broth without modifications to the titration procedure. The reagents used in the study were prepared in accordance with GOST 25794.1-83 «Methods of preparation of titrated solutions for acid-base titration».

Antagonistic activity was assessed using the agar well diffusion method as described by Schillinger and Lücke **[31]** with minor modifications. Specifically, 100 µL of the cell-free supernatant was added to 8-mm diameter wells in Nutrient agar.

Description of the Experiment

Study flow: The studies we conducted can be divided into three stages. In the first stage, we analyzed the morphological properties of the microorganisms, including cell and colony morphology. At the second stage, we performed molecular genetic analysis of the isolated microorganisms using PCR at a public laboratory at the National Center for Biotechnology (Astana, Kazakhstan). In the final stage, we studied the antagonistic properties of the microorganisms against the causative agents of bread diseases.

Determination of morphological and cultural characteristics

To determine the morphological and cultural characteristics of the studied microorganisms, cultures were plated on solid nutrient media. For this, 0.1 mL of the microbial suspension was applied to Petri dishes containing solidified medium and distributed evenly using a sterile spreader. The plates were then incubated in a thermostat at 37°C for lactic acid bacteria and at 28°C for yeast cultures.

For bacteria of the *Lactobacillus* genus, MRS medium (TM Media, pH 6.2–6.6) was used, with the following composition (g/L): yeast extract – 5.0; meat extract – 10.0; peptone – 10.0; glucose – 20.0; ammonium citrate – 2.0; sodium acetate – 5.0; Tween 80 – 1.0; Na_2HPO_4 – 2.0; K_2HPO_4 – 2.0; $MgSO_4 \cdot 7H_2O$ – 0.2; $MnSO_4 \cdot 4H_2O$ – 0.005; agar – 20.0.

For yeast cultures, Sabouraud medium (TM Media, pH 6.5 ± 1) was used, with the following composition (g/L): tap water – 1.0; glucose – 40.0; peptone – 10.0; agar – 18.0. Media were sterilized at 0.5 atm, 121°C for 20 minutes.

Analysis of the 16S rRNA Gene and ITS Region Nucleotide Sequences

To assess lysis quality and conduct further research, DNA concentration was measured spectrophotometrically with a NanoDrop 1000 at 230 nm (Table 2), followed by qualitative assessment by electrophoresis.

Genetic identification of lactic acid bacteria (LAB) was conducted by sequencing the 16S rRNA gene. For amplification of the 16S rRNA gene, universal primers were used: Reverse – 5′ ACG GCT ACC TTG TTA CGA CTT 3′ and Forward – 5′ AGA GTT TGA TCC TGG CTC AG 3′.

The PCR reaction mixture contained 2 µL of 10× reaction buffer (Fermentas), 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 10 pmol of each primer, 1 unit of Maxima Hot Start Taq DNA Polymerase (Fermentas), and 150 ng of genomic DNA as the template.

For identification of the ITS region sequence in yeast cells, PCR was performed using primers ITS1 – 5′ GGAAGTAAAAGTCGTAACAAGG 3′ and ITS4 – 5′ TCCTCCGTTATTGATGC 3′ in a total volume of 20 µL. The PCR mix contained 100 ng of DNA, 0.2 U of Taq Polymerase (Syntol), 0.2 mM of each dNTP, 1× PCR buffer (ThermoFisher), 2.5 mM $MgCl_2$, and 10 pmol of each primer.

The PCR amplification program included: an initial denaturation at 94°C for 5 minutes; 30 cycles of 95°C for 30 seconds, 52°C for 30 seconds, and 72°C for 1 minute; and a final elongation step at 72°C for 7 minutes. PCR was performed using a VeritiPro Thermal Cycler (Applied Biosystems).

Table 2 DNA concentration and purity of the samples.

Sample name	ng/μL	A260	A280	260/280	260/230
B-157	519.79	10.396	5.109	2.03	2.05
B-302	869.64	17.393	8.421	2.07	2.24
B-449	100.97	2.019	1.032	1.96	1.39
B-27	561.74	11.235	5.482	2.05	2.11
B-319	409.13	8.183	3.972	2.06	2.09
B-329	517.40	10.348	5.058	2.05	2.09
LVg	264.54	5.291	2.618	2.02	1.96
A-28g	125.60	2.512	1.211	2.07	1.83

Amplification products were separated in 1% agarose gel stained with ethidium bromide. Electrophoresis was conducted using a Bio-Rad SubCell GT horizontal electrophoresis chamber and a Bio-Rad PowerPac Basic power supply. A 1× TAE buffer was used as the running buffer.

In the samples, the size of DNA fragments was estimated by comparing their electrophoretic mobility with that of a molecular weight marker. The DNA ladder “Step 100 S-8100” from Biolabmix was used as the molecular weight marker, suitable for estimating the size and quantity of double-stranded DNA fragments ranging from 100 to 1000 base pairs in agarose gel.

PCR products were purified from residual oligonucleotides using dephosphorylation with shrimp alkaline phosphatase (SAP) and exonuclease I.

Sequencing of the 16S rRNA gene fragments of the identified LAB was performed on an automatic 3730xl DNA Analyzer (Applied Biosystems) using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), following the manufacturer's recommendations.

The nucleotide sequences of the 16S rRNA gene were analyzed and assembled into consensus sequences using SeqMan software (DNASTAR). Terminal fragments, including primer sequences and low-quality regions, were removed.

Screening of cultures for antagonistic activity

The antagonistic activity of lactic acid bacteria and yeast cultures was assessed using the agar diffusion method. *Bacillus mesentericus* FS-15, *Bacillus subtilis* 18, and *Penicillium notatum* 79 were used as test cultures. The studied strains were cultured in liquid nutrient media: MRS at 37°C for lactic acid bacteria and Sabouraud at 28°C for yeast cultures, for 48 hours.

An aqueous suspension of *B. mesentericus* was applied to Nutrient Agar plates (0.1 mL per plate) and evenly distributed using a sterile spreader. An aqueous suspension of *P. notatum* was similarly applied to Sabouraud agar plates and also distributed evenly using a sterile spreader. Wells (d = 8 mm) were made in the inoculated agar using a sterile borer, and the wells were filled with the lactic acid bacteria or yeast culture suspensions. The Petri dishes were then incubated for 120 hours. After incubation, zones of inhibition—areas without growth of *B. mesentericus* and *P. notatum*—around the wells were recorded as indicators of antibacterial activity. Sterile water was used as a negative control.

Determination of Acidity

The active acidity (pH) of the lactic acid bacteria (LAB) culture fluid was measured using a BIOBASE Benchtop pH/ORP/Ion Meter PH-920, with each sample analyzed in triplicate.

Titratable acidity was determined by the Turner titration method. For titration, 10 mL of the test medium was mixed with 20 mL of distilled water and 2 drops of 1% alcoholic phenolphthalein solution (Sigma-Aldrich). The mixture was then titrated with 0.1 M NaOH solution under constant stirring until a faint pink color appeared and persisted for at least 1 minute.

Determination of yeast rising power

To evaluate the fermentative activity of the yeast isolates, their rising power was determined using an express method (the "dough ball rising" test) in accordance with the GOST 171-81 standard. For comparative analysis, four commercial samples of baking yeast (*Saccharomyces cerevisiae*), purchased from local retail outlets, were included in the study.

To ensure the comparability of results, an equalization procedure was performed, as the experimental isolates were grown in a liquid phase (Sabouraud Dextrose Broth), while the commercial samples were in dry form. The dry yeast samples were reactivated and inoculated into Sabouraud liquid medium and cultured to accumulate

biomass. Both the experimental strains and the control samples were harvested from the culture medium by centrifugation at 4000 rpm for 15 minutes. After discarding the supernatant, the resulting biomass was used for the analysis.

A biomass sample (0.31 g) was thoroughly mixed with 4.8 mL of sodium chloride solution (2.5%) and 7.0 g of second-grade wheat flour until a homogenous dough was obtained. The dough was shaped into a ball and placed in a beaker containing distilled water preheated to 35 ± 2 °C. The beaker was then kept in an incubator to maintain a constant temperature.

The leavening power (measured in minutes) was defined as the time interval from the moment the dough ball was submerged in the water until it floated to the surface due to the accumulation of evolved carbon dioxide.

Limitation: The described method of sample preparation and equalization of samples is experimental and is not included in the methodology described in GOST 171-81.

Quality Assurance

Number of repeated analyses and experiment replication: Each experiment (except 16S rRNA sequencing) was performed in at least three independent biological replicates, and all measurements in each experiment were performed in triplicate.

Reference materials: pH calibration buffer solutions: HI 7004 L (pH 4.01), HI 7007 L (pH 7.01), and HI 7010 L (pH 10.01) (HANNA Instruments).

pREF-BDT control standard provided within the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems).

Calibration: The pH measurements were performed using a PH-920 benchtop pH/ORP meter (BIOBASE), which was calibrated prior to each set of experiments using a three-point calibration method with HANNA Instruments (USA) buffer solutions: HI 7004 L (pH 4.01), HI 7007 L (pH 7.01), and HI 7010 L (pH 10.01).

For DNA sequencing quality control, the pREF-BDT control standard provided within the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) was used during each run on the 3730xl DNA Analyzer. This reference material served as a positive control to verify sequencing resolution and base-calling accuracy.

Laboratory accreditation: Experiments were not performed in the accredited laboratory.

Data Access

The genetic sequences of the 16S rRNA gene and ITS region for the studied samples have been submitted to the GenBank database under the following accession numbers: B-27 – PV819315; B-329 – PV819323; B-449 – PV819329; B-319 – PV819335; LVg – PV826795; A-28g – PV826796.

Statistical Analysis

Sequence alignment was performed using the ClustalW algorithm, and evolutionary trees were constructed by the Neighbor-Joining method using Kimura's two-parameter (K2P) model and 1000 bootstrap replications in the MEGA12 software. Reference sequences from the GenBank database showing both high (100%) and moderate (<100%) similarity based on BLAST comparison were included for topological resolution.

Statistical significance was determined using one-way analysis of variance (ANOVA) to compare the biotechnological properties across different microbial strains. Prior to ANOVA, the normality of data distribution was verified using the Shapiro-Wilk test, and the homogeneity of variances was assessed by Levene's test. A p-value < 0.05 was considered statistically significant. All statistical calculations were performed using G*Power (ver. 3.1.9.7).

RESULTS AND DISCUSSION

Morphological and Cultural Characteristics

It was established that representatives of the genus *Lacticaseibacillus* are Gram-positive, non-spore-forming microorganisms. The cells are non-motile (lack flagella) and catalase-negative. They are primarily straight or slightly curved rods of varying lengths and may be arranged singly, in pairs, or in short chains—reflecting the tendency of some lactic acid bacteria to form chains during division (Figure 2). This group includes both cocci (genera *Lactococcus*, *Streptococcus*, and *Pediococcus*) and rods (genus *Lacticaseibacillus* and others). **[32]**, **[33]**, and **[34]**.

The morphological and cultural characteristics of the *Lactobacillus* genus collection strains are presented in Tables 3 and Table 4 below.

Table 3 Description of morphological characteristics of cells from the studied strains of lactic acid bacteria and yeasts.

Strain name	Cell size	Cell shape	Presence of motility and sporulation
		Lactic acid bacteria	
B-449	2-2.7x0.7-0.9 µm	Sticks arranged in pairs, in short chains	Non-motile, do not form spores
B-319	1-2.7x0.5-0.7 µm	Sticks arranged singly or in short chains	Non-motile, do not form spores
B-27	3-5x0.7-1.0 µm	Sticks arranged singly or in chains of different lengths	Non-motile, do not form spores
B-302	2.3-4x1-1.5 µm	Sticks arranged singly or in short chains	Non-motile, do not form spores
B-329	2-4x0.7-1.0 µm	Sticks arranged singly or in short chains	Non-motile, do not form spores
B-157	1.7-2x0.7-0.8 µm	Sticks arranged singly	Non-motile, do not form spores
		Yeast	
LVg	8-10 µm	Round, arranged singly	They are immobile, they do not form spores on the Sabouraud medium.
A-28g	8-10 µm	Round, arranged singly	They are immobile, they do not form spores on the Sabouraud medium.

Table 4 Description of morphological characteristics of colonies of the studied lactic acid bacteria strains grown on solid MRS Medium and yeast strains grown on Sabouraud Medium.

Strain name	Form	Size and diameter	Surface type	Profile type	Colony transparency	Color or pigment	Edge Features	Colony structure	Type of colonies
Lactic acid bacteria									
B-449	Round	Average 2 to 3 mm	Smooth	Round, domed	Opaque, matte	White	Smooth edges	Grainy	S-type
B-319	Round	Average from 2.5 to 3 mm	Smooth	Round, domed	Opaque, matte	White	Smooth edges	Grainy	S-type
B-27	Round	Average from 2.5 to 3 mm	Smooth	Round, domed	Opaque, matte	White	Smooth edges	Grainy	S-type
B-302	Round	Average from 2.5 to 3 mm	Smooth	Round, domed	Opaque, matte	White	Smooth edges	Grainy	S-type
B-329	Round	Average from 2 to 3 mm	Smooth	Round, domed	Opaque, matte	White	Smooth edges	Grainy	S-type
B-157	Round	Small, up to 1 mm	Smooth	Round, domed	Opaque, matte	White	Smooth edges	Grainy	S-type
Yeast									
LVg	Round	Average from 2 to 3 mm	Smooth	Round, domed	Opaque, slightly shiny	White with a cream tint	Smooth edges	Grainy	S-type
A-28g	Round	Average from 2 to 3 mm	Smooth	Round, domed	Opaque, slightly shiny	White with a cream tint	Smooth edges	Grainy	S-type

On solid nutrient media (MRS agar), lactic acid bacteria form small, round-shaped colonies. Typically, the colonies are convex, smooth-edged, opaque, and milky-white or creamy. The diameter of the colonies generally does not exceed 1–2 mm after 1–2 days of incubation; for instance, *Lacticaseibacillus* colonies on MRS agar often reach approximately 1 mm in diameter and have a smooth, glossy surface (Table 4, Figure 1).

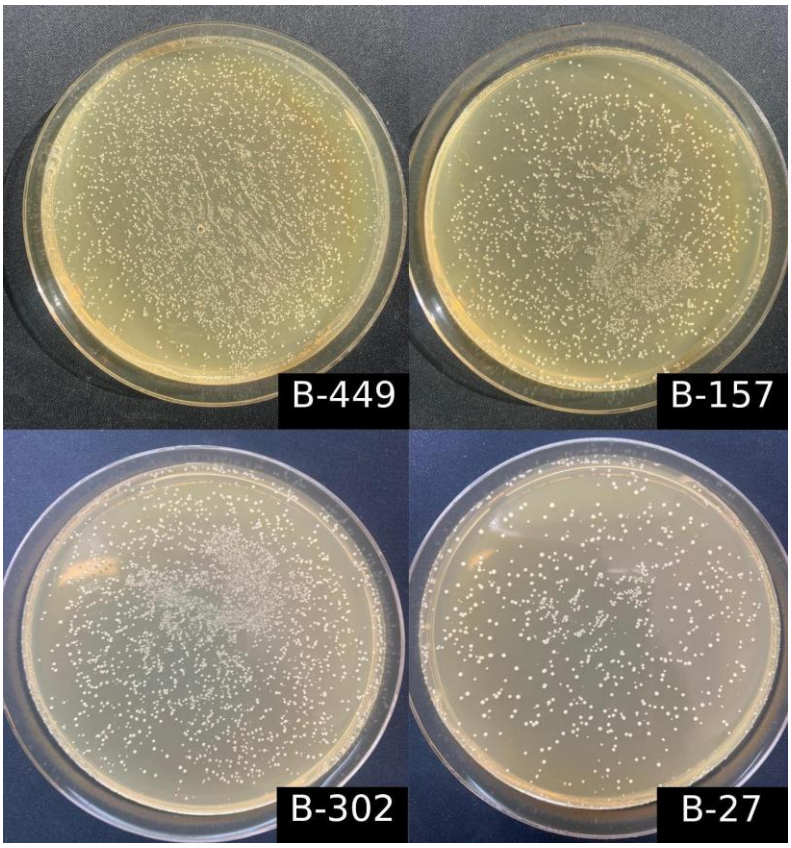
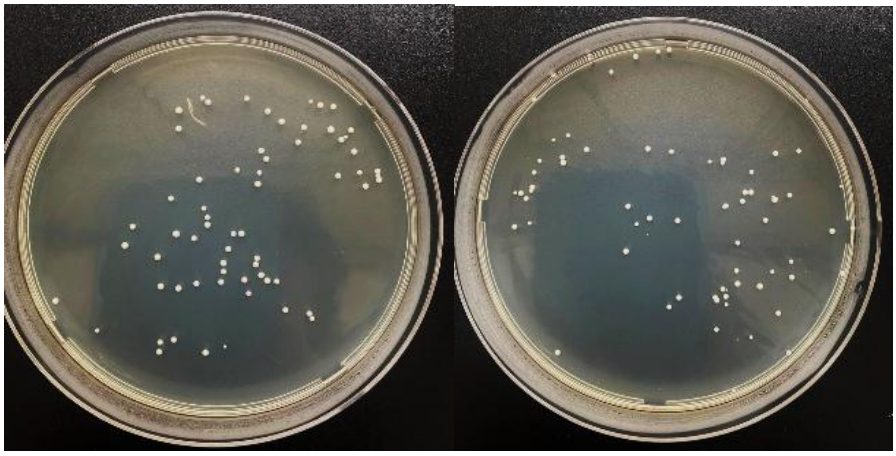


Figure 1 Colonies of lactic acid bacterium strains on MRS nutrient medium. Note: Drigalsky's seeding. Growth of microbial cultures after 48 hours of thermostating at 37 °C.

The colonies are usually creamy-pasty (buttery), without aerial mycelium. Different species exhibit distinctive colony features; for example, some LAB strains can produce exopolysaccharides, which impart a slimy (mucoid) texture to colonies **[24]**.

The two yeast isolates we examined, LVg and A-28g, had round cells measuring 8–10 μm. When cultured on solid nutrient medium, they formed round, smooth, convex colonies with a dull-glossy surface, white in color with a creamy tint (Figure 2).



LVg A-28g

Figure 2 Colonies of Yeast Strains LVg and A-28g on Sabouraud Agar Medium.Note: Drigalsky's seeding. Growth of yeast cultures after 48 hours of thermostating at 28 °C.

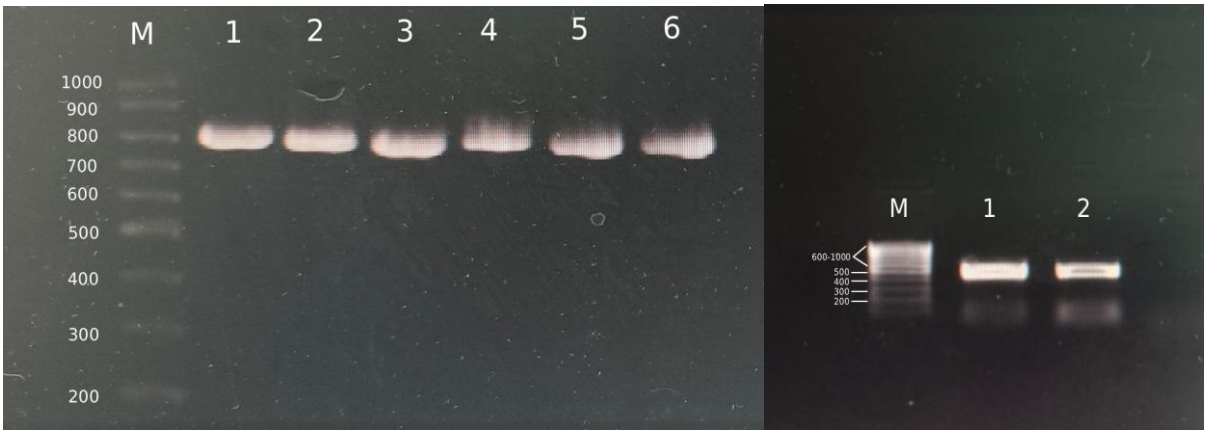
Reproduction occurs predominantly by budding—an outgrowth (bud) forms on the surface of the mother cell, grows, and eventually separates, becoming an independent cell. Under favorable conditions, budding is multipolar, and daughter cells fully detach.

Several authors, including Chavez, C. M. et. al. (2023) and Winans, M. J. (2022), have noted that on solid nutrient media (e.g., wort agar, Sabouraud agar), *S. cerevisiae* forms colonies that visually resemble bacterial colonies but are generally larger. Their color is usually white or cream (milky yellow); most baking and brewing yeasts do not produce pigments, so the colonies remain light in color. The diameter of yeast colonies after 2–3 days of incubation may reach several millimeters. *Saccharomyces cerevisiae* is characterized by the formation of opaque, pasty colonies of a creamy color with a distinct yeast odor. In cases where pseudomycelium is formed, the colony structure changes: they become drier, wrinkled, with radial furrows or "bristly" edges **[35]**, **[36]**, and **[37]**.

From a regulatory perspective, yeasts in food products are identified based on a combination of features, including growth patterns on nutrient media and cell morphology. According to GOST 10444.12-2013 "Microbiology of food products and animal feed. Methods for the detection and enumeration of yeasts and molds," yeast colonies are distinguished from molds by their smooth, moist appearance and lack of aerial mycelium. Under microscopic examination, budding round cells are visible in yeast, while mold fungi are characterized by the formation of well-developed mycelium **[38]**.

Analysis of the 16S rRNA Gene and ITS Region Nucleotide Sequences

The results of PCR reactions using specific primers were visualized using a Bio-Rad Gel Doc XR+ Gel Documentation System (Figure 3).



A B

Figure 3 Agarose gel image of PCR Amplification Products of 16S rRNA Gene and ITS Region Samples. Note: A) Agarose gel image of PCR Amplification Products of 16S rRNA Gene Samples. M – Molecular weight marker (Biolabmix) (100–1000 bp, 100 bp increments); 1 – B-27; 2 – B-302; 3 – B-449; 4 – B-157; 5 – B-319; 6 – B-329. B) Agarose gel image of PCR Amplification Products of ITS Region Samples M – Molecular weight marker (Biolabmix) (100–1000 bp, 100 bp increments); 1 – LVg; 2 – A-28g.

Analysis of amplification results from the ITS regions of yeast strains and the 16S rRNA gene in lactic acid bacteria showed high amplification efficiency and specificity. The agarose gel image of the 16S amplicons (Figure 3A) shows distinct bands with clear definition of approximately 750-800 base pairs, corresponding to the expected size of the 16S rRNA gene amplicon. The absence of nonspecific products or smearing indicates clean reactions and confirms the suitability of the amplified fragments for subsequent sequencing. Similarly, the agarose gel image of the ITS amplicons (Figure 3B) displays distinct single bands corresponding to the expected fragment size of approximately 450-550 base pairs, typical for the ITS region in yeasts.

Initial screening using the GenBank database revealed that the studied LAB strains belong to the following taxonomic group: Bacteria; Firmicutes; Lactobacillales; Lactobacillaceae; *Lacticaseibacillus*. Yeast strains belong to the following taxonomic group: Fungi, Ascomycota, Saccharomycetaceae, *Saccharomyces*. The results of the molecular identification, including GenBank accession numbers and percentage identity with closest strains, are summarized in Table 5.

Table 5 GenBank accession numbers and percentage identity of studying strains with other closest strains.

Sample name	Assigned Species	GenBank Accession (This study)	Closest Strain	Accession No. (Reference)	Identity (%)	Query Coverage (%)
B-319	*L. paracasei*	PV819335	TMPC 46C72	OM758234.1	100.00	100.00
B-27	*L. paracasei*	PV819315	TMPC 46C72	OM758234.1	100.00	100.00
B-329	*L. paracasei*	PV819323	TMPC 46C72	OM758234.1	100.00	100.00
B-449	*L. rhamnosus*	PV819329	TSGB1252	MN255548.1	100.00	100.00
LVg	*S. cerevisiae*	PV826795	HBUAS61729	OM348724.1	100.00	100.00
A-28g	*S. cerevisiae*	PV826796	RC012	MH266046.1	96.64	98.00

To confirm the species identity of the isolated lactic acid bacteria strains, phylogenetic analysis of the 16S rRNA gene nucleotide sequences was conducted (Figure 4).

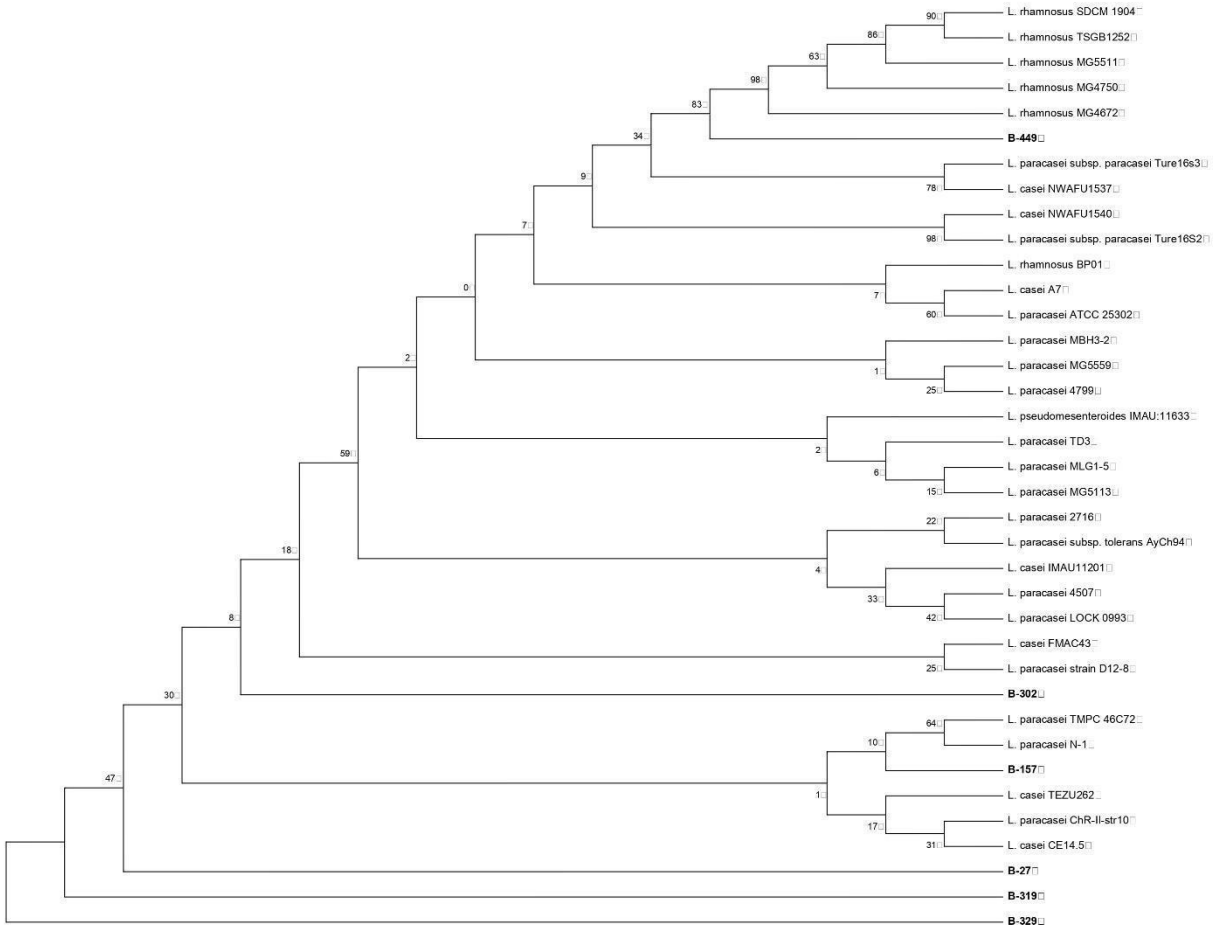


Figure 4 Phylogenetic tree based on the analysis of 16S rRNA gene fragment sequences of lactic acid bacteria strains. Note: The tree was constructed using the Neighbor-Joining method with the Kimura 2-parameter model. Bootstrap values (1000 replications) are shown at branch nodes. The scale bar represents the number of substitutions per site.

Phylogenetic analysis revealed that the studied lactic acid bacteria isolates belong to the genus *Lacticaseibacillus* and predominantly cluster with representatives of *L. paracasei*.

Strains B-27, B-157, B-302, B-319, and B-329 grouped into a single cluster with high bootstrap support values (≥64) alongside reference strains *Lacticaseibacillus paracasei* TMPC 46C72 and N-1, indicating a high degree of homology. This supports the BLAST results, which showed ≥99% sequence identity between these isolates and the listed strains.

Isolate B-449 formed a separate branch, clustering with a group of *Lacticaseibacillus rhamnosus* strains (e.g., SDCM 1904, TSGB1252, and MG5511) with bootstrap values between 83–90, suggesting closer phylogenetic affinity to *L. rhamnosus* and indicating a different species origin compared to the other isolates.

The identified phylogenetic groupings are consistent with previously published data showing that *L. paracasei*, *L. rhamnosus*, and *L. casei* form a genetically close complex within the *Lactobacillaceae* family. Type strains of *L. casei* (e.g., TEZU262, IMAU11201) and *L. pseudomesenteroides* (IMAU11633) were used as additional references to clarify species-level assignment of the studied isolates.

Thus, the phylogenetic analysis confirmed the species identity of isolates B-27, B-157, B-302, B-319, and B-329 as *L. paracasei*, while isolate B-449 was identified as *L. rhamnosus*.

To determine the species identity of the two yeast strains (A-28g and LVg), phylogenetic analysis of the ITS region was conducted (Figure 5).

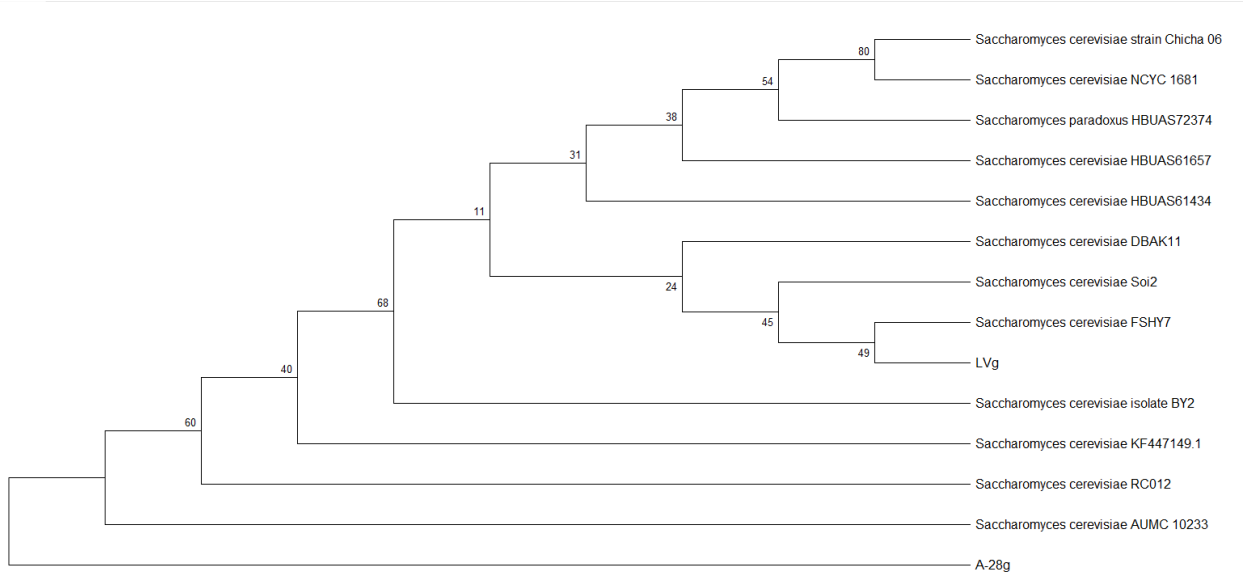


Figure 5 Phylogenetic tree based on the analysis of ITS region sequences of *Saccharomyces* yeast strains. Note: The tree was constructed using the Neighbor-Joining method with the Kimura 2-parameter model. Bootstrap values (1000 replications) are shown at branch nodes. The scale bar represents the number of substitutions per site.

Both studied isolates clearly cluster with representatives of *Saccharomyces cerevisiae*. Strain A-28g forms a distinct yet stable cluster with *S. cerevisiae* isolates AUMC 10233, RC012, and KF447149.1, with bootstrap support ranging from 68 to 80. Strain LVg also clusters with *S. cerevisiae* strains FSHY7, Soi2, and DBAK11, demonstrating ITS-region sequence similarity. More distantly related taxa, such as *S. paradoxus* HBUAS72374, form separate branches, confirming the genetic independence of the studied isolates from other species of the genus.

The phylogenetic analysis indicated that both strains (A-28g and LVg) belong to the species *Saccharomyces cerevisiae*.

Screening of Cultures for Antagonistic Activity

In the baking industry, one of the most important properties of starter cultures is their antagonistic activity against spoilage organisms. Lactic acid bacteria synthesize a wide spectrum of inhibitory metabolites, including lactic and acetic acids, hydrogen peroxide, diacetyl, phenolic compounds (e.g., 3-phenyllactic acid), and bacteriocins **[39]**. These substances acidify the dough environment, inhibit the germination of heat-resistant *Bacillus* spores, and delay the growth of molds such as *Penicillium* and *Aspergillus*. Yeasts also contribute to bioprotection by producing killer toxins, accumulating ethanol and organic acids, and competing with undesirable

microbiota for nutrients. Together, these mechanisms provide a strong bioprotective effect in bakery products **[40]**.

The mechanisms of antagonism primarily involve the production of organic acids (lactic, acetic, etc.) that acidify the environment, as well as the secretion of other inhibitory compounds. It is well known that LAB produce a wide range of antibacterial substances: hydrogen peroxide, carbon dioxide, fatty acids, diacetyl, phenolic compounds (e.g., 3-phenyllactic acid), and even low-molecular-weight bacteriocins **[41]**. The combined action of these compounds inhibits the development of many pathogenic and opportunistic microorganisms. For instance, *Lactiplantibacillus plantarum* strains produce bacteriocins that suppress the growth of *Bacillus subtilis*, a known causative agent of rope spoilage ("potato disease") in bread **[42]**.

Yeasts also contribute to biopreservation: some strains synthesize so-called killer toxins—proteinaceous factors that inhibit sensitive yeasts and bacteria. Furthermore, yeasts compete with spoilage microbiota for nutrients and accumulate significant amounts of ethanol and organic acids, thereby lowering pH and inhibiting the growth of molds and bacteria **[43]**.

Rope spoilage is primarily caused by spore-forming bacteria such as *Bacillus mesentericus*, *B. subtilis*, and closely related species including *B. licheniformis*, *B. pumilus*, *B. megaterium*, and *B. cereus* **[44]**. Spores of these bacteria survive baking temperatures and germinate during storage under warm and humid conditions, resulting in bread spoilage: the crumb becomes sticky and stringy, with an unpleasant odor of overripe fruit **[45]**.

Fungal spoilage of bread is mainly caused by molds of the genera *Penicillium* and *Aspergillus*, which form visible colonies on the surface of products. For example, *Penicillium expansum* forms bluish-green mold, while *Aspergillus niger* produces black colonies. Mold growth not only affects the taste and aroma of bread but also poses health risks by producing mycotoxins such as ochratoxin A and aflatoxins **[40]**.

In this regard, we conducted a screening of the collection strains of LAB and yeasts for their antagonistic activity against key spoilage organisms of bakery products.

In this study, we tested LAB and yeast cultures against two causative agents of rope spoilage—*B. subtilis* 18 and *B. mesentericus* FS-15—as well as the mold *Penicillium notatum* 79.

The results showed that among the 6 LAB cultures and 2 yeast cultures, only strain B-449 exhibited antagonistic activity against *P. notatum*. The inhibition zone diameter was 35.7 ± 1.0 mm (95% CI [33.22, 38.18]), indicating a pronounced antifungal effect of this strain (Figure 6).

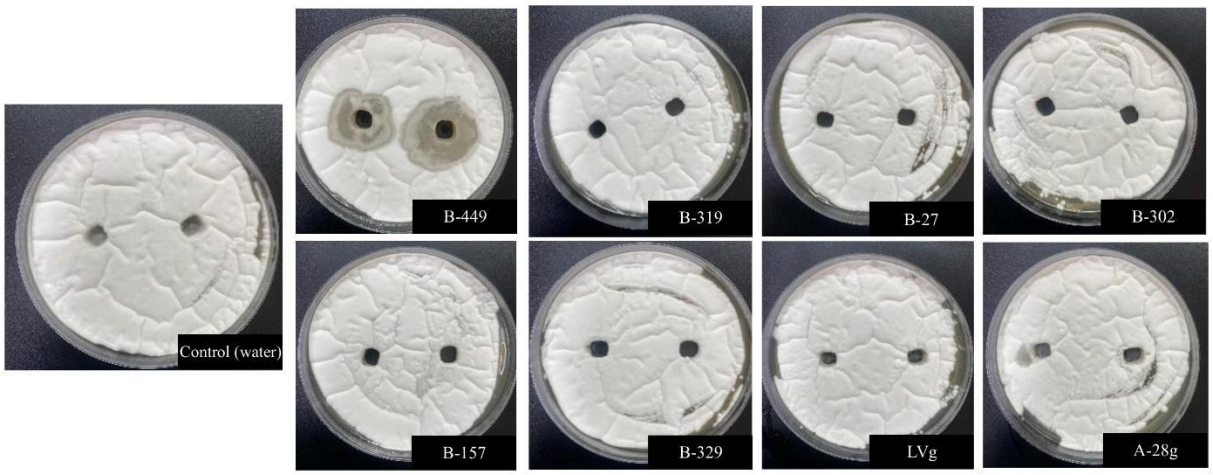


Figure 6 Antagonistic Activity of Samples Against *Penicillium notatum.* Note: The leftmost photo is the control sample (sterile water), and the photos on the right are the samples being tested.

The study of the antagonistic activity of lactic acid bacteria and yeast cultures against *Bacillus mesentericus* showed that the tested strains generally lacked such activity. The exception was strain B-27, which demonstrated a growth inhibition zone of 15.0 ± 1.3 mm (95% CI [11.77, 18.23]) *against Bacillus mesentericus* (Figure 7).

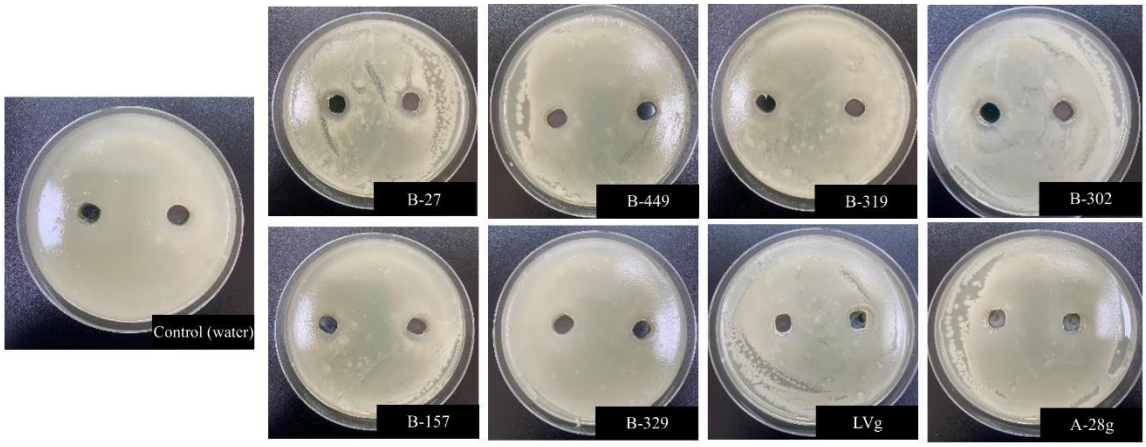


Figure 7 Antagonistic Activity of Samples Against *Bacillus mesentericus.* Note: The leftmost photo is the control sample (sterile water), and the photos on the right are the samples being tested.

Against the *Bacillus subtilis* strain, four tested lactic acid bacterial isolates exhibited varying degrees of growth inhibition of the test culture (Table 5), whereas no antagonistic activity was observed in the yeast cultures.

Table 5 Investigation of the Antagonistic Activity of Lactic Acid Bacteria and Yeasts Against *Bacillus subtilis* ($p < 0.05$).

Strain name	Growth inhibition zone, mm
Lactic acid bacteria	
B-449	19±0.8
B-319	17±0.6
B-27	15±1.0
B-302	0
B-329	19±0.4
B-157	0
Yeast	
LVg	0
A-28g	0

The evaluation of antimicrobial activity revealed significant differences among the tested isolates. A one-way ANOVA indicated a statistically significant difference in the inhibition zone diameters among the active strains ($F(3, 8) = 20.37$, $p < 0.001$). Strains B-449 and B-329 demonstrated the highest antagonistic activity, producing inhibition zones of 19 ± 0.8 mm (95% CI [17.01, 20.99])) and 19 ± 0.4 mm (95% CI [18.01, 19.99])), respectively. Strain B-319 showed lower activity (17 ± 0.6 mm (95% CI [15.51, 18.49])), followed by strain B-27 (15 ± 1.0 mm (95% CI [12.52, 17.48])). The remaining strains (B-302, B-157, LVg, and A-28g) did not exhibit any detectable inhibition zones.

Determination of Acidity

Lactic acid is the main metabolic product of lactic acid bacteria, which produce it through the enzymatic breakdown of carbohydrates, primarily glucose, via the Embden–Meyerhof–Parnas glycolytic pathway or the heterofermentative pathway. Depending on the species of lactic acid bacteria, either the L- or D-isomer of lactic acid is predominantly synthesized, while some strains produce a racemic mixture of both isomers **[46]**.

In the baking industry, lactic acid formation plays a crucial technological and organoleptic role. It lowers the pH of the dough, providing antibacterial protection against undesirable microbiota. Dough acidification also activates proteolytic enzymes, improving the texture of the bread crumb, its elasticity, and porosity structure. Moreover, lactic acid and its salts positively influence the flavor and aroma of the final bakery products, forming characteristic tangy notes that are highly appreciated by consumers.

Table 6 presents the results of the analysis of active acidity (pH) and titratable acidity of the culture fluid of 6 studied lactic acid bacterial strains.

Table 6 Acidity Indicators of the Studied Lactic Acid Bacteria ($p < 0.05$).

Strain name	Active acidity (pH)	Titratable acidity, °T
B-449	4.79±0.10	203±2.4
B-319	4.55±0.09	223±2.7
B-27	4.64±0.06	210±1.8
B-302	4.64±0.05	220±3.1
B-329	4.76±0.07	224±3.0
B-157	4.72±0.07	205±1.9

Statistical analysis performed by one-way ANOVA of the titrated acidity of the samples (Table 6) revealed significant differences in titratable acidity among all six studied strains ($F(5, 12) = 4.25$). For the observed effect size ($f = 1.33$), the statistical power (1-β) reached 1.000 at an α level of 0.05, significantly exceeding the recommended threshold of 0.80. This confirms that the study is sufficiently powered to detect significant differences between the microbial strains.

The results of active acidity (pH) measurement presented in Table 6 indicate that the studied lactic acid bacterial (LAB) strains exhibit a relatively high acidity (pH 4.5–4.7), which is typical of this group. The most pronounced acidification ability was observed in strain B-319 (pH 4.55 (95% CI [4.33, 4.77])), suggesting that it may be a highly active lactic acid producer.

The analysis of active acidity (pH) revealed statistically significant differences among the studied strains ($F(5, 12) = 4.29$). The adequacy of the sample size ($n = 3$) for the pH assessment was confirmed by calculating the statistical power (1-β): for the observed effect size $f = 1.17$, the power (1-β) reached 0.883, which exceeds the minimum recommended scientific threshold of 0.80.

Although, at first glance, the difference between pH values of 4.55 (B-319) and 4.79 (B-449) (95% CI [4.54, 5.04])) may seem insignificant, it is worth noting that the minimum pH thresholds for the growth of some pathogenic and opportunistic microorganisms may vary by 0.1 units. For example, *Clostridium botulinum* requires a minimum pH of 4.6 for growth, while *Staphylococcus aureus* requires a pH of less than 4.5 for growth inhibition. Therefore, even such small pH differences are important when it comes to maximizing food preservation.

Determination of yeast rising power

The rising power of yeast is one of the most important technological indicators that determine its enzymatic activity and suitability for use in baking. It characterizes the ability of yeast cells to intensively release carbon dioxide, which saturates the dough, forming its porous structure and providing the necessary volume and elasticity of the finished product's crumb. In industrial and craft production, determining this indicator is essential for predicting the duration of the dough proofing process and ensuring the consistent quality of bread.

In our research, four types of commercial dry yeast brands were used as comparison samples: Yuva, Omega spices, Podravka, and Dr. Bakers (Figure 8).

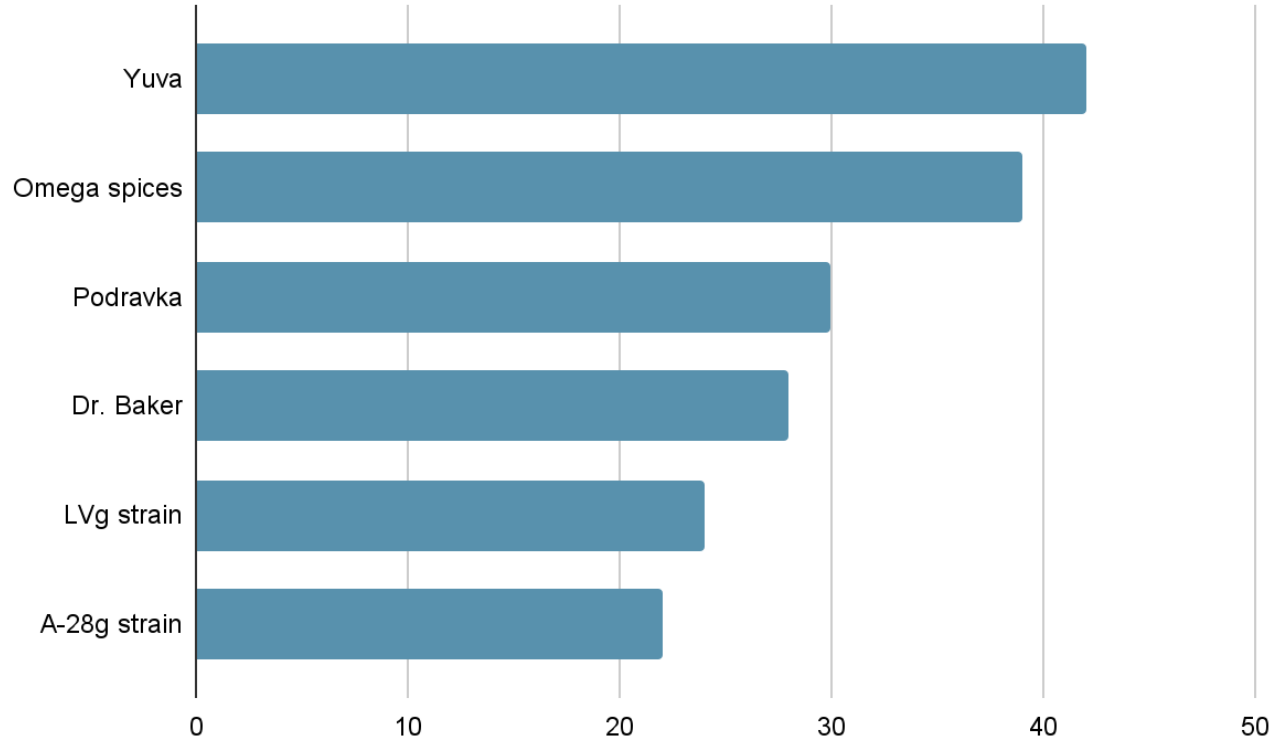


Figure 8 Leavening power of commercial and investigational yeast strains in minutes.

Although the experimental method is not very accurate, it clearly demonstrates the behavior of the yeast in the dough. As can be seen in Figure 8, the A-28g and LVg strains achieved the best results (22 minutes and 24 minutes, respectively), surpassing the commercial counterparts in rising speed. In particular, the A-28g strain achieved a result 1.9 times faster than the commercial sample "Yuva" (42 minutes). The best commercial sample in this experiment was "Dr. Bakers," which had a rising time of 28 minutes, which is 14.28% slower than the LVg strain and 21.42% slower than the A-28g strain.

CONCLUSION

The modern baking industry requires efficient biological strategies to combat microbial spoilage caused by *Bacillus spp.* and molds, which lead to significant economic losses and safety concerns. While spontaneous starters are widely utilized, their inherent microbiological variability and unpredictable fermentation kinetics pose risks to product consistency and safety. Furthermore, commercial and imported cultures often fail to perform optimally due to their sensitivity to regional raw materials and specific environmental conditions. Therefore, there is a critical need for specialized starter cultures that employ a 'multi-barrier' approach—integrating moderate acidification with the production of specific antimicrobial metabolites. The biotechnological potential of our isolates in this context lie in their demonstrated properties: in our experiments, strains B-449 and B-27 exhibited pronounced antagonistic activity against key bread spoilage agents (*Bacillus subtilis*, *Bacillus mesentericus*, *Penicillium notatum*), while B-319 demonstrated good acid-forming activity (pH ≈ 4.5). The identified properties of the studied strains suggest their potential as a basis for developing protective starter cultures. Further research will focus on validating their efficacy within actual breadmaking processes and assessing their impact on the microbiological stability of the final products. A limitation of our study is that the functional characteristics of the microorganisms studied were evaluated in vitro rather than in complex food systems such as dough or bread. Although screening provided a reliable basis for selecting isolates with high acidity and antagonistic potential, the effects of these strains on dough rheology, bread volume, and organoleptic characteristics remain to be verified in future studies.

REFERENCES

1. Rizzello, C. G., Portincasa, P. , Montemurro, M., Di Palo, D. M., Lorusso, M. P., De Angelis, M., Bonfrate, L., Genot, B., & Gobbetti, M. (2019). Sourdough Fermented Breads are More Digestible than Those Started with Baker's Yeast Alone: An In Vivo Challenge Dissecting Distinct Gastrointestinal Responses. Nutrients, 11(12), 2954. https://doi.org/10.3390/nu11122954
2. Ribet, L., Dessalles, R., Lesens, C., Brusselaers, N., & Durand-Dubief, M. (2023). Nutritional benefits of sourdoughs: A systematic review. Advances in Nutrition, 14(1), 22–29. https://doi.org/10.1016/j.advnut.2022.10.003
3. Brodie, J., & Godber, J. (2007). Bakery Processes, Chemical Leavening Agents. In Kirk-Othmer Encyclopedia of Chemical Technology. Wiley. https://doi.org/10.1002/0471238961.0308051303082114.a01.pub2
4. Gallagher, E., Gormley, T. R., & Arendt, E. K. (2004). Recent advances in the formulation of gluten-free cereal-based products. Trends in Food Science & Technology, 15(3–4), 143–152. https://doi.org/10.1016/j.tifs.2003.09.012
5. Garcia-Hernandez, A., Roldan-Cruz, C., Vernon-Carter, E. J., & Alvarez-Ramirez, J. (2021). Effects of leavening agent and time on bread texture and in vitro starch digestibility. Journal of Food Science and Technology, 59(5), 1922–1930. https://doi.org/10.1007/s13197-021-05206-1
6. Martirosyan, V., Tyurina, O., Karabinskaya, Y., Borisova, A., & Peshkina, I. (2023). Application of textured wheat flour in bakery technology. BIO Web of Conferences, 64, 01002. https://doi.org/10.1051/bioconf/20236401002
7. Isabekova, M. S., & Umiraliyeva, L. B. (2022). Studying the physiological and biochemical properties of lactic bacteria cultures in the creation of sourdough for bread. Bulletin of the Karaganda University "Biology Medicine Geography Series," 108(4), 60–67. https://doi.org/10.31489/2022bmg4/60-67
8. Medical and biological requirements and sanitary standards for the quality of food raw materials and food products. Moscow, 1992. Retrieved from https://meganorm.ru/Index2/1/4293738/4293738070.htm [In Russian].
9. Suchintita Das, R., Tiwari, B. K., & Garcia-Vaquero, M. (2023). The Fundamentals of Bread Making: The Science of Bread. In Traditional European Breads (pp. 1–40). Springer International Publishing. https://doi.org/10.1007/978-3-031-23352-4_1
10. Pihurov, M., Cotârleț, M., Borda, D., & Bahrim, G. E. (2025). The Effects of Non-Viable Probiotic Lactobacillus paracasei on the Biotechnological Properties of Saccharomyces cerevisiae. Applied Sciences, 15(16), 9221. https://doi.org/10.3390/app15169221
11. De Vuyst, L., Van Kerrebroeck, S., Harth, H., Huys, G., Daniel, H.-M., & Weckx, S. (2014). Microbial ecology of sourdough fermentations: Diverse or uniform? Food Microbiology, 37, 11–29. https://doi.org/10.1016/j.fm.2013.06.002
12. Microbial Ecology and Process Technology of Sourdough Fermentation. (2017). In Advances in Applied Microbiology (pp. 49–160). Elsevier. https://doi.org/10.1016/bs.aambs.2017.02.003
13. Reffai, Y. M., & Fechtali, T. (2025). A Critical Review on the Role of Lactic Acid Bacteria in Sourdough Nutritional Quality: Mechanisms, Potential, and Challenges. Applied Microbiology, 5(3), 74. https://doi.org/10.3390/applmicrobiol5030074
14. Plessas, S. (2021). Innovations in Sourdough Bread Making. Fermentation, 7(1), 29. https://doi.org/10.3390/fermentation7010029
15. Warburton, A., Silcock, P., & Eyres, G. T. (2022). Impact of sourdough culture on the volatile compounds in wholemeal sourdough bread. Food Research International, 161, 111885. https://doi.org/10.1016/j.foodres.2022.111885
16. De Vuyst, L., González-Alonso, V., Wardhana, Y. R., & Pradal, I. (2023). Taxonomy and Species Diversity of Sourdough Lactic Acid Bacteria. In Handbook on Sourdough Biotechnology (pp. 97–160). Springer International Publishing. https://doi.org/10.1007/978-3-031-23084-4_6
17. Brouns, F. (2021). Phytic Acid and Whole Grains for Health Controversy. Nutrients, 14(1), 25. https://doi.org/10.3390/nu14010025
18. Chaoui, A., Faid, M., & Belahsen, R. (2006). Making bread with sourdough improves iron bioavailability from reconstituted fortified wheat flour in mice. Journal of Trace Elements in Medicine and Biology, 20(4), 217–220. https://doi.org/10.1016/j.jtemb.2006.04.002
19. Lopez, C. M., Rocchetti, G., Fontana, A., Lucini, L., & Rebecchi, A. (2022). Metabolomics and gene-metabolite networks reveal the potential of Leuconostoc and Weissella strains as starter cultures in the

manufacturing of bread without baker's yeast. Food Research International, 162, 112023. https://doi.org/10.1016/j.foodres.2022.112023

20. Mendonça, A. A., Pinto-Neto, W. de P., da Paixão, G. A., Santos, D. da S., De Morais, M. A., Jr., & De Souza, R. B. (2022). Journey of the Probiotic Bacteria: Survival of the Fittest. Microorganisms, 11(1), 95. https://doi.org/10.3390/microorganisms11010095
21. Pérez-Alvarado, O., Zepeda-Hernández, A., Garcia-Amezquita, L. E., Requena, T., Vinderola, G., & García-Cayuela, T. (2022). Role of lactic acid bacteria and yeasts in sourdough fermentation during breadmaking: Evaluation of postbiotic-like components and health benefits. Frontiers in Microbiology, 13. https://doi.org/10.3389/fmicb.2022.969460
22. Salminen, S., Collado, M. C., Endo, A., Hill, C., Lebeer, S., Quigley, E. M. M., Sanders, M. E., Shamir, R., Swann, J. R., Szajewska, H., & Vinderola, G. (2021). The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. Nature Reviews Gastroenterology & Hepatology, 18(9), 649–667. https://doi.org/10.1038/s41575-021-00440-6
23. Nataraj, B. H., Ali, S. A., Behare, P. V., & Yadav, H. (2020). Postbiotics-parabiotics: the new horizons in microbial biotherapy and functional foods. Microbial Cell Factories, 19(1). https://doi.org/10.1186/s12934-020-01426-w
24. Păcularu-Burada, B., Georgescu, L. A., Vasile, M. A., Rocha, J. M., & Bahrim, G.-E. (2020). Selection of Wild Lactic Acid Bacteria Strains as Promoters of Postbiotics in Gluten-Free Sourdoughs. Microorganisms, 8(5), 643. https://doi.org/10.3390/microorganisms8050643
25. Galli, V., Venturi, M., Pini, N., Guerrini, S., Granchi, L., & Vincenzini, M. (2019). Liquid and firm sourdough fermentation: microbial robustness and interactions during consecutive backsloppings. LWT, 105, 9–15. https://doi.org/10.1016/j.lwt.2019.02.004
26. Corsetti, A. (2012). Technology of Sourdough Fermentation and Sourdough Applications. In Handbook on Sourdough Biotechnology (pp. 85–103). Springer US. https://doi.org/10.1007/978-1-4614-5425-0_4
27. Li, H., Jin, Y., Li, H., Zhao, J., Stanton, C., Ross, R. P., Chen, W., & Yang, B. (2025). Understanding Exopolysaccharides from Lactic Acid Bacteria: Synthesis, Functions, and Applications. Journal of Agricultural and Food Chemistry, 73(36), 22110–22132. https://doi.org/10.1021/acs.jafc.5c04613
28. Wilson, K. (2001). Preparation of Genomic <scp>DNA</scp> from Bacteria. Current Protocols in Molecular Biology, 56(1). https://doi.org/10.1002/0471142727.mb0204s56
29. Rapley, R. (2000). Nucleic Acid Protocols Handbook, The. Humana Press. https://doi.org/10.1385/1592590381
30. GOST 10444.11-89. (1991). Food products. Methods for the determination of lactic acid microorganisms (7 p.). Moscow: Standartinform. https://meganorm.ru/Data2/1/4294840/4294840294.pdf [In Russian]
31. Schillinger, U., & Lucke, F. K. (1989). Antibacterial activity of Lactobacillus sake isolated from meat. Applied and Environmental Microbiology, 55(8), 1901–1906. https://doi.org/10.1128/aem.55.8.1901-1906.1989
32. Dwi Ludfiani, D., Asmara, W., Endang Tri Hastuti Wahyuni, A., & Astuti, P. (2021). Identification of Lactobacillus spp. on Basis Morphological, Physiological, and Biochemical Characteristic from Jawa Super Chicken Excreta. BIO Web of Conferences, 33, 06012. https://doi.org/10.1051/bioconf/20213306012
33. Senjaliya, D. P., & Georrge, J. J. (2023). Comparative study on biochemical and molecular identification approaches of Lactobacillus species. International Food Research Journal, 30(5), 1105–1124. https://doi.org/10.47836/ifrj.30.5.03
34. Mokoena, M. P. (2017). Lactic Acid Bacteria and Their Bacteriocins: Classification, Biosynthesis and Applications against Uropathogens: A Mini-Review. Molecules, 22(8), 1255. https://doi.org/10.3390/molecules22081255
35. Chavez, C. M., Groenewald, M., Hulfachor, A. B., Kpurubu, G., Huerta, R., Hittinger, C. T., & Rokas, A. (2023). The cell morphological diversity of Saccharomycotina yeasts. FEMS Yeast Research, 24. https://doi.org/10.1093/femsyr/foad055
36. Winans, M. J. (2022). Yeast Hybrids in Brewing. Fermentation, 8(2), 87. https://doi.org/10.3390/fermentation8020087
37. Chen, A., Si, Q., Xu, Q., Pan, C., Qu, T., & Chen, J. (2025). Evaluation of Stress Tolerance and Fermentation Performance in Commercial Yeast Strains for Industrial Applications. Foods, 14(1), 142. https://doi.org/10.3390/foods14010142
38. GOST 10444.12-2013. (2015). Microbiology of food products and animal feed. Methods for detection and enumeration of moulds and yeasts. Moscow: Federal Agency on Technical Regulation and Metrology (Rosstandart). Retrieved from https://meganorm.ru/Data2/1/4293773/4293773820.pdf [In Russian]

39. Sarita, B., Samadhan, D., Hassan, M. Z., & Kovaleva, E. G. (2025). A comprehensive review of probiotics and human health-current prospective and applications. Frontiers in Microbiology, 15. https://doi.org/10.3389/fmicb.2024.1487641

40. Mani-López, E., Arrioja-Bretón, D., & López-Malo, A. (2021). The impacts of antimicrobial and antifungal activity of cell-free supernatants from lactic acid bacteria in vitro and foods. Comprehensive Reviews in Food Science and Food Safety, 21(1), 604–641. https://doi.org/10.1111/1541-4337.12872

41. Banicod, R. J. S., Tabassum, N., Javaid, A., Kim, Y.-M., & Khan, F. (2025). Lactic Acid Bacteria–Derived Secondary Metabolites: Emerging Natural Alternatives for Food Preservation. Probiotics and Antimicrobial Proteins. https://doi.org/10.1007/s12602-025-10672-6

42. Iosca, G., Fugaban, J. I. I., Özmerih, S., Wätjen, A. P., Kaas, R. S., Hà, Q., Shetty, R., Pulvirenti, A., De Vero, L., & Bang-Berthelsen, C. H. (2023). Exploring the Inhibitory Activity of Selected Lactic Acid Bacteria against Bread Rope Spoilage Agents. Fermentation, 9(3), 290. https://doi.org/10.3390/fermentation9030290

43. Rahman, M., Islam, R., Hasan, S., Zzaman, W., Rana, M. R., Ahmed, S., Roy, M., Sayem, A., Matin, A., Raposo, A., Zandonadi, R. P., Botelho, R. B. A., & Sunny, A. R. (2022). A Comprehensive Review on Bio-Preservation of Bread: An Approach to Adopt Wholesome Strategies. Foods, 11(3), 319. https://doi.org/10.3390/foods11030319

44. Rumeus, I. (2023). BREAD ROPE SPOILAGE DEVELOPMENT. Journal of Engineering Science, 30(2), 185–195. https://doi.org/10.52326/jes.utm.2023.30(2).16

45. Pacher, N., Burtscher, J., Johler, S., Etter, D., Bender, D., Fieseler, L., & Domig, K. J. (2022). Ropiness in Bread—A Re-Emerging Spoilage Phenomenon. Foods, 11(19), 3021. https://doi.org/10.3390/foods11193021

46. Yarullina, D. R., & Fakhrullin, R. F. (2014). Bacteria of the genus Lactobacillus: General characteristics and methods of working with them: Educational and methodological guide (51 p.). Kazan: Kazan University. Retrieved from https://kpfu.ru/staff_files/F_903664652/2025_Metodichka_Yarullina_po_pravilam_09_04_2025.pdf

Funds:

This work was supported under the project "Development of biopreparations for various types of bread based on domestic highly active cultures of microorganisms that ensure the production of high-quality and safe bakery products" within the framework of the scientific and technical program BR22886613 "Development of innovative technologies for the processing and storage of agricultural plant products and raw materials", budget program 267 "Improving the accessibility of knowledge and scientific research", subprogram 101 "Program-targeted financing of scientific research and activities" of the Ministry of Agriculture of the Republic of Kazakhstan for the years 2024-2026.

Acknowledgments:

We would like to thank the Scientific and Production Center for Microbiology and Virology and the National Center for Biotechnology, for their assistance in conducting research and providing a research base.

Competing Interests:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

AI Statement:

Grammarly, were used for performing English grammar proofreading.

Contact Address:

Lazat Umiraliyeva
Affiliation: Kazakh Research Institute of Processing and Food Industry, 050060, 238G Gagarin Ave., Almaty, Kazakhstan,
Tel.: +77077289625
E-mail: l.umiraliyeva@rpf.kz
ORCID: https://orcid.org/0000-0002-8256-0161
Author contribution: conceptualization, project administration.

Elvira Ismailova
Affiliation: Research and Production Center for Microbiology and Virology, 050010, 105 Bogenbay Batyr Str., Almaty, Kazakhstan,
Tel.: +77760141248
E-mail: elya7506@mail.ru
ORCID: https://orcid.org/0000-0002-2774-9164
Author contribution: validation, methodology, investigation.

Ivan Filatov
Affiliation: International Engineering Technological University 050060, 89/21 Al-Farabi avenue, (93G/5 Al-Farabi avenue), Almaty, Kazakhstan,
Tel.: +77473989649
E-mail: filatov-vanya@inbox.ru
ORCID: https://orcid.org/0000-0003-4855-3950
Author contribution: writing - original draft, investigation, visualization.

Asan Ospanov
Affiliation: Kazakh Research Institute of Processing and Food Industry, 050060, 238G Gagarin Ave., Almaty, Kazakhstan,
Tel.: +77015230075
E-mail: a.ospanov@rpf.kz
ORCID: https://orcid.org/0000-0003-2396-3419
Author contribution: resources, formal analysis.

Corresponding author: **Filatov Ivan**

Copyright notice: