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ANTIMICROBIAL ACTIVITY OF LACTIC ACID BACTERIA ISOLATED FROM SPONTANEOUS SOURDOUGH BASED ON ECOLOGICAL FLOURS

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Abstract

This study aimed to isolate and identify lactic acid bacteria (LAB) strains from spontaneously fermented sourdoughs prepared from different ecological flour types: einkorn wheat, corn flour, and rye flour, manufactured according to the traditional type I sourdough protocol. The study also evaluated their antibacterial and antifungal properties, which are key properties for industrial applications. Twenty LAB strains were isolated, and eight strains were identified to the species level using a semi-automated Biolog® Microbial Identification System. Three isolates (*Lactobacillus brevis* LM6, *Lactobacillus fermentum* LM3, and *Lactobacillus coryniformis* subs. *coryniformis* LM8) demonstrated versatile carbohydrate metabolism by producing gas when grown at 30 °C, thus revealing their heterofermentative characteristics. The strains were tested for antimicrobial properties using the agar well diffusion method against pathogenic bacteria Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, and *Bacillus spizizenii.* Most LAB strains exhibited antibacterial activity against the test microorganisms, particularly against *B. spizizenii*. The antifungal effects of LAB strains against *Aspergillus brasiliensis*, *Penicillium crysogenum*, and *Mucor racemosus* were also investigated. Only four LAB strains showed an inhibitory effect on the growth and development of fungi. *P. chrysogenum* was the most susceptible to the action of LAB strains, especially *L. plantarum LM2*, *P. acidilactici LM5*, and *L. coryniformis* LM8. These results revealed that LAB isolated from the spontaneous sourdough have promising antimicrobial and antifungal properties, making the ecological flour sourdough a good source of LAB with high potential to be used for obtaining single or mixted starter cultures.

Keywords: spontaneous ecological flour sourdough; lactic acid bacteria, antimicrobial activity; Biolog® system

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Introduction

The use of the sourdough technological process as the basis of fermentation and leavening is one of the oldest biotechnological processes in the production of cereal foods (Chavan and Chavan, 2011). Sourdough is defined as an acidic, sharp-tasting mixture of flour and water, fermented with the help of yeast and lactic acid bacteria (LAB) (Siepmann *et al.*, 2018), mainly heterofermentative strains, which produce metabolites such as lactic acid and acetic acid, but also other acids in a smaller quantity and therefore resulting in pleasant sour-tasting products (Chavan and Chavan, 2011).

The metabolic activity of the dough microbiota significantly impacts the technological performance of the dough, as well as its nutritional properties, sensory profile, shelf life, and overall quality of the bakery product (Rocha, 2011). LAB are generally accepted as safe microorganisms (GRAS) and play important roles in food fermentation and preservation, either by the presence of natural microbiota or by the addition of starter cultures (in single cultures or as consortia of several microbial species) under controlled conditions (Sanpa *et al*., 2019).

Food/feed biopreservation refers to the strategies and procedures used for preserving food/feed using selected, non-pathogenic, and safe microorganisms (*i.e*., protective microbial cultures) (Bartkiene *et al*., 2019). Such protective microorganisms are used to prevent the development of unwanted microorganisms (by inhibiting growth or by lethal effects), thereby protecting food from spoilage caused by molds and bacteria, increasing shelf life, reducing food loss, and substantially improving food safety. Such strategies are considered natural and effective methods of controlling foodborne pathogens. Among bio-preservation strategies, LAB are considered good candidates because they produce natural antimicrobial and antioxidant metabolites (Bartkiene *et al*., 2019), which can be classified as low molecular mass compounds such as hydrogen peroxide, carbon dioxide, diacetyl, uncharacterized compounds, and high molecular mass compounds like bacteriocins (Ammor *et al*., 2006).

Each antimicrobial compound produced during fermentation provides an additional hurdle for pathogens and spoilage bacteria to overcome before they can survive and/or proliferate in a food or beverage, from the time of manufacture to the time of consumption. Antimicrobial-producing lactic acid bacteria (LAB) may be used as protective cultures to enhance the microbial safety of foods, and they also play a crucial role in preserving fermented foods (Şanlıbaba and Güçer, 2015).

This study aimed to isolate, identify, and characterize LAB strains from spontaneous ecological flour sourdough based on their growth and carbohydrate metabolism, and to evaluate their antimicrobial and antifungal properties.

Materials and methods

Materials used for sourdough preparation

The raw materials used in experiments were procured from Romanian manufacturers: corn (*Zea mays*) flour from SC Paradisul Verde SRL, Brasov, Romania, obtained from a 100% organic corn crop produced in Romania, Einkorn wheat (*Triticum monococum*) flour, rye (*Secale cereale*) flour from Biofarmland Manufactura SRL, Arad, Romania.

Spontaneous sourdough preparation and sampling

Two types of sourdough were selected for the experiment based on the result of the preliminary tests, showing that the most productive sourdoughs were those obtained with Einkorn wheat flour (100%) and the 1:1 ratio of rye flour and corn flour, after fermentation of over 72 h at a temperature of 25 °C.

Thus, these two sourdoughs were used as biological materials to obtain the population of lactic bacteria, subjected to the stages of isolation, identification and characterization. Aliquots of the resulting sourdough were used for the microbiological analysis of LAB isolation.

Culture media, microbiological growth and enumeration

For the enumeration and isolation of LAB, de Man, Rogosa and Sharp agar (MRS, Oxoid, Ltd., Basingstoke, UK) was used. Duplicates of 10 g samples of sourdough were suspended in 90 mL of peptone physiological solution prepared in the laboratory using the ingredients bacteriological peptone and NaCl (Oxoid, Ltd., Basingstoke, UK). Appropriate dilutions were made, and 1 mL inoculum was plated on MRS agar, followed by incubation at 30 °C for 72 h. The colonies were numbered, and the interpretation of results was performed using the following equation:

$$N = (\sum C)/(n1 + 0.1\ n2) \times d \qquad (1)$$

where N = number of colony-forming units (CFU) from two serial dilutions; ∑C = sum of colonies counted in all retained plates; n1 = number of plates retained at first dilution; n2 = number of plates retained at the second dilution; d = first retained.

The results were expressed as log of CFU per gram of sample. Analysis was performed following ISO 15214:2001.

The grown isolates were further purified on MRS agar using the continuous stroke method and incubated for 24-48 hours. Purification was repeated on the MRS agar to obtain a single colony. The pure isolate was then grown on MRS media, slanted as a stock culture, and stored in a refrigerator at 4 °C (Ibrahim *et al*., 2017).

Biochemical characterization of lactic acid bacteria

The Gram staining test was used to determine whether LAB isolates were Gram-positive. The appearance of the purple bacteria under the microscope was defined as Gram-positive.

Gas production was detected by the Durham tube method (Harley and Prescott, 2008) in MRS broth for 24 h at 30 °C. The fermentative capacity of the isolated lactic acid bacteria strains was assessed by evaluating their ability to ferment the culture medium (MRS Broth) and the production of gas in the Durham tube.

LAB isolation and identification using the Biolog® microbial identification system

The Biolog® Microbial Identification System (Biolog, Inc., USA) identifies microorganisms based on redox reactions that grow in the wells of a microplate. The identification system applies to both Gram-positive and Gram-negative bacteria, as well as yeasts and fungi. The equipment analyzes the ability of the microbial cell to metabolize the main classes of biochemical substances while also determining other important physiological properties of the analyzed microorganism, such as pH, tolerance to lactic acid, and chemical sensitivity. The biochemical reactions are based on the use of different carbon sources, such as sugars, carboxylic acids, amino acids, or peptides, which serve to biochemically discriminate and characterize the studied microorganisms.

The Biolog GEN III MicroPlate was used to analyze the microorganism in 94 phenotypic tests, comprising 71 carbon sources and 23 chemical sensitivity tests.

The unique metabolic footprint generated by the microorganism was recorded and compared with the appropriate GEN III Biolog® database (Biolog, Inc., USA). The MicroLog software (Biolog, Inc., USA) was used to interpret the reactions that occurred in the 96-well plates.

The identification of the microorganisms involved multiple stages. Thus, each LAB isolate was transferred from MRS agar using a sterile loop onto the surface of BUGTM agar (Biolog Universal Growth Agar, Hayward, USA) and incubated for 24 h at 33 °C. After the incubation period, the colony was scraped from the surface using a sterile cotton swab, and the swab was dipped into Biolog IF-A inoculating fluid until a turbidity of 90-98% was achieved. The turbidity was assessed using a Biolog turbidimeter, model number 21907 (Biolog, Inc., USA). A volume of 100 µL of inoculum was pipetted into each of the 96 wells of a single Biolog® GEN III plate for each LAB isolate. In total, 20 LAB isolates were recovered. The resulting plates were incubated at 33 °C for 24 h, and the biochemical reactions were recorded using the MicroStation™ Reader (Biolog, Inc., SUA) with a 590 nm wavelength filter at 24 h, using protocol A, as suggested by the manufacturer.

The microplate reader calculates a similarity index for every strain in the database that is most closely related to the test strain. At the 24 h reading, if the index is below 0.50, the instrument reports "no identification." If the index is between 0.50 and 0.74 at 24 h, a "good identification" is reported, along with a genus and a species name. "Excellent identification" is reserved for indices of 0.75 or greater.

Antimicrobial activity testing of the lactic acid bacteria strains by the agar well diffusion technique

All the LAB strains were assessed for their antimicrobial activities against a variety of pathogenic and spoilage bacteria, *Bacillus spizizenii* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, and filamentous fungi, *Aspergillus brasiliensis* ATCC 16404, *Mucor racemosus* ATCC 42647, *Penicillium chrysogenum* ATCC 10106.

The tested LAB strains were inoculated in MRS broth and incubated at 30°C for 24 hours. After incubation, 2 mL of the MRS broth (v/v), in which the LAB strains were multiplied, was inoculated into fresh MRS broth and propagated at 30 °C for 18 h. Afterwards, the multiplied LAB was used to determine their antimicrobial activities against the pathogenic bacteria and filamentous fungi listed above.

The agar well diffusion assay was used to test the antimicrobial activity of the LAB supernatants. For this purpose, a 0.5 McFarland unit density suspension of each pathogenic bacteria strain was inoculated onto the surface of TSA medium (Oxoid, Ltd., Basingstoke, UK) using sterile cotton swabs. Wells of 6 mm diameter were punched in the agar and filled with 50 µL of test LAB culture. The inoculated Petri dishes were incubated for 24 hours at 37 °C. The antimicrobial activities against the tested bacteria were determined by measuring the diameter of the inhibition zone (DIZ, mm). The evaluation of the antifungal activity of LAB isolates was conducted using the same working protocol as for antibacterial testing. The culture medium used was PDA (Potato Dextrose Agar), and the incubation period was 5 days at 25 °C.

To ensure the reproducibility of the results, two repetitions of the same sample were performed and the measurement results (mm of inhibition zone) were expressed as an average mean (± standard deviation). In parallel, positive control samples were made for the evaluation of microbial growth and reference controls represented by disks impregnated with chloramphenicol solution (50 mg/ml).

Data analysis

Antimicrobial tests were performed in duplicate for each microorganism (n = 2). Microbiological data were expressed as logarithms of the number of colony-forming units (cfu mL^{-1}). Descriptive statistics (average and standard deviation) of these results have been employed in data analysis. Data analysis for metabolic profiling of bacteria was conducted using BioTek Gen5 software (Biolog, Inc., USA). Evaluation of significant differences was performed using one-way analysis of variance (ANOVA) in Minitab statistical software version 20, followed by Tukey’s test at a 0.05 level of confidence.

Results and discussion

Biochemical characterization of lactic acid bacteria strains isolated from spontaneous sourdough

Twenty selected strains, when cultivated on MRS agar, formed isolated, drop-shaped colonies that were shiny and had smooth edges (type S). The colonies were small, with a diameter of up to 1 mm, lenticular in depth, creamy white in color, and pasty in consistency. Evaluation of the bacteria's morphology is a crucial stage in the final identification process. The morphological properties of lactic acid bacteria were determined based on the shape of the cells, their mobility, size, and characteristic color in Gram staining (Table 1).

Microscopic analysis of the LAB isolates revealed that they are composed of bacilli, cocci, and coccobacilli, arranged in chains of varying sizes. A preponderance of lactic acid bacteria with a cocci appearance was noted. The microscopic morphological characteristics of the bacteria were examined by the Gram staining test. In this study, all the isolated bacteria exhibited purple staining on the microscope slide, indicating that the tested bacteria were Gram-positive.

Table 1. Phenotypic characterization of lactic acid bacteria isolated from spontaneous sourdough

Strain no.	Gram straining	Colony morphology	Cell morphology
1	+	Creamy, circular colonies, smooth-edged	Rod-shaped, non-motile, non-sporulated, singular
2	+	Milky white, big colonies, rounded, smooth.	Rod-shaped, non-motile, non-sporulated, short chain
3	+	Creamy, circular colonies, smooth-edged	Rod-shaped, non-motile, non-sporulated**,** singular
4	+	Creamy, circular colonies, convex elevation, smooth-edged	Rod-shaped, non-motile, non-sporulated, form chain
5	+	Creamy, circular colonies, smooth-edged	Rod-shaped, non-motile, non-sporulated**,** form a chain
6	+	Creamy, circular colonies, convex elevation, smooth-edged	Rod-shaped, non-motile, non-sporulated, form a chain
7	+	Milky white, big colonies, rounded, smooth.	Rod-shaped, non-motile, non-sporulated, short chain
8	+	Creamy, circular colonies, convex elevation, smooth-edged	Rod-shaped, non-motile, non-sporulated, form a chain
9	+	Creamy colonies, rounded, smooth-edged	Rod-shaped, non-motile, non-sporulated, form a chain
10	+	Creamy, circular colonies, smooth-edged	Rod-shaped, non-motile, non-sporulated, singular
11	+	White colonies, star shape	Rod-shaped, non-motile, non-sporulated, singular
12	+	Milky white, big colonies, rounded, smooth.	Coccus-shaped, non-motile, form short chains
13	+	White colonies, star shape	Rod-shaped, non-motile, non-sporulated, short-chain
14	+	Creamy, circular colonies, smooth-edged	Rod-shaped, non-motile, non-sporulated, singular
15	+	White colonies, star shape	Coccobacilli are non-motile, non-sporulated, and form chains
16	+	Creamy, circular colonies, smooth-edged	Rod-shaped, non-motile, non-sporulated, singular
17	+	White colonies, star shape	Rod-shaped, non-motile, non-sporulated, form a chain
18	+	Milky white, big colonies, rounded, smooth	Rod-shaped, non-motile, non-sporulated, short chain
19	+	Creamy, circular colonies, smooth-edged	Rod-shaped, non-motile, non-sporulated, form a chain
20	+	White colonies, star shape	Rod-shaped, non-motile, non-sporulated, singular

(+) positive reaction

Identification of lactic acid bacteria strains isolated from spontaneous sourdough using the Biolog® microbial identification system

Our study confirmed that most of the isolated LAB strains, using the semi-automated Biolog® Microbial Identification System, belong to the genus *Lactobacillus*. Only one LAB strain was identified as *Pediococcus acidilactici* LM5. Eight LAB strains were identified (Table 2) from 20 colonies isolated. The acceptable instrument readings after 24 h incubation noted levels of similarity index in the range of 0.695 – 0.890 (good and excellent identification).

Table 2. Biolog identification of the selected bacterial isolates

Sourdough type	Isolate No	Isolate ID	Similarity index
Einkorn wheat flour	LM1	*Lactobacillus bifermentas_1*	0,792
Einkorn wheat flour	LM2	*Lactobacillus plantarum*	0,845
Corn Flour + Rye flour 1:1	LM3	*Lactobacillus fermentum*	0,790
Corn Flour + Rye flour 1:1	LM4	*Lactobacillus casei*	0,890
Corn Flour + Rye flour 1:1	LM5	*Pediococcus acidilactici*	0,850
Corn Flour + Rye flour 1:1	LM6	*Lactobacillus brevis*	0,820
Einkorn wheat flour	LM7	*Lactobacillus bifermentas_2*	0,711
Einkorn wheat flour	LM8	*Lactobacillus coryniformis subs. coryniformis*	0,695

Sourdough LAB typically belongs to the genus Lactobacillus, but it also includes strains belonging to the genera *Leuconostoc*, *Pediococcus*, and *Enterococcus* (De Vuyst and Neysens, 2005). In general, heterofermentative *Lactobacillus* species predominate in the sourdough microbiota, particularly in spontaneous sourdough, which comprises natural mixed cultures of lactic acid bacteria (LAB) and/or yeast. Our results revealed that, out of the isolated strains, three LAB isolates were heterofermentative, represented by *L. coryniformis subs. coryniformis* LM8, *L. casei* LM4, *L. fermentum* LM3.

L. coryniformis subs. coryniformis LM8 isolated from Einkorn wheat sourdough, along with *L. bifermentas* LM1 and LM7, were among the natural microbiota but are not frequently found in wheat sourdoughs (Sekwati-Monang *et al*., 2012). These LAB strains were identified by Biolog® System based on the metabolic footprint generated and recorded, and compared with GEN III Biolog® database. *Pediococcus acidilactici* is a Gram-positive lactic acid fermenting bacterium that belongs to the *Lactobacillaceae* family. Fu *et al*. (2021) demonstrated a synergistic effect between *P. acidilactici XZ31* and yeast-degrading wheat allergens.

The sourdough type I obtained from ecologic Einkorn wheat flour, ecologic corn flour and ecologic rye flour, produced using a fermentation process at low temperature (25 °C), is primarily dominated by *Lactobacillus* species.

Gas production and viability of lactic acid bacteria strains isolated from spontaneous sourdough

Based on the CO_2 gas production test performed, it was found that the identified isolates *L. coryniformis subs. coryniformis* LM8, *L. casei* LM4, *L. fermentum* LM3 were able to produce CO_2. The results of the CO_2 production test can be used to discriminate the lactic acid bacteria species as heterofermentative or homofermentative types (Abedi and Hashemi, 2020). The results are indicated in Table 3.

Table 3. Fermentative characteristics and viable counts* (log CFU/ml) of the identified LAB strains

Isolate name	Gas production	Log CFU/ml
L. bifermentans_1 LM1	-	9.72 ± 0.25^a
L. plantarum LM2	-	9.30 ± 0.22^a
L. fermentum LM3	+	8.17 ± 0.29^b
L. casei LM4	-	9.40 ± 0.17^a
P. acidilactici LM5	-	9.47 ± 0.35^a
L. brevis LM6	+	9.33 ± 0.15^a
L.bifermentans_2 LM7	-	9.97 ± 0.21^a
L. coryniformis LM8	+	9.70 ± 0.10^a

*(+) positive reaction; (-) negative reaction; *means ± standard deviation log CFU/mL*
Means that do not share a letter are significantly different.

Thus, the CO_2-forming isolates were classified as heterofermentative bacteria. In addition to lactic acid, heterofermentative lactic acid bacteria also produce alcohol and CO_2. On the other hand, if lactic acid bacteria do not produce gas, they can be classified as a homofermentative group.

Heterofermentative lactic acid bacteria play a crucial role in the fermentation process of dough, contributing to both its nutritional profile and sensory characteristics. These bacteria, including species such as *Lactobacillus brevis*, *Lactobacillus fermentum*, and *Lactobacillus sanfranciscensis*, are distinguished by their ability to produce a diverse range of metabolites during fermentation (Wang *et al*., 2021).

Heterofermentative LAB is characterized by its ability to ferment carbohydrates via the phosphoketolase pathway, leading to the production of lactic acid, acetic acid, ethanol, and carbon dioxide (CO_2) (Wang *et al*., 2021). This contrasts with homofermentative lactic acid bacteria (LAB), which primarily produce lactic acid. The production of CO_2 by heterofermentative lactic acid bacteria (LAB) contributes to the leavening of the dough, resulting in the characteristic structure of sourdough bread. The gas production is essential not only for the texture but also for the overall sensory value of the bread (Islam and Islam, 2024).

Based on the analysis of the growth performance of the isolated LAB, the highest concentration of viable cells after 24 h incubation was found in the case of *L. bifermentas* LM7 (9.97 ± 0.21 log CFU/mL) isolated from Einkorn wheat sourdough. Good microbial viability was also observed for the remaining LAB strains; the concentration of viable cells varied between 9.30 and 9.72 log CFU/mL, while *L. fermentum* LM3 presented a concentration of 8.17 log CFU/mL (Table 3).

Antimicrobial activity of the isolated lactic acid bacteria strains

All eight identified LAB strains were assessed for their antimicrobial activities against a variety of pathogenic and opportunistic bacterial strains, *Bacillus spizizenii,* ATCC 6633, *Staphylococcus aureus*, ATCC 25923, *Enterococcus faecalis*, ATCC 29212, *Escherichia coli*, ATCC 25922, and fungi *Aspergillus brasiliensis* ATCC 16404, *Mucor racemosus* ATCC 42647, *Penicillium chrysogenum* ATCC 10106, by using the agar well diffusion method.

The diameter of the inhibition zones of the sourdough LAB strains against pathogenic bacteria is shown in Table 4. *L. casei* LM4, *L. plantarum* LM2, *L. brevis* LM6, and *P. acidilactici* LM5 exhibited inhibitory properties against all four tested pathogenic bacterial strains. The highest DIZ of 30.5 ± 0.7 mm against *Bacillus spizizenii* was observed in the case of *P. acidilactici* LM5.

Table 4. Diameter inhibition zones (DIZ, mm) of the isolated sourdough lactic acid bacteria (LAB) strains against pathogenic bacteria

Strains	*E. coli*	*S. aureus*	*B. spizizenii*	*E. faecalis*
L. bifermentans_1 **LM1**	13.5±2.1[a]	11.0±1.4[b]	16.0±1.4[b]	18.5±2.1[a]
L. plantarum **LM 2**	14.5±0.8[a]	14.5±0.5[a,b]	25.5±0.7[a]	12.8±0.5[a,b,c]
L. fermentum **LM3**	8.0±0.0[a]	11.5±0.9[a,b]	26.5±2.1[a]	11.0±1.4[a,b,c]
L. casei **LM4**	11.0±0.4[a]	24.5±0.4[a]	27.0±2.8[a]	11.5±2.1[a,b,c]
P. acidilactici **LM5**	14.0±0.2[a]	9.5±0.7[b]	30.5±0.7[a]	18.0±0.2[a,b]
L. brevis **LM6**	6.0±0.0[a]	18.5±2.1[a,b]	24.0±2.8[a]	9.0±1.4[b,c]
L.bifermentans **LM7**	17.5±0.7[a]	13.5±2.1[a,b]	13.5±0.7[b,c]	19.5±0.7[a]
L. coryniformis_2 **LM8**	7.5±0.7[a]	11.0±1.4[b]	8.0±0.0[c]	8.5±0.7[c]

Means that do not share a letter are significantly different.

The isolated lactic acid bacteria strains showed strong inhibition properties against *Bacillus spizizenii* (*Bacillus subtilis subsp. spizizenii* derived from ATCC 6633). The exception is *L. coryniformis* LM8, which had low antimicrobial activity, producing an inhibition zone of only 8.0 ± 0.0 mm.

The incidence of spoilage of wheat flour bread caused by *Bacillus* spp. has probably increased because more bread is manufactured without preservatives and with raw materials such as bran and seeds. Bread rope, which is the most important spoilage of bread after mold, occurs mainly in summer under warm and humid conditions and is mainly caused by *B. subtilis*. The dominance of *B. subtilis* in bread could be explained by the high heat resistance of this species – some spores can survive during the baking process, as the maximum temperature during baking in the center of the bread is 97°C to 100°C for a few minutes (Vaiciulyte-Funk *et al.*, 2015).

The largest zone of inhibition against *B. spizizenii* was recorded in the case of *Pediococcus acidilactici* LM5 (30.5 ± 0.7 mm), followed by *Lactobacillus casei* LM4 (27.0 ± 2.8 mm), *Lactobacillus fermentum* LM3 (26.5 ± 2.1 mm), *Lactobacillus brevis* LM6 (24.0 ± 2.8 mm), and *Lactobacillus plantarum* LM2 (25.5 ± 0.7 mm) (Figure 1).

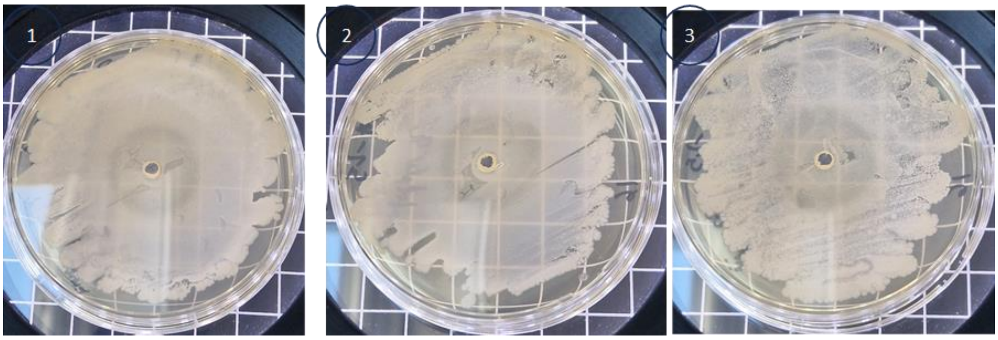

Figure 1. Antimicrobial activity of *L. plantarum* LM2 (1), *L. casei* LM4 (2) and *P. acidilactici* LM5 (3) on the growth of *Bacillus spizizenii.* Visualization of the inhibition zone around agar well.

The Gram-positive bacteria (*S. aureus, B. spizizenii, E. faecalis*) showed a greater susceptibility to the action of metabolites produced by the tested LAB strains. Gram-negative bacteria tend to be more resistant to antimicrobial agents than Gram-positive bacteria due to the presence of additional protection provided by the outer membrane. In addition to the outer membrane, both Gram-positive and Gram-negative bacteria have a cell wall that protects the cytoplasmic membrane (Burt, 2004). In this study, the Gram-positive bacteria, *E. coli*, showed minimal susceptibility to the action of LAB strains, only *L. bifermentas* LM1 and LM7, *L. plantarum* LM2 and *P. acidilactici* LM5 had a stronger inhibitory effect on its growth on the surface of the nutrient medium.

Thus, as in the case of other antimicrobial compounds, it can be concluded that Gram-positive bacteria are much more susceptible to the action of active antimicrobial compounds than Gram-negative bacteria (Burt, 2004). Growth inhibition effects, with varying intensities, were observed for the LAB strains L. bifermentas LM1 and LM7, which exhibited similar bacterial growth inhibition zones in the case of the test microorganisms, with notable efficiency against E. faecalis (18.5 ± 2.1 mm and 19.5 ± 0.7 mm, respectively).

The selection of appropriate LAB strains for producing antimicrobial compounds through fermentation and their application as bio-preservatives in bakery products is promising, particularly when isolated and tested LAB strains are used. In this context, isolated LAB strains can be used to design specific starter cultures or to produce antimicrobial metabolites, among many other compounds of high added value, from a commercial perspective. The antifungal activity of the isolated LAB strains against *Aspergillus brasiliensis*, *Mucor racemosus*, and *Penicillium chrysogenum* is shown in Table 5.

Table 5. Diameter inhibition zones (DIZ, mm) of the isolated sourdough lactic acid bacteria (LAB) strains against fungi strains

Strains	***A. brasiliensis***	***M. racemosus***	***P. chrysogenum***
L. bifermentans_1 **LM1**	6.0 ± 0.0[b]	6.0 ± 0.0[a]	6.5 ± 0.7[a]
L. plantarum **LM 2**	10.5 ± 2.1[a]	6.0 ± 0.0[a]	8.0 ± 2.8[a]
L. fermentum **LM3**	6.0 ± 0.0[b]	6.5 ± 0.7[a]	7.0 ± 1.4[a]
L. casei **LM4**	6.5 ± 0.7[b]	7.0 ± 1.4[a]	8.0 ± 0.0[a]
P. acidilactici **LM5**	6.0 ± 0.0[b]	6.5 ± 0.7[a]	9.5 ± 0.7[a]
L. brevis **LM6**	11.0 ± 0.7[a]	6.5 ± 0.4[a]	7.0 ± 0.7[a]
L.bifermentans **LM7**	6.0 ± 0.0[b]	6.0 ± 0.0[a]	6.0 ± 0.0[a]
L. coryniformis_2 **LM8**	6.0 ± 0.0[b]	6.0 ± 0.0[a]	6.0 ± 0.0[a]

Means that do not share a letter are significantly different

A valuable benefit offered by *P. acidilactici* is its ability to produce a phenolic compound that has demonstrated antifungal activity against filamentous fungi (Shen *et al*., 2022). Bartkiene *et al*. (2019) found that *L. coryniformis* LUHS71, a sourdough-isolated lactic acid bacteria strain, presented a high inhibition of the growth of the tested fungal species *A. nidulans*, *P. oxalicum*, *P. funiculosum*, and *F. poae* in agar well diffusion tests.

Initial screening for antifungal activity of LAB isolates against spoilage fungi revealed that only four out of eight isolates exhibited an inhibitory effect on the growth and development of fungi. *P. chrysogenum* was the most susceptible to the action of lactic acid bacteria, especially *L. plantarum* LM2, *P. acidilactici* LM5, *L. casei* LM4. *Mucor racemosus* was more resistant to the inhibitory action of the tested LAB strains, showing sensitivity only to *L. casei* LM 4 (inhibition diameter 7.0 ± 1.4 mm).

An excellent inhibition of the growth and sporulation of the mycelium of *A. brasiliensis* was found upon inoculation with *L. plantarum* LM2 and *L. brevis* LM4. LAB isolates exhibit inhibition of conidial formation, as suggested by the presence of a white zone adjacent to the mycelium inhibition zone. The inhibitory effect of sporulation induced by the studied LAB has an additional impact on the ability of the fungus to propagate.

L. bifermentas strains LM1, LM7 and *L. fermentum* LM3, had the lowest antifungal activity against the tested mold strains, the zone of inhibition being represented by the diameter of the well in the medium (6 mm).

The production of organic acids during dough fermentation provides significant protection against pathogens and spoilage microorganisms, as their undissociated forms exhibit strong microbial antagonistic effects, as demonstrated in this study. Because the fungistatic effects are mainly due to the production of acetic acid, rather than lactic acid (acetic acid has a higher dissociation constant than lactic acid), heterofermentative LAB exhibits the broadest spectrum of antifungal activity (Bartkiene *et al*., 2019), which is in agreement with current experimental data, especially regarding *L. brevis* LM6.

Studying interactions between lactic acid bacteria and fungi is no simple matter, mainly since bacteria produce fermentation end products that themselves are active to a certain extent or act synergistically with specific antifungal compounds. In nature, this is of importance for the outcome of microbial interactions. Each antimicrobial compound produced during lactic acid bacteria fermentation provides an additional hurdle for spoilage organisms to overcome in food or other biotechnological applications (Schnurer and Magnusson, 2005).

The obtained results are highly promising because the use of sourdough fermented with LAB, which exhibits antifungal activity, offers a natural alternative to chemical preservatives in bakery products (Axel *et al*., 2016).

Antimicrobial and antifungal properties, as well as the metabolic capacity to ferment a wide range of carbohydrate sources, are just a few examples of the high industrial techno-economic potential of LAB sourdough cultures or sourdough starters based on individual cultures or distinct consortia (Bartkiene *et al*., 2019).

Conclusions

Spontaneous sourdough is an excellent source of lactic acid bacteria (LAB) with a high potential to address multiple needs and overcome technical limitations faced by the industry.

In this study, eight strains of lactic acid bacteria with potential industrial applications, isolated and identified from two types of spontaneously fermented sourdoughs, were tested for their *in vitro* antimicrobial activities. All strains showed a viability between 8.17 ± 0.29 and 9.97 ± 0.21 log CFU/mL. Three strains out of eight isolated strains showed the ability to produce CO_2, namely *L. brevis LM6*, *L. fermentum* LM3, and *L. coryniformis* LM8.

Most of the LAB strains showed antibacterial activity against the test microorganisms, especially against *B. spizizenii*.

According to the results of this study, *L. casei* LM4, *L. plantarum* LM2, and *P. acidilactici* LM5 strains are generally considered potentially important starter culture organisms due to their antimicrobial activity against pathogenic bacteria and mold strains. These results unveiled that LAB isolated from spontaneous ecological flour sourdough are promising antimicrobial and antifungal ingredients.

Such protective microorganisms are used to prevent the growth of unwanted microorganisms (by inhibiting growth or by lethal effects), thereby protecting food from spoilage caused by molds and bacteria, increasing shelf life, reducing food losses, and substantially improving food safety.

In future studies, these strains could be used to obtain starter sourdough for bread making, due to their antimicrobial profile and preservation capacity.

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References

Abedi, E., Hashemi, S.M.B. 2020. Lactic acid production – producing microorganisms and substrates sources-state of art, *Heliyon*, **6**(10), e04974.

Ammor, S., Tauveron, G., Dufour, E., Chevallier, I. 2006. Antibacterial activity of lactic acid bacteria against spoilage and pathogenic bacteria isolated from the same meat small-scale facility 1-Screening and characterization of the antimicrobial compounds, *Food Control*, **1**(6), 454-461.

Axel, C., Brosnan, B., Zannini, E., Furey, A., Coffey, A., Arendt, E.K. 2016. Antifungal sourdough lactic acid bacteria as biopreservation tool in quinoa and rice bread, *International Journal of Food Microbiology*, **239**, 86-94.

Bartkiene, E., Lele, V., Ruzauskas, M., Domig, K.J., Starkute, V., Zavistanaviciute, P., Bartkevics, V., Pugajeva, I., Klupsaite, D., Juodeikiene, G., Mickiene, R., Rocha, J.M. 2019. Lactic acid bacteria isolation from spontaneous sourdough and their characterization, including antimicrobial and antifungal properties evaluation. *Microorganisms*, **8**(1), 64.

Burt, S., 2004. Essential oils: their antibacterial properties and potential application in food, *Journal of Applied Microbiology*, **94**(3), 223-53;

Chavan, R.S., Chavan, S.R. 2011. Sourdough Technology—A Traditional way for wholesome foods: A Review. *Comprehensive Reviews in Food Science and Food Safety*, **10**(3), 169–182.

De Vuyst, L., Neysens, P., 2005. The sourdough microflora: biodiversity and metabolic interactions, *Trends in Food Science & Technology*, **16**(1), 43-56.

Fu, W., Liu, C., Meng, X., Tao S., Xue, W. 2021. Co-culture fermentation of *Pediococcus acidilactici* XZ31 and yeast for enhanced degradation of wheat allergens, *International Journal of Food Microbiology*, **347**, 109190.

Harley, J.P., Prescott, L.M.M. 2008. Laboratory exercises in microbiology, 7th ed.; McGraw-Hill higher education: New York City, NY, USA.

Ibrahim, A., Fridayanti, A., Delvia, F. 2017. Isolasi dan identifikasi bakteri asam laktat (bal) dari buah mangga (*Mangifera indica* L.), *Jurnal Ilmiah Manuntung*, **1**(2), 159.

Islam, M.A., Islam, S. 2024. Sourdough bread quality: Facts and factors. *Foods*, **13**(13), 2132.

Rocha, J.M. 2011. Microbiological and lipid profiles of broa: contributions for the characterization of a traditional Portuguese bread, *Superior School of Agriculture, University of Lisbon* (ISA-UL): Lisbon, Portugal.

Sanpa, S., Sanpa, S., Suttajit, M. 2019. Lactic acid bacteria isolates from Pla-som, their antimicrobial activities and fermentation properties in Pla-som., *Journal of Food Health and Bioenvironmental Science* (January-April 2019), **12**(1), 36-43.

Schnurer, J., Magnusson, J., 2005. Antifungal lactic acid bacteria as biopreservatives – review, *Trends in Food Science & Technology*, **16**(1–3), 70-78.

Sekwati-Monang, B., Valcheva, R., Gänzle, M.G. 2012. Microbial ecology of sorghum sourdoughs: effect of substrate supply and phenolic compounds on composition of fermentation microbiota. *International Journal of Food Microbiology*, **159**(3), 240-246.

Siepmann, F.B., Ripari, V., Waszczynskyj, N., Spier, M.J.R. 2018. Overview of sourdough technology: from production to marketing. *Food Bioprocess Technol*ogy, **11**(1),1-29.

Shen, J., Shi K., Dong, H., Yang, K., Lu, Z., Lu, F., Wang, P. 2022. Screening of sourdough starter strains and improvements in the quality of whole wheat steamed bread. *Molecules*, **27**(11), 3510.

Şanlıbaba, P., Güçer, Y., 2015. Antimicrobial activity of lactic acid bacteria. *Journal of International Scientific Publications*, **3**, 451-457.

Vaiciulyte-Funk, L., Žvirdauskiene, R., Šalomskiene, J., Šarkinas, A. 2015. The effect of wheat bread contamination by the *Bacillus* genus bacteria on the quality and safety of bread, *Zembirbyste-Agriculture*, **102**(3), 351–358.

Wang, Y., Wu, J., Lv, M., Shao, Z., Hungwe, M., Wang, J., Bai, X., Xie, J., Wang, Y., Geng, W. 2021. Metabolism characteristics of lactic acid bacteria and the expanding applications in food industry, *Frontiers in Bioengineering and Biotechnology*, **9**, 612285.