

Characterization of functional effects of different LAB isolated from sourdoughs in Turkey

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Abstract

In this study, the technological properties of five different lactic acid bacteria (LAB) isolated from sourdough collected from three cities of Turkey (Gümüşhane, Manisa, Ankara) and Cyprus were investigated. For this purpose, antimicrobial, antifungal, phytase, and proteolytic activities of these bacteria and their effect on pH were examined. The pH of the prepared solutions decreased to 3.8 and 4.4 from 6.3 by LAB addition following 24 h incubation. *Lactiplantibacillus plantarum* O6F-25 strain showed the best inhibitory effect against four gram-positive and four gram-negative bacteria. In terms of proteolytic activity, *Lactiplantibacillus plantarum* 45MK-32 was the most effective strain. The antifungal effects of LAB were tested against *Aspergillus flavus*, *Aspergillus niger*, and *Penicillium carneum*. *Levilactobacillus brevis* KCO-48 was the most effective LAB strain. Phytase activities (710.40 – 840.37 U.mL⁻¹) of LAB studied except *Limosilactobacillus fermentum* (29GT-19), which has the lowest phytase activity, were not significantly different ($P < 0.05$). This study revealed that sourdough LABs have the potential to be used as biopreservative and produce functional food products.

Introduction

Sourdough technology is one of the earliest fields of biotechnological applications, mainly originated from the fermentation of grain matrices by natural lactic acid bacteria (LAB) (Gobbetti *et al.* 2014). However, the trend bringing about the consumption of health-promoting foods has increased the production of functional and nutritional products (Teleky *et al.* 2020). Sourdough bread meets this demand of conscious consumers because it offers several important health benefits such as lowering glycemic index, increasing mineral bioavailability, antimicrobial agent, and bioactive peptide formation, thus promoting clean label bread production (Rizzello *et al.* 2017; Tsafraquidou *et al.* 2020).

Internal and external factors influence sourdough production. Flour type and quality, fermentation temperature and time, water activity, relative humidity, dough yield, pH and acidification, oxygen tension, and backslapping dynamics determine sourdough characteristics (De Vuyst *et al.* 2017). Since traditional spontaneous sourdough is time-consuming and trying, it needs skilled labour, and causes differences in quality of final bread, liquid or dried starter cultures are chosen for industrial processes (Siepmann *et al.* 2018). The demand for the stable composition of starter cultures in the modern sourdough-making industry encouraged various studies on the selection of starter cultures with superior functionality (Gänzle and Ripari 2016). The sourdough microbiota is the primary influencer of the technological and

nutritional value of the sourdough bread (Sakandar *et al.* 2019). Sourdough microbiota consists mainly of LAB and yeast of various genera and species. Most LAB species isolated from sourdoughs belong to the genus *Lactobacillus* with more than 60 species (De Vuyst *et al.* 2017). *Lb. brevis*, *Lb. fermentum*, *Lb. paralimentarius*, and *Lb. plantarum* are among the most prevalent LAB present in sourdough (Minervini *et al.* 2014).

Many of the studies have focused on specific technological and nourishing effects of sourdough LAB such as proteolytic activity (Ozturkoglu-Budak *et al.* 2016; Melini and Melini 2018), phytase activity (Cizeikiene *et al.* 2015; Karaman *et al.* 2018), antibacterial activity (Cizeikiene *et al.* 2013; Petkova *et al.* 2021), antifungal activity (Demirbaş *et al.* 2017; Quattrini *et al.* 2019). In addition, low pH resulting from LAB's acid production increases the activities of amylases and proteases present in the flour, thus contributing to bread rheology and flavor via influencing gluten structure (Yildirim-Mavis *et al.* 2019). Furthermore, antimicrobial and antifungal activities sourced from LAB metabolites improve the shelf life and safety of the final sourdough bread (Demirbaş *et al.* 2017). Therefore, antifungal starter cultures have been successfully applied in bread (Axel *et al.* 2016). Another essential feature of sourdough fermentation is the stimulating impact on phytic acid degrading enzymes. Phytic acid degradation leads to increased mineral, free amino acid, and protein bioavailability (Gobbetti *et al.* 2014).

Many studies revealed strain dependency of LAB characteristics (Cizeikiene *et al.* 2013; Melini and Melini 2018; Cizeikiene *et al.* 2015; Ozturkoglu-Budak *et al.* 2016; Demirbaş *et al.* 2017). In this study, five LAB species among 17 isolates of sourdoughs were selected via 16S rDNA gene sequencing and FT-IR spectroscopy. The study aims were to assess the technological and nutritional characteristics of selected LAB. For this purpose, antibacterial, antifungal, proteolytic, phytase activities and effects of these LABs were analysed.

Experimental

Bacterial strains and growth conditions

LAB strains used within the scope of this study were isolated from sourdoughs collected from four different regions of Turkey. The type of flour, dough yield and depository used in the collected sourdoughs were given in Table 1. Each strain was grown in De Man Rogosa Sharpe (MRS, Merck KGaA, Darmstadt, Germany) medium for 24 h at 37 °C and under anaerobic conditions. Five LAB were chosen among 17 isolated bacteria based on 16S rDNA gene sequencing and FT-IR spectroscopy for further analysis.

Table 1. The type of flour, dough yield and depository used in the collected sourdoughs.

Sourdough code	Flour Type	Depository	Efficiency of sourdough
29GT-19	Wheat and rye	Bakery	250
06B-2	Wheat	Bakery	250
O6F-25	Wheat	Bakery	250
KCO-48	Wheat	Home made	150
45MK-32	Wheat	Bakery	250

FT-IR measurements

The isolated sourdough LAB strains were anaerobically cultivated on All Purpose Tween Agar (APT) at 34 °C for 24 h. 25 µL of cell suspension of grown cells suspended and homogenized in 100 µL distilled water was placed on ZnSe table and then dried at 40 °C. The spectral measurements were practiced by a Tensor 27 spectrophotometer (Bruker Optic GmbH & Co. KG, Ettlingen, Germany) equipped with an HTS-XT unit in the range of 600 – 4,000 cm⁻¹ wavenumbers. The spectra of the isolates were processed using OPUS software (Karaman *et al.* 2018).

Proteolytic activity of lactic acid bacteria

Milk agar plates, which include 28 g.L⁻¹ skim milk powder, 5 g.L⁻¹ casein peptone, 2.5 g.L⁻¹ yeast extract, 1 g.L⁻¹ glucose, and 15 g.L⁻¹ agar were prepared to evaluate the proteolytic activity of LAB strains. Then, four 20 µL cell spots of LAB strains were placed on the prepared agar, and plates were incubated anaerobically for 16 h at 37 °C. Following 48 h incubation, proteolytic activity was indicated as clear zone surrounding LAB cell spots (Axel *et al.* 2016).

Quantitative determination of extracellular phytase activity of LAB strains

The phytase activities of LAB isolated from sourdough samples were determined by adding 0.1 mL of the broth, including LAB strains previously grown in fresh MRS medium into 4.9 mL modified MRS medium with 0.1 % Na-phytate and 0.2 % glucose. Following incubation at 30 °C for 24 h, the LAB cells were separated from the medium by centrifugation at 9,000 rpm at 4 °C. Then, 250 µL cell-free supernatant and 250 µL substrate prepared by solving 2 mmol.L⁻¹ Na-phytate in 0.1 mol.L⁻¹ sodium acetate-acetic acid were mixed. The mixture was allowed for the reaction at 50 °C for 15 min. In order to cease the reaction, 500 µL of a 10 % (w/v) trichloroacetic acid (TCA) solution was used. A mixture of 2.5 % (w/v) ammonium molybdate solved in a 5.5 % (v/v) sulfuric acid solution and 2.5 % (w/v) ferrous sulfate solution in the ratio of 4 : 1 was used to obtain the color reagent. KH₂PO₄ at diverse concentrations were used as phosphorous source to form calibration curve. The absorbance values of samples were measured at 700 nm (Yildirim and Arici 2019).

Determination of the antibacterial activity of sourdough LAB strains

The antimicrobial activities of LAB sourdough strains were determined by the method adapted from Demirbaş *et al.* (2017). The inhibitory effects of LAB were tested against four gram-negative pathogens; *Salmonella typhimurium* ATCC 0402, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 06023 and four gram-positive pathogens; *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 13932, *Bacillus cereus* ATCC 11778, and *Bacillus subtilis* ATCC 6633. The isolated sourdough strains were grown overnight with 1 % inoculation in 10 mL of MRS broth to determine antibacterial activity. The grown cells in the culture were separated at 14,000 × g for 5 min, 4 °C by centrifugation. After the centrifuge, with a sterile 0.22 µm syringe filter, the supernatant was filtered to remove all bacteria cells that had remained in the supernatant. Cell-free supernatant (CFS) was adjusted to pH 6.0 with NaOH then treated with catalase (Merck KGaA, Darmstadt,

Germany) at 25 °C for 30 min. Thus, possible inhibition effects of organic acids with H₂O₂ were eliminated. 20 µL supernatant was poured into the wells punched in Tryptic Soy Agar plates where pathogen strains previously spread and allowed to incubate at 37 °C for 24 h. Clear zones were measured as mm around the wells.

Determination of antifungal activities of sourdough isolates

The lactic acid bacteria isolated from sourdough were grown at 37 °C for 24 h with 1 % inoculation in 10 mL of MRS broth. The suspensions were arranged at concentration 10⁷ CFU.mL⁻¹, and following the overnight growth, two 5 µL cell spots of LAB strains were placed on the MRS agar plates, and plates were incubated anaerobically for 48 h at 37 °C. Afterward, 10 mL of soft malt extract agar (2 % glucose, 0.8 % agar) at suitable temperature comprising 10⁴ CFU.mL⁻¹ *Penicillium carneum*, *Aspergillus flavus*, *Aspergillus niger* were inoculated on lactic acid bacteria in plates and incubated for 2 – 3 d at 25 °C. After that, the antifungal activity was classified as no inhibitory (-), delayed spore formation with no clear zone of inhibition (+), delayed spore formation with the small clear zone of inhibition (++) , delayed spore formation with the good clear zone of inhibition (+++), and broad inhibition on spore formation and mycelial growth with remarkable zones around a colony (++++) (Demirbaş *et al.* 2017).

pH decrease by lactic acid bacteria metabolites

To determine the effect of metabolites produced by LAB strains and pH decrease, cultures were grown with 1 % inoculation in 10 mL of MRS broth. The pH measurement was fulfilled after 3, 6, 9, and 24 h by pH meter (Hanna Instruments Deutschland GmbH, Vöhringen, Germany).

Statistical analysis

One-way analysis of variance (ANOVA) followed by Tukey's test ($P < 0.05$) was used to determine the difference between strains depending on the measured characteristics. Minitab version 17.3.1 (Minitab, LLC, State College, USA) and JMP 9 version was used for this purpose. Each calculation

was given as mean ± standard deviation of three replicates.

Results

FT-IR spectra of LAB isolates

FT-IR microspectroscopy was exploited to identify the microorganisms. This technique is particularly utilized to characterize LAB in the microbiological field. This study provided a quick description of LAB biodiversity in sourdough samples. LAB identification was accomplished thanks to both 16S rDNA gene sequencing and FT-IR spectroscopy at HTS-XT module. As demonstrated in Fig. 1, 16 LAB isolates were clustered and obtained

dendrogram gave five different groups. The groups have included 6 *Lactiplantibacillus plantarum*, 5 *Levilactobacillus brevis*, 2 *Limosilactobacillus fermentum*, 2 *Bacillus haynesii*, 1 *Lactiplantibacillus pentosus*, respectively. 16S-rDNA based phylogenetic tree was formed as shown in Fig. 2 attributed to the dendrogram. Therefore, 5 different *Lactobacillus* species, which are *Levilactobacillus brevis* KCO-48, *Lactiplantibacillus plantarum* 06F-25, *Companilactobacillus paralimentarius* 06B-2 and *Limosilactobacillus fermentum* 29GT-19 and *Lactiplantibacillus plantarum* 45MK-32 were selected. Then, these species were tested for their technological characteristics.

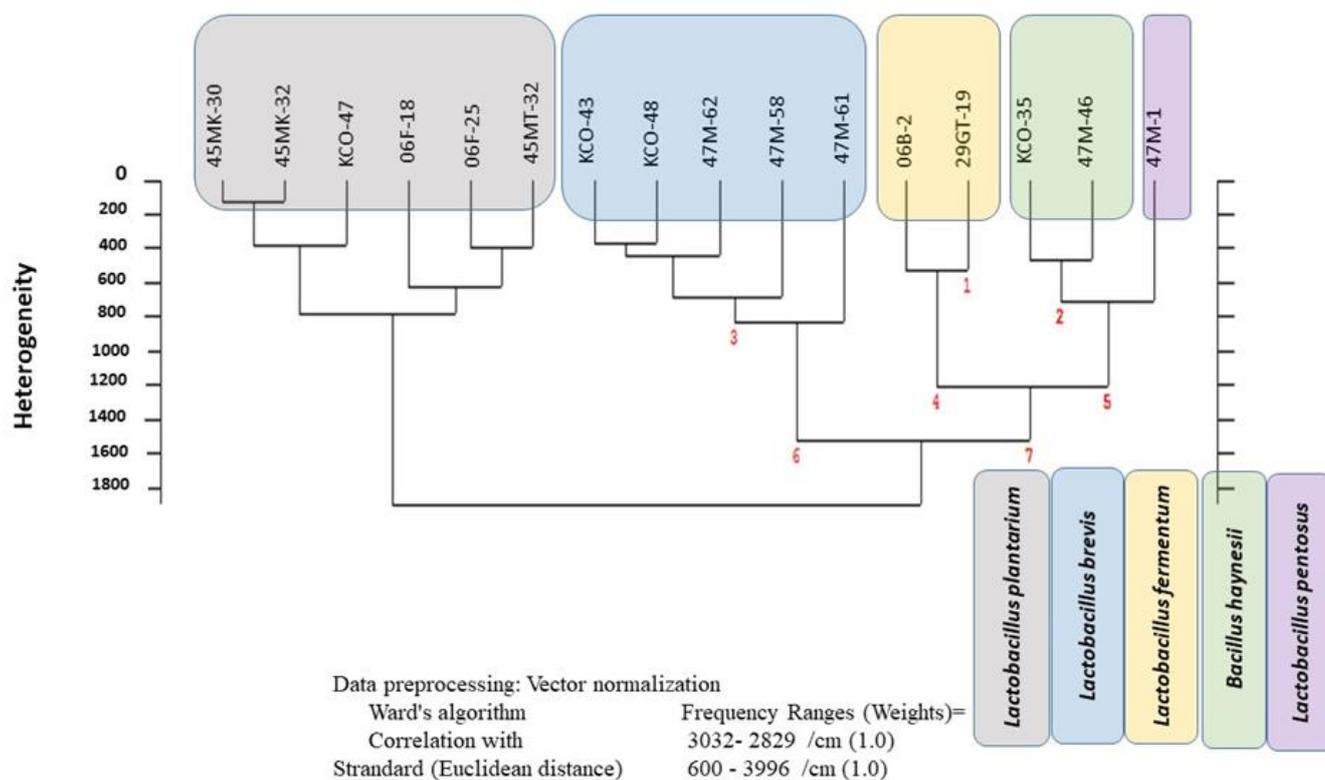


Fig 1. Dendrogram obtained based on FTIR spectra of the isolated LAB. Spectral ranges: 600 – 3,996 and 3,032 – 2,829 cm⁻¹. Ward's algorithm and correlation with standard (Euclidean distance).

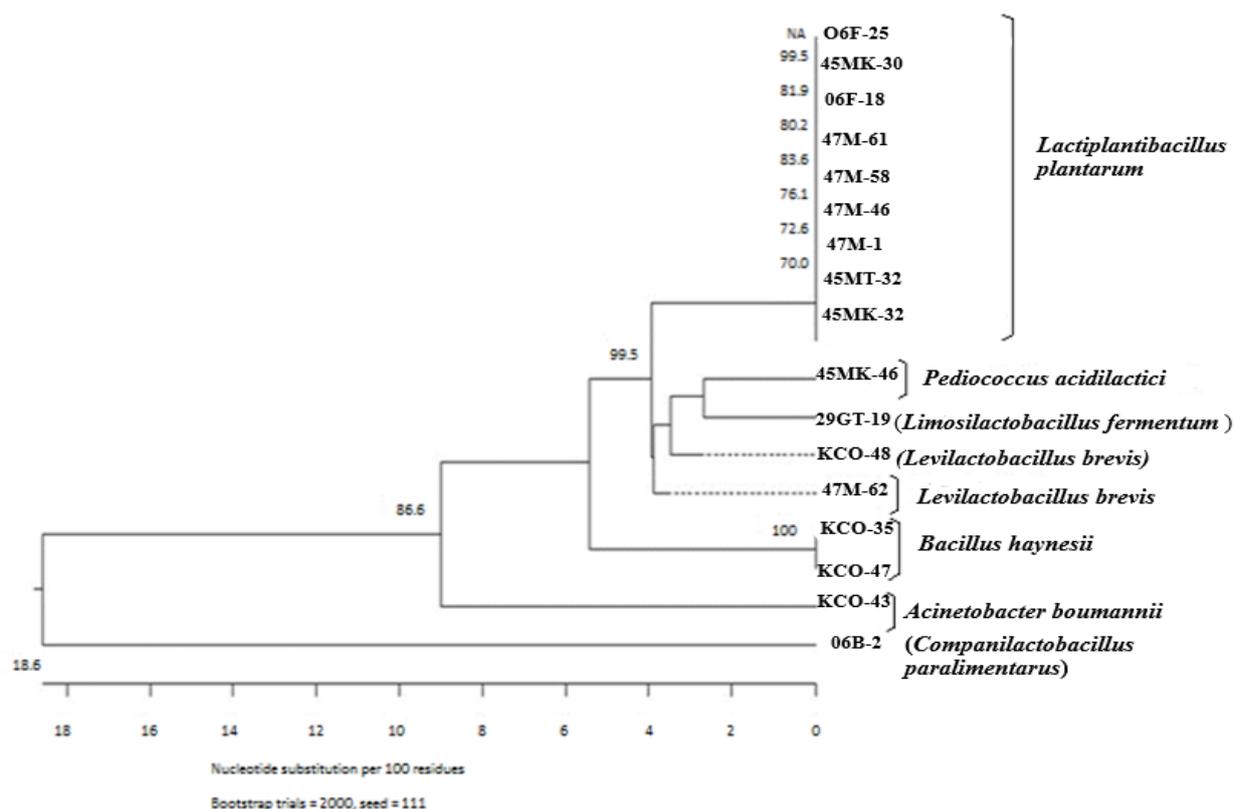


Fig 2. Phylogenetic tree for 17 isolates based on 16S rDNA gene sequencing. Phylogenetic reconstruction was generated via Neighbor-Joining method with a length of 406 nucleotides, 16S rDNA fragments. The count of bootstrap replicates is 2,000. Bootstrap values higher than 70 % are displayed. The bar shows the length corresponding to 0.1 nucleotide substitution.

Determination of proteolytic activity

Five LAB were screened for their protease activity on casein-containing plates. The proteolytic activities of LAB isolates were determined by measuring clear zone diameter derived from protease enzymes produced by LAB on skim milk agar. LAB isolates studied except *Levl. brevis* KCO-48 demonstrated proteolytic activity. Clear zone diameters ranged from 9.97 to 12.60 mm as stated in Table 2.

Table 2. Proteolytic activity diameters of clear zones.

Microorganisms	Clear zone diameter [mm]
<i>Levl. brevis</i> KCO-48	-
<i>Liml. fermentum</i> 29GT-19	10.0 ± 0.00 ^b
<i>Lpb. plantarum</i> 06F-25	10.0 ± 0.00 ^b
<i>Coml. paralimenterius</i> 06B-2	10.0 ± 0.00 ^b
<i>Lpb. plantarum</i> 45MK-32	12.6 ± 0.29 ^a

^{a-b}Different letters indicates the significant differences between the selected strains ($P < 0.05$).

The highest proteolytic activity was produced by *Lpb. plantarum* 45MK-32. Differences in

proteolytic activities of *Liml. fermentum* 29GT-19, *Lpb. plantarum* 06F-25, *Coml. paralimenterius* 06B-2 were negligible ($P > 0.05$).

Extracellular phytase activity

Extracellular phytase activity of LAB isolated from sourdough were determined exploiting the addition of sodium phytate as substrate to MRS broth and release of phosphorous compound by phytase activity produced by LAB. As shown in Fig. 3, the phytase activities were found between 710 U.mL⁻¹ and 841 U.mL⁻¹. The LAB strain with the highest phytase activity is *Levl. brevis* KCO-48 with the value of 840.6 U.mL⁻¹ whereas the lowest phytase activity occurred by *Liml. fermentum* 29GT-19 with the value of 710.4 U.mL⁻¹. The phytase activities of *Lpb. plantarum* 45MK-32 and *Lpb. plantarum* 06F-25 were found 825.2 and 821.67 U.mL⁻¹, respectively. The phytase activity of *Coml. paralimenterius* was detected similarly to the *Lpb. plantarum* species. No significant difference was found among phytase activities of LAB studied in the study, excluding *Liml. fermentum* 29GT-19.

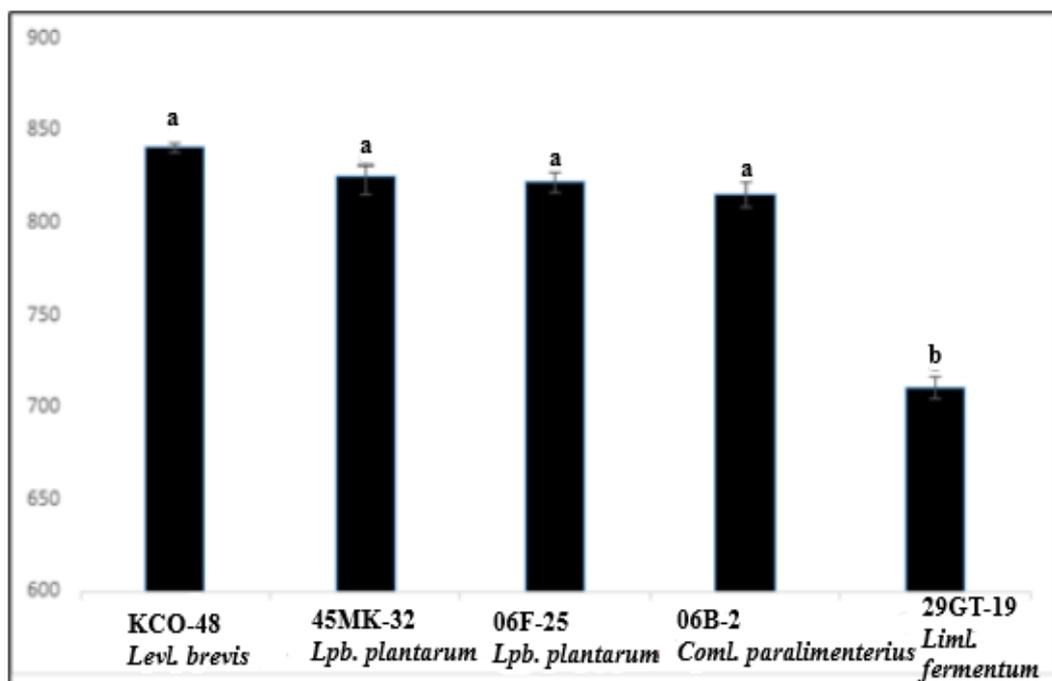


Fig. 3. Extracellular phytase activities of LAB isolates (U.mL⁻¹). ^{a-n}Within the column, the letters indicate that there is significant difference between the strains ($P < 0.05$).

Antimicrobial activity of LAB against pathogens

In this study, the antimicrobial capacity of neutralized and catalase-treated cell-free supernatants (CFS) of LAB growth media was tested against 8 pathogenic bacteria to determine the inhibitory action of bacteriocin-like inhibitory

substances (BLIS). In agar well diffusion method, clear zone diameter has been studied to determine the antimicrobial activity. The zone diameters of the inhibition area were given in Table 3 and the figure of the *Lpb. plantarum* strain that creates the best antimicrobial zone is given in Fig. 4.

Table 3. Antimicrobial activity of CFS of LAB strains against pathogenic bacteria.

Pathogens	Zone of inhibition [mm]				
	<i>Liml. fermentum</i> 29GT-19	<i>Lpb. plantarum</i> 06F-25	<i>Coml. paralimenterius</i> 06B-2	<i>Levl. brevis</i> KCO-48	<i>Lpb. plantarum</i> 45MK-32
<i>K. pneumoniae</i> ATCC 06023	10.0 ± 0.00 ^a	10.0 ± 0.00 ^a	10.0 ± 0.00 ^a	10.0 ± 0.00 ^a	10.0 ± 0.00 ^a
<i>P. aeruginosa</i> ATCC 27853	nd	10.0 ± 0.00 ^a	nd	nd	nd
<i>B. subtilis</i> ATCC 6633	10.2 ± 0.03 ^a	10.0 ± 0.00 ^a	10.0 ± 0.00 ^a	10.0 ± 0.00 ^a	10.0 ± 0.00 ^a
<i>L. monocytogenes</i> ATCC 13932	12.0 ± 0.00 ^a	11.6 ± 0.03 ^b	10.0 ± 0.00 ^e	10.2 ± 0.03 ^d	11.0 ± 0.00 ^c
<i>B. cereus</i> ATCC 11788	12.0 ± 0.00 ^d	13.2 ± 0.03 ^a	12.8 ± 0.03 ^b	10.8 ± 0.03 ^e	12.6 ± 0.03 ^c
<i>S. typhimurium</i> ATCC 0402	12.0 ± 0.00 ^a	12.0 ± 0.00 ^a	11.8 ± 0.03 ^b	11.0 ± 0.00 ^d	11.6 ± 0.03 ^c
<i>E. coli</i> ATCC 25922	10.0 ± 0.00 ^a	10.0 ± 0.00 ^a	10.0 ± 0.00 ^a	10.0 ± 0.00 ^a	10.0 ± 0.00 ^a
<i>S. aureus</i> ATCC 25923	12.2 ± 0.03 ^a	12.2 ± 0.03 ^a	11.8 ± 0.03 ^b	11.8 ± 0.03 ^b	10.0 ± 0.00 ^c

^{a-b} Different letters indicates the significant differences between the selected ($P < 0.05$). nd – no inhibition zone was detected.

All bacteria displayed mild or moderate inhibition of pathogens. The highest antimicrobial activity of the supernatants was detected against *B. cereus* ATCC 11778. *Lpb. plantarum* 06F-25 was only strain that inhibits all pathogens. *P. aeruginosa* inhibited only by *Lpb. plantarum* 06F-25 metabolites has become the most resistant bacteria. The highest zone diameters were observed against *B. cereus* ATCC 11778 by *Lpb. plantarum* 06F-25, *Coml. paralimentarius* 06B-2, *Lpb. plantarum* 45MK-32. Even though *Lpb. plantarum* 06F-25

and *Lpb. plantarum* 45MK-32 are the same species, they have differed in antibacterial effect against 6 out of 8 pathogens. LAB were found more efficient on gram-positive pathogens than gram-negative pathogens. *S. typhimurium* ATCC 0402 was the least resistant gram-negative pathogen with an average 11.7 mm zone diameter. However, the zone diameters resulted from CFS on other gram-negative bacteria ≤ 10 mm. The lowest inhibition on gram-positive pathogens was observed on *B. subtilis* ATCC 6633 with an average 10.0 mm zone diameter.

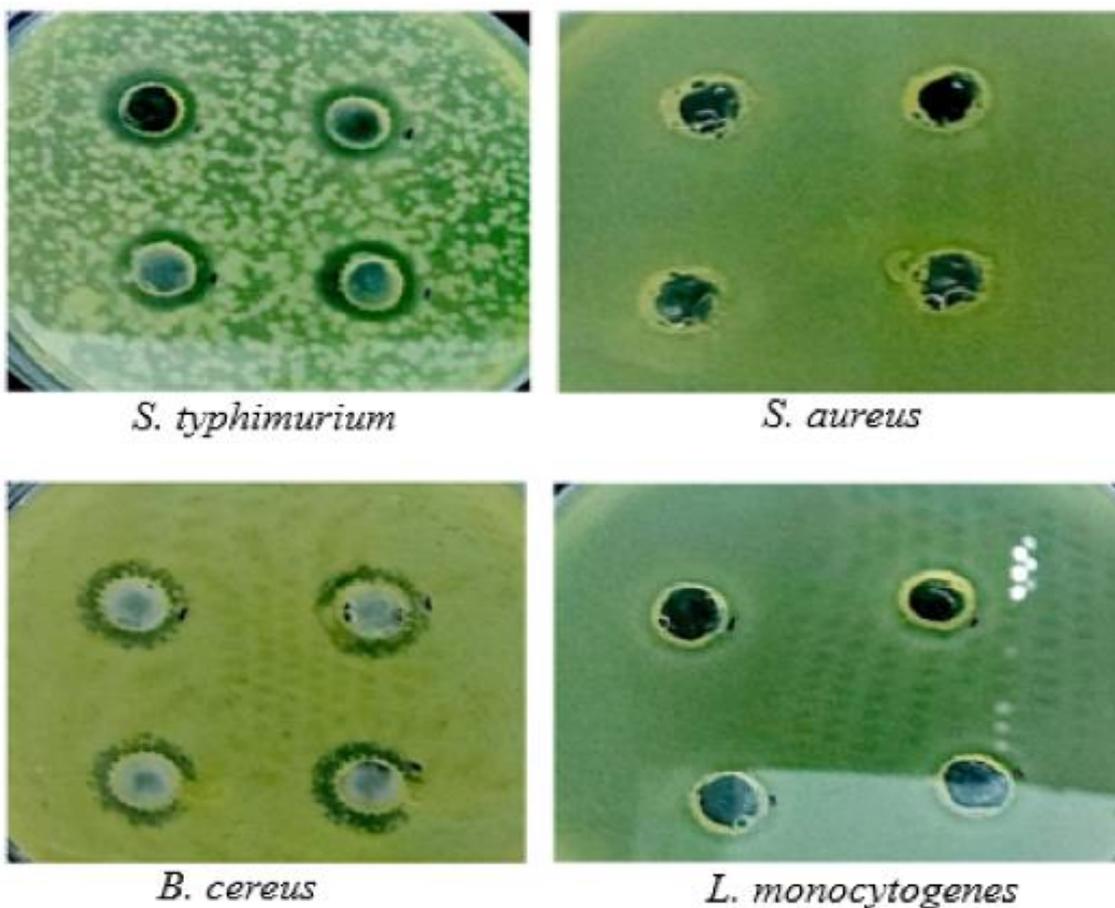


Fig. 4. Figure of best zone measurement.

Antifungal activities of lactic acid bacteria

The influence of LAB grown in MRS broth on several mould species such as *Penicillium carneum*, *A. flavus*, and *A. niger* was analyzed to test antifungal activity. Their effects on the moulds were given in Table 4. *P. carneum* was not inhibited by LAB. Even though any LAB did not have any antifungal effect on *P. carneum*, all tested LAB inhibited *A. flavus*. All LAB excluding *Coml.*

paralimentarius 06B-2 also showed inhibitory impact on *Aspergillus niger*. The highest inhibitory impact was demonstrated against *A. flavus* and *A. niger* by *Coml. paralimentarius* 06B-2 and *Liml. fermentum* 29GT-19. *Lpb. plantarum* species 45MK-32 and 06F-25 demonstrated lower inhibitory action with a small clear zone with delayed spore formation than *Liml. fermentum* and *Levl. brevis* against *A. niger*.

Table 4. Inhibition of several moulds by LAB isolates.

Microorganisms	Inhibitory spectrum of LAB supernatants		
	<i>Penicillium carneum</i>	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>
<i>Liml. fermentum</i> 29GT-19	-	++	++++
<i>Lpb. plantarum</i> 06F-25	-	++	+
<i>Coml. paralimenterius</i> 06B-2	-	++++	-
<i>Levl. brevis</i> KCO-48	-	+++	+++
<i>Lpb. plantarum</i> 45MK-32	-	++	+

The mould inhibition of LAB was classified as; (-) no inhibition, (+) spore formation delayed but no clear zone, (++) spore formation delayed with a small clear zone around the colony, (+++) spore formation delayed with good clear zone around the colony, (++++) extensive inhibition of spore formation and mycelial growth with definite clear zones around colonies.

pH decrease due to the metabolites produced by LAB

The pH values of MRS broths including LAB isolates were measured every 3 h in the first 9 h and 24th h. The measurement results were given in Table 5. All LAB have a strong impact on the pH

value of the MRS broth medium. Whereas initial pH values of broths ranged between 6.51 and 6.56, the last pH of broths varied from 3.83 to 4.41. The highest decrease in pH value by 3.83 was observed in *Lpb. plantarum* 45MK-32 containing broth. *Liml. fermentum* 29-GT-19 was found the least effective strain on pH.

Table 5. pH decreasing effect of LAB.

Microorganisms	Time [h]			
	3 h	6 h	9 h	24 h
<i>Lpb. plantarum</i> 06-F25	6.38 ± 0.02 ^{ABa}	6.18 ± 0.02 ^{Bb}	5.14 ± 0.02 ^{Dc}	4.04 ± 0.01 ^{Cd}
<i>Levl. brevis</i> K-CO48	6.40 ± 0.03 ^{ABa}	6.36 ± 0.02 ^{Aa}	6.09 ± 0.02 ^{Ab}	3.92 ± 0.03 ^{Dc}
<i>Coml. paralimenterius</i> 06-B2	6.27 ± 0.03 ^{Ca}	6.00 ± 0.03 ^{Cb}	5.31 ± 0.02 ^{Cc}	4.33 ± 0.03 ^{Bd}
<i>Liml. fermentum</i> 29-GT19	6.29 ± 0.03 ^{BCa}	6.04 ± 0.02 ^{Cb}	5.39 ± 0.03 ^{Cc}	4.41 ± 0.02 ^{Ad}
<i>Lpb. plantarum</i> 45-MK32	6.41 ± 0.03 ^{Aa}	6.37 ± 0.02 ^{Aa}	5.70 ± 0.02 ^{Bb}	3.83 ± 0.01 ^{Ec}

* Capital letters – differences in uppercase letters indicate significant difference ($P < 0.05$) in a row.

* Small letters – differences in lowercase letters indicate significant difference ($P < 0.05$) in a column.

Discussion

FT-IR analysis is a thought reliable method to identify bacteria and yeast with chemometric techniques (Wenning and Scherer 2013). FT-IR analysis was conducted on 17 sourdough LAB isolates brought from 7 different regions of Turkey. First derivatives of the obtained spectra included in 1,500 – 1,200 cm^{-1} , 1,200 – 900 cm^{-1} , 900 – 700 cm^{-1} spectral regions were utilized to differentiate spectra of *Lactobacillus* species that had been studied before (Akman et al. 2021). In this study, LAB isolated from sourdough samples was chosen exploiting FT-IR cluster analysis, which gives faster results than RAPD-PCR. According to the cluster analysis, 16 LAB isolates were genotypically identified. Based on 16S rDNA sequencing, among 17 LAB, 9 *Lpb. plantarum*, 2 *Levl. brevis*, 1 *Liml. fermentum*, 1 *Coml. paralimenterius*, and 4 *Bacillus haynesii* were

determined. In addition, 16S rDNA-based phylogenetic tree of LAB isolates using the neighbor-joining method presented the conformable phylogenetic cluster. Whereas 45MK-32 and 06F-25 isolates were clustered on the same branch, 29GT-19, KCO-48, and 06B-2 isolates were clustered on distinct branches. The similarity of *Lpb. plantarum* isolates were specified by phylogenetic clustering. It was concluded that cluster analysis of 17 LAB isolates was consistent with grouping attributed to 16S rDNA and separated these isolates into different groups phylogenetically.

Proteolytic activity has an important role in the quality of bread. Proteolysis derived from LAB increases amino acid amount in sourdough; hence, it affects flavor development by reaction between amino acids and reducing sugar during baking (Gil-Cardoso et al. 2021), and Atanasova et al. (2014) found that the antimicrobial-active peptides were

formed by LAB grown in goat milk. LAB isolated from yogurt samples and described by biochemical and morphological analysis for their proteolytic activities were studied, and it was found that all LAB isolates have proteolytic activities (Phyu *et al.* 2015). Moreover, Axel *et al.* (2016) investigated LAB isolated from sourdough samples and the lowest proteolytic activity were detected in *Levl. brevis* incompatible with the finding in our research. Two different *Lpb. plantarum* strains 45MK-32 and 06F-25 have different clear zone diameters displaying that proteolytic activity of LAB is strain-specific. Microbial acidification provides favorable environment (pH 3.5 – 4.0) for aspartic proteinases (Gänzle *et al.* 2008). Hence, extracellular proteinases of LAB are influenced by medium pH. *Lpb. plantarum* 45MK-32, which has the highest proteolytic activity in our study, was the most efficient to alter the pH of MRS medium leading to the formation of favourable conditions for proteinase activity.

Whole meal bread consumption has remarkably increased since awareness of its nutritionally valuable components, such as dietary fibers, vitamins, minerals, complex carbohydrates, and proteins (Mohammadi-Kouchesfahani *et al.* 2019). However, phytic acid, the major phosphorus form in cereal grains including wheat, impedes the absorption of Fe^{+2} , Zn^{+2} , Ca^{+2} , Mg^{+2} , Cu^{+2} , and Mn^{+2} ; thus, mineral deficiencies are promoted (Ficco *et al.* 2009; Liu *et al.* 2019). LAB does not degrade phytate directly; however, it presents favourable conditions by decreasing pH for extracellular phytase activity (Reale *et al.* 2007). Nevertheless, some studies have been conducted which indicate intracellular phytase activity of LAB (Nuobariene *et al.* 2015; Yildirim and Arici 2019). In the study conducted by Karaman *et al.* (2018) the extracellular phytase activities of LAB strains obtained from sourdough were found to range from 703.1 U-mL⁻¹ to 1153.8 U-mL⁻¹. Yildirim and Arici (2019) also examined 64 LAB isolates from sourdough in terms of extracellular phytase activity. The results varied between 725.58 U-mL⁻¹ and 803.95 U-mL⁻¹ in accordance with the results in our study. Sourdough bread consumption offers health-promoting effect since LAB can degrade phytate in whole wheat flour which contains high amount of phytate. Therefore, the decrease in mineral uptake can be prevented (Lopez *et al.* 2003; Rizzello *et al.* 2017).

Furthermore, LAB's phytase activity benefits the small intestine via decomposing the remaining phytate (Haros *et al.* 2009). Moreover, the usage of phytase-active LAB enables to minimize anti-nutritional characteristics of animal feeding (Humer *et al.* 2015).

It is a fact that LAB's antimicrobial characteristics can be exploited to prevent spoilage of foods and kill pathogenic bacteria and the benefits in the large intestine as probiotic. LAB produces organic acids, hydrogen peroxide, diacetyl, and bacteriocin-like inhibitory substances which inhibit bacterial growth. Bacteriocins can promote invasion of beneficial strains in the gastrointestinal tract via inhibiting competing or pathogen strains; hence, the microbiota and host immune system are regulated (Arqués *et al.* 2015). In our study, almost all LAB showed an inhibitory effect on common pathogens following neutralization and catalase treatment. Bacteriocin-producing ability of 437 LAB isolated from 70 sourdough samples were tested (Petkova *et al.* 2021). Whereas 85 of these strains are effective on indicator organisms without removing acids and other antimicrobial agents in cell-free supernatant (CFS), only five LAB strains inhibited indicator organisms after neutralization, catalase treatment and sterilization by filtration. Venkadesan *et al.* (2015) investigated the antimicrobial effect of 9 LAB isolated from milk products on different pathogens. *Liml. fermentum* strains led to moderate inhibition on *E. coli*, *S. aureus* and *S. typhi* and *L. monocytogenes* ATCC 13932 while *Lpb. plantarum* showed weak inhibition. Cizeikiene *et al.* (Cizeikiene *et al.* 2013) analyzed five LAB isolated from rye sourdough to specify their antimicrobial effect against 23 pathogenic bacteria, including *B. cereus*, *B. subtilis*, various *Pseudomonas spp.*, *Staphylococcus aureus*, *Listeria monocytogenes*, *E. coli*, and *Salmonella spp.* strains. The results showed that the BLIS of all LAB studied inhibited *B. subtilis* strains whereas no inhibitory effect was detected on other studied pathogens. Moreover, our study is consistent with the studies maintaining mild, low, or no inhibitory impact of LAB metabolites on well-recognized pathogens. Consequently, the antimicrobial capacity of LAB supernatants without acid and H₂O₂ removal is considerable; however, catalase treated and neutralized CFS influence on pathogenic bacteria is limited.

Mould growth is a major spoilage reason for bakery products. The most prevalent mould species are *Aspergillus*, *Fusarium*, and *Penicillium*. *Biopreservation techniques have been considered one of the main strategies to prevent spoilage owing to the chemical-free food demand of society (Melini and Melini 2018)*. LAB produces various antifungal compounds such as organic and carboxylic acids, reuterin, fatty acids, and cyclic dipeptides (Crowley *et al.* 2013). Sevgi and Tsveteslava (2015) reported that six different *Lpb. plantarum* strains retarded *A. niger* growth. In our study, *Lpb. plantarum* strains showed mild inhibitory effect by delaying spore formation on *A. niger* growth and their antifungal impacts were expressed as clear zone diameter around the LAB colonies. Moreover, Demirbaş *et al.* (2017) found that *Lpb. plantarum* has a moderate impact on *A. niger* with a small clear zone. Kivanc *et al.* (2011) analyzed 45 different LAB against 7 mould species by means of dual agar overlay and well method, *Levl. brevis* strains studied have clear inhibition zone around the colonies against *A. flavus* in accordance with *Levl. brevis* KCO-48 inhibitory impact on *A. flavus*. Antifungal action of LAB depends on mould species. Although *Coml. paralimentarius* showed the highest antifungal effect on *A. flavus*, it did not demonstrate any inhibiting impact on *Penicillium carneum* and *Aspergillus niger*. It was suggested that the metabolites produced by LAB inhibited mould growth and extended shelf life, thus demonstrating that LAB could be exploited as biopreservative (Muhialdin *et al.* 2011). Similarly, *Liml. fermentum* 29GT-19 showed extensive inhibition on *A. niger* with a good clear zone and delayed spore formation and moderate inhibition on *A. flavus*. The results showed that LAB inhibits *A. niger* and *A. flavus* differently depending on mould and LAB species. *Penicillium carneum* is the most resistant mould against all studied LAB. Antifungal metabolites producing LAB could be utilized as biopreservative in bakery products.

Conclusion

In summary, LAB isolates isolated from sourdough samples were tested for some technological and nutritional properties. These strains had antagonistic effects against pathogenic bacteria.

They tend to degrade and multiply phytic acid that binds minerals. Therefore, they have the potential to improve human health and product quality. It can be said that bioactive peptides and aroma compounds inhibit mould growth and lower the pH as much as preventing the survival of pathogens and spoilage microorganisms. The superior properties of LAB differentiate from each other. *Levl. brevis* KCO-48 demonstrated the highest phytase activity, *Lpb. plantarum* 06F-25 was only LAB that inhibits all tested pathogens. *Lpb. plantarum* 45MK-32 have the highest proteolytic activity and highest pH decreasing effect. *Liml. fermentum* 29GT-19 and *Levl. brevis* KCO-48 were most effective to hinder mould growth and spore formation considering total effects on studied moulds. The studied LAB can be exploited to promote human health and develop/preserve food quality when taking all their characteristics into account. Moreover, the utilization of these LAB as combination have promised functional products since they may meet different requirements in terms of product quality.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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