Journal of Food Measurement and Characterization (2020) 14:1646–1655
https://doi.org/10.1007/s11694-020-00412-5

ORIGINAL PAPER

The molecular and technological characterization of lactic acid bacteria in einkorn sourdough: effect on bread quality

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Received: 20 September 2019 / Accepted: 10 February 2020 / Published online: 19 February 2020
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Abstract

This study aimed to investigate the characterization of lactic acid bacteria strains (LABs) in spontaneous sourdough produced from einkorn flour as a new sourdough source and to determine some quality characteristics of sourdough bread. Thirty-two different LABs were isolated from spontaneously fermented einkorn sourdough under laboratory conditions. Seven different lactic acid bacteria strain (LAB) species, *Lb. crustorum* (dominant species 10), *Pediococcus* (4), *Lb.* brevis (6), *Lb. paraplantarum* (1), *Lb. plantarum* (5), *Lb. fermentum* (4), and *Lb. curvatus* (2) were identified by PCR (16S). *Lb. paraplantarum* and *P. acidilactici* showed high antibacterial activity against *B. subtilis* ATCC6633, *B. cereus* ATCC11778, and *E. coli* ATCC25922, and *Lb. crustorum MN047* and *L. brevis* R-1 showed an antifungal effect on *P. carneum, A. flavus*, and *A. niger.* At the same time, all strains showed an acid-tolerant effect, because they showed a survival ratio higher than 70% at pH 2.5. The highest phytase activity was observed from *L. paraplantarum* 2815 and *L. plantarum* AAS3. The study aimed to investigate *Lb. brevis* R-1 and *Lb. paraplantarum* 7285 starter culture effects and bread quality parameters on dough made solely with einkorn or with a mixture of 25%, 50%, or 75% einkorn flour, compared to a solely wheat flour sourdough. The sourdough bread containing 25% and 50% einkorn flour had better volume, specific volume, and textural properties than bread containing 75% and 100% einkorn flour; they were scored as good as 100% wheat dough bread in terms of sensory properties. This study recommends that einkorn sourdough could be utilized as a beneficial source, having a diverse range of LABs and strong technological properties.

Keywords Lactic acid bacteria (LAB) · PCR · Sourdough · Einkorn bread

Introduction

Due to an increase in consumer demand for high quality, healthy food, the importance of cereals with good nutrition and sensory properties has also increased [1]. The trend to use sourdoughs in bread production has increased due to the high sensorial and nutritional quality and shelf-life properties of the sourdough bread. The use of wheat or rye flour in producing sourdough has a long tradition in bread technology, but in recent years, the interest in other flours in sourdough fermentation has increased. Other flours are used in sourdough preparation mainly due to improved functional properties and nutritional values. Another reason for using other flours instead of wheat flour in sourdough production is special diets, for example those that require gluten-free bread [2].

Einkorn (*Triticum monococcum* L.) promises growth thanks to its specific quality and high nutritional value. Einkorn is a diploid species of wheat grown in the Mediterranean and in small areas of Europe [3]. Compared with other types of wheat, einkorn wheat contains more protein, lipids (unsaturated fatty acids), phosphorus, potassium, pyridoxine, lutein, and beta carotene [4]. Einkorn also has a lower content of beta-amylase and lipoxygenase to prevent the decay of compounds such as carotenoids, tocopherols, conjugated phenolics, and phytosterols and to protect the nutritive properties of food.

Sourdough can be produced both spontaneously and commercially. It has been reported that sourdough having single- or multiple-stage fermentation processes improves the texture, volume, and shelf life of bread [5–7]. Spontaneous sourdough, depending on the microflora of the flour,

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is started by the fermentation of the flour and water mixture. LABs and yeasts become the dominant species in the sourdough microbiota after it is fed a few times throughout the week [8, 9]. Commercial sourdough is produced using starter cultures that consist of pure LABs and yeasts [7]. The origin of the flour, the storage conditions, the hygienic conditions, and the technological factors of the fermentation process affect the microflora of the spontaneous sourdough [7, 10].

While there are many studies on the nutritional value of the einkorn flour products [11–13], this is the first study to determine lab species in fermented Turkish einkorn sourdough. The primary objective of this research was to characterize LABs in spontaneous sourdough produced from einkorn flour and to explore the diversity of the LABs. Another aim of this study was to explore the technological characteristics of LABs and to investigate the potential of making sourdough bread formulated with 25%, 50%, 75%, and 100% einkorn flours by preparing a starter culture from bacteria with the best properties.

Materials and methods

Materials

Einkorn flour was provided by the Istanbul Halk Ekmek (İstanbul, Turkey). All chemicals used in this study were obtained from Merck (Darmstadt, Germany).

Preparation of spontaneous sourdough

Spontaneous sourdough was formed by fermenting a flour and water mixture without the addition of an external starter culture. Einkorn wheat flour and water were mixed by a dough blender (KitchenAid, Antwerp, Belgium) at a ratio of 1:1 to form sourdough. The dough was kneaded for 4 min for homogenization and was left to ferment at 28 °C for 24 h. Every 24 h, 100 g of flour and 100 mm of water were added to the dough; the fermentation process was continued for 4 days.

Isolation and identification of LABs from sourdough samples

LABs were isolated and counted using ModMRS, MRS, Rose Agar, and MRS-5 supplemented with cycloheximide. For each sourdough batch, serial tenfold dilutions were commonly performed using peptone solutions for sourdough samples. They were plated to the corresponding agar using the spread-plate procedure. After the plating, different colonies were selected from the plates containing the highest sample dilutions (10^{-6}). Stock cultures were prepared from selected colonies and were stored at − 80 °C with glycerol, as described by [14]. FTIR and sequence identifications of the isolates were also performed.

Identification of LABs by FTIR

LABs were grown on APT agar under anaerobic conditions at 34 °C for 48 h. The suspension prepared by a growing colony and 100 µL sterile distilled water was transferred to an Eppendorf tube. Of the resulting solution, 25 µL was transferred to the slices on the ZnSe FTIR plate and dried at 40 °C for 45 min. Measurements were conducted using an HTS-XT FTIR spectrometer in the region between 400 and 4000 cm^{-1} (Bruker, Karlsruhe, Germany). For data processing, OPUS software v.6 (Bruker) was used [15].

DNA extraction

For total DNA isolation, the colonies were transferred to sterile Eppendorf tubes (1.5 mL) from the isolates that were purified in the appropriate agar in the previous step. After centrifugation at 13,000×*g* for 10 min at 4 °C, pellets were frozen at − 80 °C for 15 min and were resuspended in 95 ml of 1X PCR buffer [16].

Bacterial identification by 16S rDNA gene sequencing

PCR was performed on LABs selected from different groups according to comparison results from FTIR. Identification was performed with 16S rDNA gene sequencing. Genes of isolates were amplified using three different primers, PEU7-DG74, p806-p8FPL, and 27F-1492R. A PCR mixture within the 25 µL final reaction volume contained 10 × PCR buffer, 2.5 mM of dNTP mixture, 0.5 µM each of forward and reverse primers (PEU7 and DG74), and 5 U of Taq DNA polymerase (Sigma, USA) [17]. PCR was performed using the following cycling conditions: 95 °C for 5 min (initial denaturation), 30 cycles at 94 °C for 15 s/52 °C for 30 s/72 °C for 2 min, and a final extension at 72 °C for 5 min; p806-p8FPL cycling conditions were 94 °C for 5 min, 35 cycles at 94 °C for 1 min/75 s at 56 °C/75 s at 72 °C, and a final extension for 10 min at 72 °C. For the 27F-1492R primer pairs, DNA was denatured at 94 °C for 2 min and was amplified for 35 cycles at 94 °C for 40 s, at 45 °C for 50 s, and at 72 °C for 50 s, with a final extension applied for 2 min at 72 °C [18].

The images of gels displaying PCR products were as taken after 1.5 h of electrophoresis at 100 V on 1.2% gel. Obtained sequences were searched in the Basic Local Alignment Search Tool (BLASTn) database, and the matching microorganism sequence records with their respective similarity percentages were determined [19].

pH and TTA analysis

The pH value of the sourdough samples was measured by a pH meter (HANNA Instruments, Italy). Titratable acid analysis (TTA) was conducted according to the method described by [20].

Technological capacity characterization of LABs

Bile, pH, potassium sorbate, and NaCl tolerance

LABs were activated in the broth using the method described by [21], and their optical density (OD) on a 600 nm spectrophotometer was set to the value of 1. After their OD was set, the LAB bacteria were inoculated in an MRS broth composed of a pH value of 2.5 and 0.3% bile.

For K-sorbate and NaCl, LABs were inoculated to an MRS broth with 6% NaCl and on an MRS broth containing 100 ppm potassium sorbate; they were incubated at 37 °C for 24 h. The agar spot method was employed for plating on MRS agar. OD values were compared with those of the control group; the culture was counted after 24 h to calculate the surviving percentage as follows:

$$\text{Bile salt/pH} - \text{tolerant bacteria count (\%)} = A/B \times 100$$

where, A: Bile salt/pH broth bacteria count after 24 h and B: Initial bacteria count

$$\text{Potassium sorbate/NaCl} - \text{tolerant bacteria count (\%)} = A/B \times 100$$

where, A: Potassium sorbate/NaCl broth bacteria count after 24 h and B: Initial bacteria count.

Antimicrobial activity

The antimicrobial activity of the LABs was determined by the diffusion agar method. Pathogens were activated in the broth for 24 h at 37 °C. Pathogenic bacteria (*B. subtilis* ATCC6633, *B. cereus* ATCC11778, and *E. coli* ATCC25922, which were approximately 10^5–10^7 cfu/mL) were spread on nutrient agar. The LABs were activated at 37 °C and were inoculated in the amount of 1% into 10 mL of the MRS broth. After being centrifuged at 14,000×*g* for 5 min, 20 µL of the resulting supernatant was diffused into nutrient agar wells with a 2 mm diameter. Inhibition zones were measured after 24 h incubation at 37 °C. Diameters were measured in millimeters.

Antifungal activity

The antifungal activities of the sourdough isolates were determined against *Penicillium carneum, Aspergillus flavus*, and *Aspergillus niger*. Molds were grown in potato dextrose agar for 7 days at 30 °C. LABs were activated, scratched on the MRS agar, and grown for 24 h on MRS broth. The spot was diffused on 10 µL of MRS agar and incubated for 24 h at 34 °C. Then 10 mL of malt extract soft agar was overlaid and incubated aerobically for 48 h at 30 °C. The forming inhibition zones were examined [22].

Phytase activity

The ability of LABs to produce phytase was determined in the medium containing calcium phytate or sodium phytate. Pikovskaya's agar was prepared, and the LABs were inoculated via the drop method and were then incubated at 30 °C for 7 days. Subspecies around which transparent zones were observed were classified as phytase-positive. Subspecies around which no transparent zones observed were classified as phytase-negative. Zone diameters were measured in millimeters [23].

Preparation of sourdough

LAB and yeast isolates showing the best technological characteristics were selected to use in sourdough fermentation. Accordingly, the strains stocked at − 80 °C were first activated for 24 h on a solid medium (MRS agar), followed by the incubation of lactic acid bacteria in a 10 ml MRS broth medium for 24 h. Subsequently, the cultures were transferred to 50 mL of liquid media for a second time and were again incubated for 24 h, and the cell pellets were washed by centrifugation at 4 °C. After incubation pellets were obtained by centrifuging at 6000×*g* for 15 min, microbial cell pellets were used in preparations of einkorn and wheat dough.

Bread production

Einkorn flour was used to prepare blends with wheat flour in wheat flour/einkorn flour ratios of 0/100, 25/75, 50/50, 75/25, and 100/0. The water absorption of these flours was determined primarily on the Brabender farinograph. The farinograph values of these flours were 61%, 58.3%, 56%, 55%, and 52.5%, respectively. The sourdough samples were added to comprise 20% of the dough weight and salt (1.5% of dry matter). After 20 min at room temperature, the dough was fermented for 3 h at 30 °C and at 85% relative humidity in a fermentation cabinet (Nuve TK252, Turkey). The dough was baked in an electric oven (Fimak, 161 Turkey) for 40 min at a 190 °C top temperature. The bread was cooled at room temperature for 2 h before analysis.

Sourdough analysis, bread dough and bread analyses

After the produced pieces of bread reached room temperature, weight (g) measurements were made, and volume (mL) values were determined based on displacement with millet seeds. Specific volume (mL/g) values were found by proportioning the obtained volume values to the weight.

To determine the textural properties of the bread samples, each sample was sliced to 1.25 mm thickness; it was equipped with a 5 kg load cell and a 36 mm diameter cylindrical compression probe and was subjected to a compression test using a texture analyzer (SMS TA.XT2 Plus, UK). Hardness properties were determined. Also, the crumb and breadcrust color, Hunter L, a and b color values were determined by a color determination device (CR-100 Konica Minolta, Japan) [24].

Sensory analysis of bread samples

In the sensory analysis, crust and breadcrumb color, pore structure, taste, smell, chewability, and general acceptance were evaluated by 12 trained panelists, placing the samples in a score range from 1 (very bad) to 7 (very excellent).

Statistical analyses

One-way analysis of variance (ANOVA) was performed using JMP version 9 to determine important differences between the significance with reference to the measured properties. Significant differences were declared at $p < 0.05$. The mean and standard deviation of three replicates were also calculated.

Results and discussions

pH, acidity and microbiological analysis

The pH and total titratable acidity values of the einkorn sourdough were obtained during 4 days of fermentation. The TTA and pH of einkorn sourdoughs during the fermentation period were significantly changed ($p < 0.05$). The pH decreased from 6.18 to 3.81 for 4 days, and the TTA increased from 0.16 to 1.87%. In this study, the maximum values of LAB count varied according to agar types. LABs isolated from ModMRS, MRS-5, MRS, and Rose agars ranged from 8.56 to 9.61 log (cfu)/g. Max and min values of LAB count were obtained from ModMRS and MRS-5, respectively.

Identification of lactic acid bacteria

FTIR mathematically enables the identification of microorganisms by computer data processing, with the analysis and comparison of the whole spectra [25]. In total, 75 lactic acid bacteria strains isolated from einkorn sourdough were selected on MRS, ModMRS, Rose, and MRS-5 agars, and 42 isolates were first subjected to FTIR analysis and clustered to select genotypic identification. Thus, in our study, according to the W5-W4-W3 spectral regions, the first derivative method was applied to the spectra of the LAB analysis of the cluster analysis (Fig. 1a). According to the cluster analysis, 32 isolates were selected and were then identified genotypically. LABs isolated from einkorn sourdough were identified via 16S rDNA sequencing; the sequencing results are shown in Table 1. The sequence alignments were used to build a phylogenetic tree, using the neighbor-joining method (Fig. 1b). Six main groups were clustered as *Pediococcus, Lb. brevis, Lb. curvatus, Lb. fermentum, Lb. crustorum,* and *Lb. plantarum*. The dominant species was *Lb. crustorum.*

Bile and pH tolerance

A concentration of 0.3%, close to human bile concentration, is accepted as a critical value [26]. Thirteen subspecies of LABs isolated from einkorn survival percentages are illustrated (Table 2). All subspecies exhibited high tolerance to bile salts, with a survival ratio of over 85%. *Lb. brevis qp109*, *Lb. fermentum 1949, P. acidilactici A-4, Lb. crustorum MN047, Lb. crustorum B-481, Lb. brevis R-1, Lb. plantarum FM02, Lb. plantarum 1838,* and *Lb. plantarum GM1403* showed the highest tolerance, with a survival rate of over 90%.

Another critical property of probiotic microorganisms was the acid tolerance of LAB subspecies. These subspecies survived the acidic environment of the stomach and reached the intestinal system and formed colonies to compete with the pathogenic bacteria [27].

Thirteen subspecies tested were incubated for 24 h at 2.5 pH, and their survival rates were calculated (Table 2). The highest survival rates were observed in *Lb. paraplantarum 72,815*, *Lb. fermentum 1949, Pediococcus acidilactici A-4, Lb. crustorum MN047, Lb. crustorum B-481, Lb. brevis R-1,* and the *Lb. plantarum GM1403* subspecies, with a survival rate of over 90%.

In the study, the three strains of *Lb. plantarum SW03, Lb. plantarum SW07,* and *P. acidilactici SW05* showed the highest survival capacity when they were exposed to gastric acid, with a pH of 2–2, 5–3 [28]. In the study of [29], kefir isolates were deemed to be acid tolerant, as they survived at a rate of 70%. By using this ratio as a reference, the LAB subspecies isolated from einkorn could also be deemed to be acid tolerant.

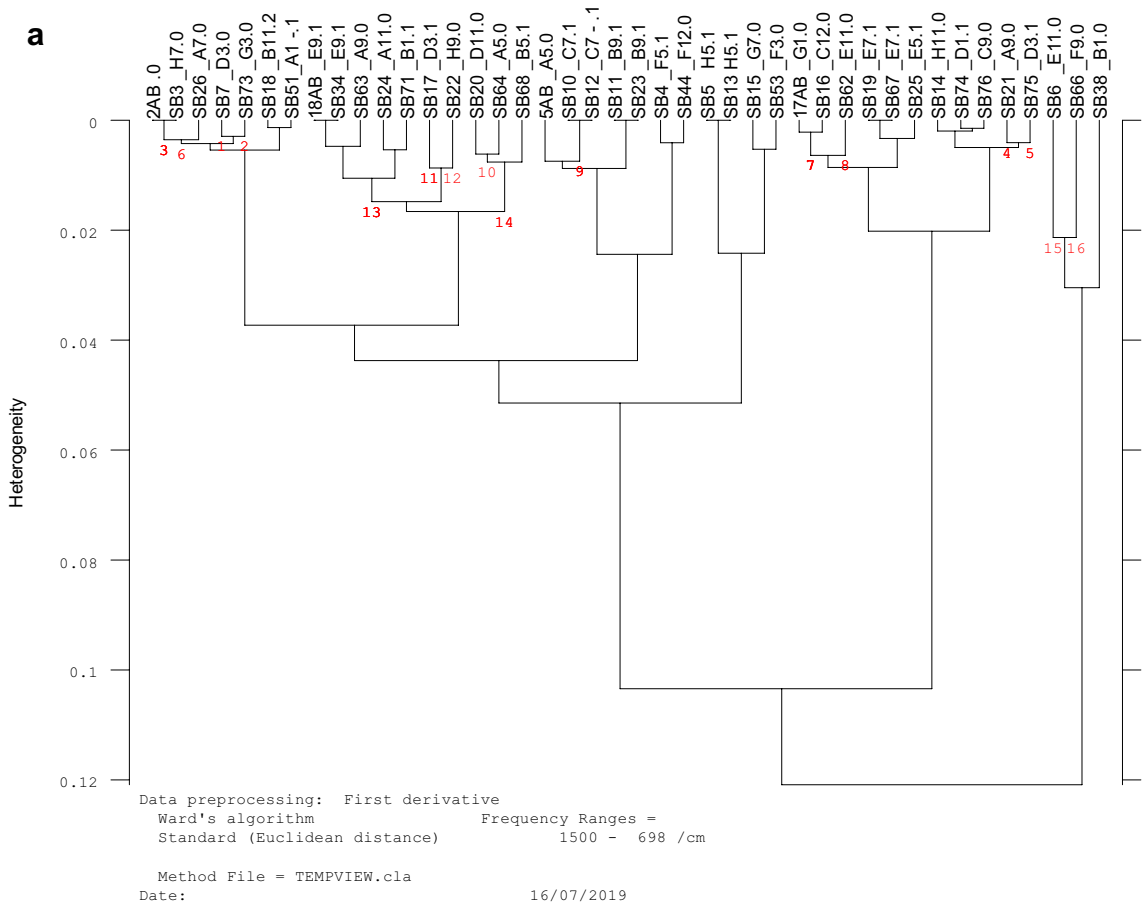


Fig. 1 **a** The FTIR fingerprinting of 42 LAB strains. Using Ward's algorithm method to form a hierarchical clustering of LAB strains. Ward; Spectral windows: W4 (1200–900 cm^{-1}), W5 (900–700 cm^{-1}), W3 (1500–1200 cm^{-1}). **b** Neighbor-joining relationship of 16S rDNA sequence of einkorn sourdough LAB isolates

Potassium sorbate and NaCl

All isolates were observed to be tolerant to 6% NaCl concentration. As shown in Table 2, isolates exhibited a survival ratio of over 85%. *Lb. brevis* qp109, *Lb. fermentum* 1949, *Pediococcus acidilactici* A-4, *Lb. paraplantarum, Lb. crustorum* MN047, *Lb. crustorum* B-481, *Lb. plantarum* AAS3, *Lb. plantarum* FM02, *Lb. plantarum* 1403, and *Lb. brevis* R-1 demonstrated a survival rate over 90%.

P. acidilactici A-4 had the lowest survival rate for K-sorbat, while the other subspecies exhibited a survival ratio of over 85%. Survival rates for K-sorbat over 90% were observed for *Lb. brevis* qp109, *L. paraplantarum, L. fermentum* 1949, *L. crustorum* MN047, *L. plantarum* AAS3, *L. crustorum* B-481, *L. plantarum* FM02, *and L. fermentum* 1301.

Potassium sorbate and salt, which are used as preservatives in fermented products, are important for bread and fermented cereal products as well. Salt, used both as a preservative and for taste, as well as potassium sorbate have great importance for bread and cereal products. These chemical additives used in fermented products to prevent the growth of pathogenic microorganisms may also inhibit the growth of starter culture; thus, species tolerant of such preservatives are preferred [30]. For this reason, the percent vitality of LABs isolated from einkorn wheat was measured (Table 2). In their study, [31] reported that no meaningful difference was observed between isolates at 15 °C in 2%, 4%, or % NaCl, and a significant decrease in alive cell count was observed for isolate *Lb. lactic* subsp. *lactis* UTNFa38 in 6% NaCl. In this study, the maximum ratio of salt used was determined as 6%, due to the observation of the LAB-killing effect of 6% NaCl.

Antimicrobial activity

There has been great interest in potential pathogen-antagonizing LABs in food processing applications, as LABs were accepted as food-grade ingredients [32, 33]. In this study, the antimicrobial activity of isolated LABs against pathogenic

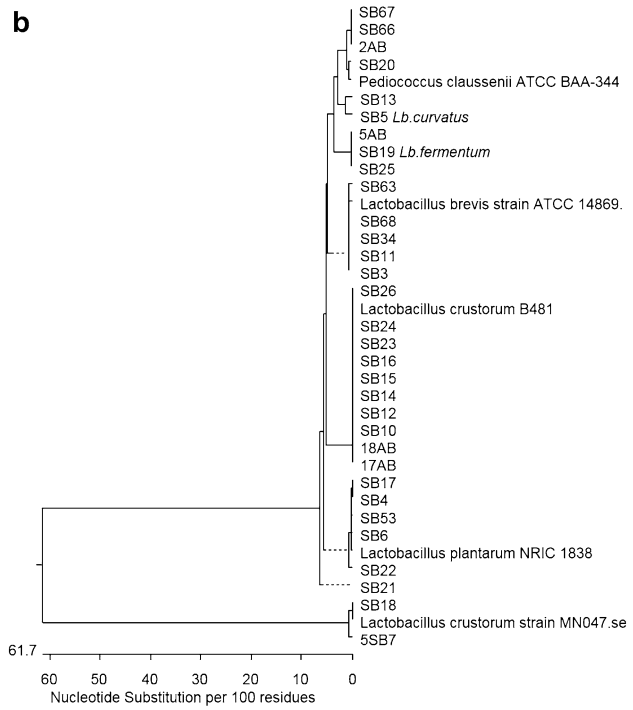


Fig. 1 (continued)

bacteria *B. subtilis* ATCC6633, *B. cereus* ATCC11778, and *E. coli* ATCC25922 was observed (Table 3).

Since *B. subtilis* causes rope spoilage in bread, antibacterial activity against *B. subtilis* is very important to increase the shelf life of bread. All LAB subspecies isolated from einkorn sourdough showed an antibacterial effect on *B. subtilis*. *Lb. paraplantarum* 72,815, and *P. acidilactici* A-4, while *Lb. crustorum* MN047 exhibited a strong resistance against *B. subtilis, B. cereus*, and *E. coli*. All subspecies were observed to exhibit antimicrobial activity against *B. subtilis ATCC6633*. Eight LAB subspecies showed antimicrobial activity against *E. coli* ATCC 25,922. The study of [34] isolated LAB *B. cereus* from baby feces, and the isolate displayed a high inhibition against *Lb. rhamnosus* and *Lb. curvatus;* in the same study, *L. curvatus* was found to be high.

Fungal spoilage is one of the basic problems for bread and other bakery products, causing economic losses and health problems [35]. In this study, the antifungal activity of LAB subspecies against *Penicillium carneum, Aspergillus flavus*, and *Aspergillus niger* was examined. As shown in Table 3, *Lb. paraplantarum 72,815, Lb. crustorum MN047, Lb. crustorum B-481,* and *Lb. brevis R-1* displayed antifungal activity against *Penicillium carneum, Aspergillus flavus*, and *Aspergillus niger molds. Lb. brevis Qp109, Lb. curvatus LII, Lb. fermentum 1949, Lb. fermentum1301,* and *Lb. plantarum GM1403* showed no antifungal activity.

Phytase activity

The phytase activity of the LAB isolates is shown in Table 3. Some LAB isolates showed phytase activity, while others did not, with phytase activity significantly changing according to the LAB isolates ($p < 0.05$). The highest phytase activity was measured in *Lb. paraplantarum* 72,815 and *Lb. plantarum* AAS3 (14 mm), and the lowest was observed in *Lb. brevis* qp109 and *Lb. plantarum* GM1403 (6 mm) (Table 3). Previous studies have shown that most minerals form a complex bond with phytic acid to produce phytates,

Table 1 Genotypic identification by 16S rDNA sequence analysis of LAB isolated during einkorn sourdoughs fermentation

	Isolate number	LAB	Identity (%)		Isolate number	LAB	Identity (%)
1	SB3	*Lb. brevis* 0138	99	17	SB21	*Lb.plantarum*GM1403	99
2	SB4	*Lb. plantarum* AAS3	99	18	SB22	*Lb. paraplantarum* 72,815	99
3	SB5	*Lb. curvatus* LII	99	19	SB23	*Lb. crustorum* B-481	99
4	SB6	*Lb. plantarum* FM02	99	20	SB24	*Lb. crustorum* B-481	99
5	SB7	*Lb. crustorum* MN047	99	21	SB25	*Lb. fermentum* IMDO 130,101	99
6	SB10	*Lb. crustorum* B-481	99	22	SB26	*Lb. crustorum* B-481	99
7	SB11	*Lb. brevis* 0138	99	23	SB34	*Lb. brevis* 0138	99
8	SB12	*Lb. crustorum* B-481	99	24	SB53	*Lb. plantarum* 1838	99
9	SB13	*Lb. LII curvatus*	99	25	SB63	*Lb. brevis* qp109	100
10	SB14	*Lb. crustorum* B-481	99	26	SB67	*Pediococcus acidilactici* A-4	100
11	SB15	*Lb. crustorum* B-481	99	27	SB66	*Pediococcus acidilactici* A-4	100
12	SB16	*Lb. crustorum* B-481	99	28	SB68	*Lb. brevis* R-1	99
13	SB17	*Lb. plantarum* AAS3	99	29	2AB	*Pediococcus acidilactici* A-4	100
14	SB18	*Lb. crustorum MN047*	99	30	5AB	*Lb. fermentum* NRIC1949	100
15	SB19	*Lb. fermentum* NRIC 1949	100	31	17AB	*Lb. crustorum* B-481	99
16	SB20	*Pediococcus pentosus 0123*	99	32	18AB	*Lb. fermentum* NRIC 1949	100

Table 2 The viable counts of 13 strains in MRS supplemented with 6% NaCl, 0.3% bile, 100 mg/kg potassium sorbate (pH 2.5)

LAB strains	Log CFU/mL Control	Log CFU/mL 6% NaCl	(%6 NaCl) Survival (%)	Log CFU/mL 0.3% Bile	%0.3 Bile Survival (%)	Log CFU/mL 100 mg/kg p. sorbate	100 mg/kg K-sorbate Survival (%)	Log CFU/mL pH 2.5	pH 2.5 Survival (%)
L. brevis qp109	9.14 ± 0.3^{d}	8.26 ± 0.2^{d}	90^{d}	8.35 ± 0.2^{c}	91^{bc}	8.56 ± 0.3^{b}	94^{a}	7.38 ± 0.7^{ef}	81^{f}
L. curvatus TW	9.26 ± 0.2^{c}	8.25 ± 0.5^{d}	89^{d}	7.86 ± 0.3^{h}	85^{f}	8.20 ± 0.3^{e}	89^{e}	7.86 ± 0.3^{cd}	85^{e}
L. praplantarum	9.03 ± 0.3^{e}	8.11 ± 0.5^{e}	90^{d}	8.07 ± 0.3^{ef}	89^{cd}	8.6 ± 0.4^{g}	89^{e}	8.40 ± 0.2^{b}	93^{b}
L. fermentum 1949	9.06 ± 0.2^{e}	8.39 ± 0.2^{bc}	93^{c}	8.36 ± 0.3^{c}	92^{b}	8.35 ± 0.6^{d}	92^{b}	7.84 ± 0.3^{cd}	91^{d}
P. acidilactic A-4	8.62 ± 0.2^{g}	8.11 ± 0.5^{e}	95^{b}	8.12 ± 0.8^{de}	94^{a}	7.21 ± 0.5^{j}	84^{g}	7.90 ± 0.2^{cd}	95^{a}
L. crustorum MN047	8.28 ± 0.5^{i}	7.88 ± 0.5^{f}	95^{ab}	7.90 ± 0.6^{h}	95^{a}	7.58 ± 0.2^{i}	92^{bc}	7.49 ± 0.3^{e}	91^{d}
L. plantarum AAS3	9.26 ± 0.5^{c}	8.34 ± 0.2^{cd}	90^{d}	8.05 ± 0.5^{efg}	87^{e}	8.76 ± 0.5^{a}	95^{a}	7.22 ± 0.2^{g}	78^{g}
L. crustorum B-481	8.38 ± 0.5^{h}	8.06 ± 0.2^{e}	96^{a}	7.91 ± 0.3^{gh}	94^{a}	7.52 ± 0.3^{i}	90^{d}	7.76 ± 0.2^{d}	93^{bc}
L. brevis R-1	8.93 ± 0.2^{f}	8.44 ± 0.3^{bc}	95^{b}	7.94 ± 0.5^{fgh}	90^{d}	7.90 ± 0.2^{h}	88^{e}	8.53 ± 0.2^{ab}	92^{bcd}
L. fermentum 1301	9.20 ± 0.3^{cd}	7.87 ± 0.6^{f}	86^{e}	8.21 ± 0.5^{d}	89^{cd}	8.47 ± 0.3^{c}	92^{b}	7.81 ± 0.5^{cd}	85^{e}
L. plantarum FM02	9.26 ± 0.2^{c}	8.45 ± 0.6^{b}	91^{c}	8.74 ± 0.2^{a}	94^{a}	8.40 ± 0.5^{d}	91^{cd}	7.25 ± 0.5^{fg}	78^{g}
L. plantarum 1838	9.47 ± 0.3^{a}	8.11 ± 0.5^{e}	86^{e}	8.49 ± 0.5^{b}	90^{cd}	8.19 ± 0.2^{ef}	86^{f}	7.93 ± 0.1^{c}	84^{e}
L. plantarum GM1403	9.39 ± 0.2^{b}	8.81 ± 0.2^{a}	94^{b}	8.81 ± 0.2^{a}	94^{a}	8.14 ± 0.3^{f}	87^{f}	7.54 ± 0.2^{a}	91^{cd}

Viable cell counts of 13 strains isolated from einkorn sourdough after incubation for 24 h in MRS broth at 6% NaCl, 0.3% bile, and 100 mg/kg potassium sorbate (pH 2.5). But, the control was only incubated in MRS broth for 24 h. Values for each pH at 24 h not sharing the same letters are significantly different from each other ($p < 0.05$)

Table 3 Antimicrobial and phytase activity of 13 identified species

LAB strain	*B. subtilis* ATCC 6633	*B. cereus* ATCC 11,778	*E. Coli* ATCC25922	*Aspergillus niger*	*Penicillium carneum*	*Aspergillus flavus*	Phytase (mm)
Lb. brevis Qp109	++	−	−	−	−	−	6 ± 0.1^{e}
Lb.curvatus LII	+	+	−	−	−	−	−
Lb. paraplantarum 72,815	++	++	++	+	+	+	14 ± 0.1^{a}
Lb. fermentum 1949	++	+	+	−	−	−	9 ± 0.1^{d}
P. acidilactic A-4	++	++	++	−	+	+	10 ± 0.1^{c}
Lb. crustorum MN047	++	+	+	+	+	+	−
Lb. plantarum AAS3	++	++	+	+	−	−	14 ± 0.0^{a}
Lb. crustorum B-481	++	−	−	+	+	+	12 ± 0.1^{b}
Lb. brevis R-1	++	++	+	+	+	+	−
Lb. fermentum 1301	++	+	−	−	+	−	−
Lb. plantarum FM02	++	−	−	−	+	−	−
Lb. plantarum 1838	++	++	−	−	+	−	−
Lb. plantarum GM1403	++	++	−	−	−	−	6 ± 0.0^{e}

The different lower case letters in the same column show statistical significance ($p < 0.05$)

Diameter of antibacterial inhibition zones (mm): $++^{>}$ 10 mm; $+ < 9$ mm; antifungal positive (+), not determined (−), Phytase (-) no activity

and the bioavailability of these minerals is reduced [36, 37]. Reduction of phytates in bread production is achieved via enzymatic degradation and endogen phytase activity increase or LABs, yeasts, and other microorganisms [38]. This study concluded that the phytase activity of isolates should be investigated to increase the bioavailability of the minerals in sourdough bread.

Properties of sourdough

To determine the changes in the doughs due to the rise of acidity, pH values and microbial load were measured after kneading and after the fermentation process. For sourdough BS1 (wheat sourdough), the measured pH decreased from 5.59 to 3.47, corresponding to a rise in acidity from 0.21% to 1.21%, while for the sourdough SS1 (einkorn sourdough), the pH values were 5.6 and 3.35, and the corresponding acidity was 0.26% and 1.43%, respectively. All samples were found to be statistically different ($p < 0.05$). For sourdough made of 100% einkorn flour, SS1, the post-fermentation pH was measured to be lower than that in the sourdough made of 100% wheat flour, BS1; however, the TTA was higher.

At the beginning of fermentation, all sourdough showed LABs in the range of 7.90 ± 0.7 log cfu/g BS1 and 7.88 ± 0.3 log cfu/g SS1; after fermentation, the LAB count was found to be a range of 8.51 ± 0.4 log cfu/g BS1 to 9.60 ± 0.6 log cfu/g. The beginning of the fermentation yeast count was found to be in the range of 6.0 ± 0.6 log cfu/g to 6.02 ± 0.9 log cfu/g; after fermentation, the yeast number was 6.65 ± 0.7 log cfu/g to 6.55 ± 0.4 log cfu/g ($p < 0{,}05$). Einkorn sourdough was found to contain more LABs than wheat sourdough, while the yeast contents were found to be statistically close. Lactic acid bacteria in the dough fermented glucose to produce lactic acid, which caused a reduction in dough pH.

Properties of bread

The weight, specific bread volume, hardness, and color values of the sourdough bread samples were as shown in Table 4. The bread volume varied to between 295 ± 7.0 cm^3 and 172 ± 3.5 cm^3; the specific volume ranged from 2.16 ± 0.1 cm^3/g to 1.37 ± 0.1 cm^3/g; and the hardness was in the range of between 3.79 ± 0.4 N and 1569 ± 0.3 N.

The volume and specific volume decreased as the ratio of einkorn flour increased. The volume of SYE4 bread (made from 100% einkorn flour) was found to be statistically similar to the volume of SYE3 bread (containing 75% einkorn flour) ($p > 0{,}001$), while the specific volume values were found to be lowest in the SYE4 bread samples. The highest specific volume was found in the control bread SKE1

Table 4 Volume, hardness and color properties of the bread samples

Bread code	Flour type	Volume (cm^3)	S.Volume (cm^3/g)	Hardness (N)	Crust clour			Crumb clour		
					L*	a*	b*	L*	a*	b*
SKE1	%100 Wheat (control)	288 ± 1.25^{a}	2.13 ± 0.03^{a}	3.79 ± 0.40^{e}	68.34 ± 0.91^{a}	6.01 ± 0.52^{d}	30.97 ± 0.82^{a}	70.50 ± 0.20^{a}	0.29 ± 0.11^{e}	18.01 ± 0.50^{b}
SYE1	%25 Einkorn %75 wheat	249 ± 4.00^{b}	1.86 ± 0.01^{b}	6.53 ± 0.01^{d}	64.82 ± 0.70^{b}	8.32 ± 0.40^{c}	27.84 ± 0.50^{b}	62.66 ± 0.10^{b}	2.45 ± 0.00^{d}	17.89 ± 0.50^{b}
SYE2	%50 Einkorn %50 Wheat	234 ± 1.75^{c}	1.73 ± 0.02^{c}	9.70 ± 0.10^{c}	60.34 ± 0.30^{c}	8.59 ± 0.44^{c}	26.31 ± 0.30^{c}	61.00 ± 0.00^{c}	3.98 ± 0.30^{c}	20.13 ± 0.23^{a}
SYE3	%75 Einkorn %25 Wheat	214 ± 4.53^{d}	1.55 ± 0.04^{d}	12.53 ± 0.43^{b}	58.73 ± 0.11^{d}	9.91 ± 0.20^{b}	25.59 ± 0.77^{cd}	58.65 ± 0.20^{d}	5.29 ± 0.25^{b}	20.35 ± 0.32^{a}
SYE4	%100 Einkorn	206 ± 1.51^{d}	1.47 ± 4.95^{e}	15.69 ± 0.30^{a}	53.88 ± 0.12^{e}	11.82 ± 0.14^{a}	25.43 ± 0.50^{cd}	55.02 ± 0.03^{e}	6.41 ± 0.20^{a}	20.7 ± 0.41^{a}

Significant differences in volume specific volume hardness and color values of einkorn bread containing different amounts of wheat flour are shown in different letters ($p < 0.05$)

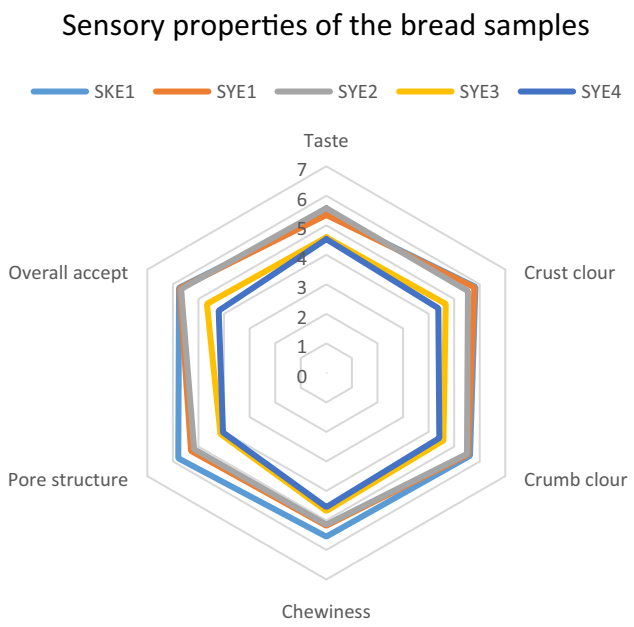


Fig. 2 Sensory properties (external appearance, crust and crumb color, consistency, flavor and taste) of wheat-einkorn bread depending on einkorn share

(containing 100% wheat flour). The specific volume of SYE1 bread was closest to that of the control bread. The volume of the SYE2 bread was lower than the volume of the control bread and 19% higher than that of the SYE3 bread.

The use of the starter culture consisting of SS1 sourdough increased the volume of the SYE4 bread, made of 100% einkorn flour. Texture analysis (Table 4) showed hardness value of the breads decreased. The least hardness was observed for the bread SYE1, which contained 25% einkorn flour. The effect of the gluten network formed by adding wheat flour to the einkorn flour and starter culture was to improve the volume and textural properties of the bread.

The brightness of the crust and crumb color was observed to be highest for ordinary wheat flour, and bread with SYE1 added was observed to have a similar brightness. As redness increased on the crusts and in the crumbs of SYE2, SYE3, and SYE4 bread, due to the ratio of einkorn flour, yellowness was reduced on the crust and increased in the crumb. Einkorn flour contains more lutein than wheat flour [39], which causes einkorn bread to be yellower. Wheat bread was brighter, while einkorn bread was observed to be yellower and redder.

The sensory properties of the bread samples were as shown in Fig. 2. Bread samples produced from SYE1 and SYE2 were close to the reference (control) bread in terms of sensory score ($p > 0.05$). Regarding taste, sourdough bread with 75% (SYE3) and 100% (SYE4) einkorn flour content showed lower scores compared to SYE1 and SYE2. Although in their study, [40] reported to have observed low volume, thick walls in the crumb, and irregular texture for bread made of 100% einkorn wheat, in this study, sourdough bread made of 100% einkorn flour and fermented with the starter was observed to have no thick wall structure either on the crust or in the crumb. When evaluated by the panelists, the texture was deemed statistically close to sourdough bread with 75% and 100% einkorn flour, and it was scored as acceptable.

Conclusion

The biodiversity of lactic acid bacteria in einkorn sourdough was determined. Thirteen different LAB strains isolated from einkorn sourdough were identified. The dominant species was *Lactobacillus crustorum*. LABs play an important role in sourdough fermentation and also have probiotic potential, thanks to their functional and technological properties. *Lb. brevis* R-1 and *Lb. paraplantarum* 7285 showed the highest technological activity and showed the possibility of being used as starter cultures in the preparation of einkorn bread. The *Lb. brevis* R-1 and *Lb. paraplantarum* 7285 combinations of starter culture and the addition of wheat flour with added proportions to the einkorn improved the sourdough structure and bread quality of the einkorn bread. Einkorn sourdough allowed the isolation of promising microorganisms. Our findings suggested that LABs from einkorn dough could potentially be considered for use in the bakery industry.

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