ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/340523158

Physicochemical and biological characterization of the EPS produced by L. acidophilus isolated from rice bran sourdough

Article *in* LWT- Food Science and Technology · April 2020

DOI: 10.1016/j.lwt.2020.109373

CITATIONS
2

READS
90

4 authors:

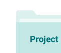
Abbas Abedfar
Institute of Food Science and Technology
15 PUBLICATIONS 32 CITATIONS
SEE PROFILE

Sepideh Abbaszadeh
Baqiyatallah University of Medical Sciences
41 PUBLICATIONS 640 CITATIONS
SEE PROFILE

Marzieh Hosseininezhad
Institute of Food Science and Technology
24 PUBLICATIONS 132 CITATIONS
SEE PROFILE

Maryam Taghdir
Baqiyatallah University of Medical Sciences
46 PUBLICATIONS 373 CITATIONS
SEE PROFILE

Some of the authors of this publication are also working on these related projects:



evaluation of thermal and osmotic stresses on some probiotics during the product manufacturing process View project

Effect of spontaneous and controlled fermentation of sourdough on microbial exopolysaccharides View project

All content following this page was uploaded by Marzieh Hosseininezhad on 23 September 2020.

The user has requested enhancement of the downloaded file.

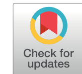
LWT - Food Science and Technology 127 (2020) 109373

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Physicochemical and biological characterization of the EPS produced by *L. acidophilus* isolated from rice bran sourdough



Abbas Abedfar[a], Sepideh Abbaszadeh[a,b,*], Marzieh Hosseininezhad[c], Maryam Taghdir[a,**]

[a] Health Research center, Life style institute,Baqiyatallah University of Medical Sciences
[b] Department of Nutrition and Food Hygiene, Faculty of Health, Baqiyatallah University of Medical Sciences, Tehran, Iran
[c] Department of Food Biotechnology, Research Institute of Food Science and Technology (RIFST), Mashhad, Iran

ARTICLE INFO

Keywords:
Spontaneous fermentation
Rice bran sourdough (RBS)
Exopolysaccharides (EPS)
L. acidophilus
Molecular tests

ABSTRACT

Spontaneously-fermented RBS contains a variety of LAB and bacterial metabolites with potential applications as sources of functional ingredients. In this work, the EPS production capacities of the dominant LAB separated from spontaneously fermented RBS was investigated. *Lactobacillus acidophilus* was identified as the dominant isolate via biochemical and molecular investigations. The total carbohydrate, protein and phosphorus contents of the 2.04×10^6 Da EPS were 254.9 g/L, 0.095 g/L, and 0.36% w/w, respectively. HPLC was used for compositional assessment in terms of monosaccharide constituents, revealing that glucose, galactose and maltose were the major components of the EPS produced by *L. acidophilus*. The EPS was identified using FT-IR, ^{1}H and ^{13}C NMR spectra. FT-IR analysis demonstrated the polysaccharide nature of the EPS; the presence of alcoholic, aromatic and carboxylic acid groups was confirmed via their respective stretching vibrations. The microbial EPS extracted from *L. acidophilus* was identified as a linear $\alpha(1 \rightarrow 6)$ polysaccharide. Also, the percentage inhibition of DPPH by the EPS was significant ($P < 0.05$). Thus, potential antioxidant characteristics were demonstrated as important aspects of the EPS produced by the strain isolated from fermented RBS.

1. Introduction

A non-aseptic, biological ecosystem exists within sourdough, comprising one of the oldest and most important cereal fermentations known to humankind. This ecosystem is based on the fermentation (both alcoholic and lactic acid) of flour and water (Gobbetti, Rizzello, Di Cagno, & De Angelis, 2014). The functioning of this ecosystem is mainly dependent on microbiota composition (particularly lactic acid bacteria (LAB)) and fermentation conditions (Lhomme et al., 2015). Sourdough fermentation has two primary functions; the first is dough leavening, through which a gaseous dough is produced, and the second is producing organic acids with favorable effects on bread quality, shelf life and sensory attributes (Aplevicz, Ogliari, & Sant'Anna, 2013). In fact, the acidification activity of the dominant LAB determines the majority of the positive characteristics related to sourdough (Manini et al., 2016). Rice bran (RB) constitutes a highly available and cheap source of protein, dietary fiber, and phytochemicals for use in the fortification of staple foods, such as bread. Milling wheat and rice results in the formation of functional byproducts named wheat bran (WB) and rice bran (RB), which can be utilized in the processing of sourdough breads. WB and RB have high concentrations of bioactive molecules and dietary fibers, which are influential on the microbial features and functional characteristics of the produced sourdough (Katina et al., 2012). Sourdoughs prepared with WB and RB are high quality products due to having nutritional, antimicrobial, functional and antistaling properties (Abedfar, Hosseininezhad, Sadeghi, Raeisi, & Feizy, 2018; Gobbetti et al., 2014). The fermentation of RBS can occur either spontaneously or non-spontaneously; the latter is conducted using specific technological parameters and conditions (De Vuyst et al., 2014). LAB have the capability of producing extracellular polymers known as microbial exopolysaccharides (EPSs) (Vu, Chen, Crawford, & Ivanova, 2009). Batch fermentation is usually used to produce EPSs on an industrial scale (Boza, Neto, Costa, & Scamparini, 2004). EPSs are produced during the growth of bacteria and have a generally recognized as safe (GRAS) status; these substances feature certain antibiotic peptides and bioactive compounds with nutraceutical properties and functions in thickening, gel production, hygroscopy, encapsulation and emulsifier stabilization (Li, Jin, Meng, Gao, & Lu, 2013; Zhou et al., 2017). Given their significant water retention ability, EPSs produced during sourdough fermentation can potentially be used as antistaling

* Corresponding author. Health Research Center, Life style institute, Baqiyatallah University of Medical Sciences, Tehran, Iran.
** Corresponding author.Health Research Center, Life Style Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran.
E-mail addresses: Abbaszade@ut.ac.ir (S. Abbaszadeh), mtaghdir@bmsu.ac.ir (M. Taghdir).

https://doi.org/10.1016/j.lwt.2020.109373
Received 24 September 2019; Received in revised form 10 March 2020; Accepted 3 April 2020
Available online 08 April 2020
0023-6438/ © 2020 Elsevier Ltd. All rights reserved.

additives to increase bread quality (Lynch, Coffey, & Arendt, 2018). Biological significance and application of microbial EPS created by lactobacilli in situ during sourdough fermentation could substitute hydrocolloids commonly used as texturizing and prebiotic supplement in bread production (Tieking et al., 2003). Subsequent technological functions of EPS from LAB has been proposed: water absorption of the dough, dough rheology and machinability, dough stability during frozen storage, loaf volume and bread staling (Galle & Arendt, 2014).

Previously, few studies have focused on the production of EPSs by LAB isolated from sourdough. The objective of the present study was to characterize the physicochemical and biological properties of the microbial EPS isolated from *L. acidophilus* which is native in the traditional rice bran (specific varieties in northern Iran) with appropriate dough yield and the potential ability of producing exogenous metabolites as well as high oxidant capacity that reacts with biomolecules. These metabolites were structurally characterized by using, ^{1}H, ^{13}C NMR and FT-IR spectra in order to determine their different functional groups. Thereby, application of this bacterial strain along with its microbial EPS isolate facilitate controlled fermentation of RBS in order to improve the technological characteristics.

2. Materials and methods

2.1. Raw materials

Rice bran was purchased from Dastas Factory (Iran) and chemically analyzed according to the standard methods described by the American Association of Cereal Chemists (AACC) (AACC International, 2010). The remaining chemicals as well as the media used (MRS Broth, Agar and Nutrient Broth) were purchased from Sigma-Aldrich (St Louis, USA) or Merck.

2.2. Methods

2.2.1. Spontaneous fermentation of RBS

To prepare the spontaneous fermentation of RBS, a mixture of rice bran flour and table water was used with a dough yield ($\frac{\text{dough weight}}{\text{Flour weight}} \times 100$) of 360. The produced RBS was then fermented at 37 °C for 24 h on the first day (Katina et al., 2012). Back-slopping/refreshment process (adding the previously prepared sourdough to the fresh sourdough formulation) was performed on the second day; freshly formulated sourdough was supplemented with one-fourth of the of sourdough from the previously baked batch (DY = 360). The back-slopping process was performed repetitively on the subsequent days until roughly constant pH and total titratable acidity (TTA) values were achieved (Di Cagno et al., 2014). The pH (pH meter Inolab D-82362, Germany) and TTA (Katina, Heinio, Autio, & Poutanen, 2006) of the samples were determined after each back-slopping cycle. Finally, bacterial growth was determined quantitatively in CFU.g^{-1} via the colony-forming unit assay (Venturi, Guerrini, Granchi, & Vincenzini, 2012).

2.2.2. Isolation and screening 16S rDNA identification of dominant LAB

Once the back-slopping cycles had completed, the dominant LAB isolated from RBS were determined by spread on the surface of MRS agar plates. Phenotypic tests, biochemical assays, and PCR were used to evaluate the nature of pure single colonies from the streak plate of LAB isolates RBS (Sadeghi, Ebrahimi, Mortazavi, & Abedfar, 2019). Subsequently, The genomic DNA of pure single colonies of the selected LAB isolate was extracted with a DNA Extraction Kit (Bioneer's AccuPrep®, South Korea) and then subjected to specific PCR according to Leite et al. (2015). The amount of PCR reagents in an ultimate volume of 30 μl included a unit of standard buffer PCR, 25 picamol/microlitre of each primer, a mixture of dNTP with 0.2 M mol concentration, 25 μg of serum albumin, Taq polymerase with an activity of 2.5 units (Roboset, French), and 3 μl of DNA with a concentration of 100 ng. A total of 1500 base pairs (bp) were amplified through the PCR process, with different regions of 16 rDNA in LAB being the target sequence. The forward specific primer had a base sequence of 27F: 5′-AGAGTTTGAT CCTGGCTCAG-3', while the reverse specific primer had a sequence of 1492R:5'-GGTTACCTTGTTACGACTT-3'. Following electrophoresis in agarose gel (1.5% w/v in TBE buffer), PCR product sequencing was conducted (MWG, Germany); the sequences were then edited and aligned by ClustalX algorithm in BioEdit, and the BLASTn procedure was used to compare the results with the genomic data provided by NCBI (http://www.ncbi.nlm.nih.gov/genbank/). The neighbor-joining (NJ) technique (bootstrap replicates = 1000) was used to construct phylogenic trees and estimate the phylogenic relationships; the Tamura-Nei model was followed for all phylogenetic analyses, which were conducted using MEGA 7 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013).

2.2.3. Preparation of bacterial suspension and conditions for EPS production

Pure cultures of *L. acidophilus* (LC_155899.1) from RBS were inoculated in MRS broth, to which 10% glucose had previously been added. Incubation then occurred in facultative anaerobic conditions at 37 °C for 24 h to 10^{8} CFU/ml. The highest amount of biomass produced at the stationary phase's initial and middle parts was subjected to centrifugation (15000*g*, 4 °C, 30 min) (Hermle, Z36 HK, Germany) before being suspended again in sterile water (Dal Bello et al., 2007). The supernatants were added to the MRS broth, while the EPSs were isolated using the pellets (Bajpai et al., 2015). Using criteria related to mucoid phenotype, the LAB strains that produced EPSs were screened initially via observation while being cultured in MRS agar at 37 °C for 48 h (Tallon, Bressollier, & Urdaci, 2003).

2.2.4. Microbial EPS preparation

Samples (10 ml) of the LAB strains that had previously been cultured were subjected to centrifugation (15000g, 4 °C, 30 min). Then, the pellets were washed using 5 ml of physiological solution before being centrifuged again (15000*g*, 4 °C, 15 min). The viscous pellets were then added to 5 ml of 0.05 M ethylenediaminetetraacetic acid (EDTA), before being incubated at 4 °C for 4 h with gentle agitation (Pars Azma, Iran); the samples were then centrifuged for 30 min at 6000*g* and 4 °C. The EPS was precipitated from the supernatant by adding two volumes of cold ethanol (96%) and incubating at 4 °C. Furthermore, after centrifugation at 6000*g* and 4 °C for 30 min, deionized water was used to dissolve the EPS-containing precipitate, and this solution was subjected to dialysis (Spectra/Por dialysis membrane) for 24–48 h with water refreshment thrice per day. Finally, the purified EPS was kept at −80 °C until further experimentation (Tallon et al., 2003).

2.2.5. Chemical analysis of EPS

The phenol-sulfuric acid method (Goh, Haisman, Archer, & Singh, 2005) was used to determine the carbohydrate concentration with glucose as the standard (Tamani, Goh, & Brennan, 2013), and the absorbance of EPS (0.1 mg/10 ml) was measured at 485 nm. The Bradford assay (1976) was used to determine the total protein content with the aid of a bovine serum albumin (BSA) standard curve. Under constant vortex, 5 ml of Bradford solution (Coomassie Brilliant Blue 250G, 0.01% w/v, ethanol 47% v/v, phosphoric acid 8.5% v/v) and 300 μl of soluble EPS (0.5 g in 500 μl of Milli-Q water) were mixed at room temperature for 30 min. The concentration of protein (mg/100 ml) was determined via absorbance at 595 nm (Tallon et al., 2003). The Phosphate Beer-Lambert Plot was used to identify the concentration of phosphate (mg/ml) with the help of a standard Na_2HPO_4 solution (100 mgP/ml) and the Vanadate-Molybdate reagent. A 10 ml sample of the EPS solution (0.2 g EPS hydrolyzed at 120 °C for 120 min using 2 M trifluoroacetic acid), 10 ml of Vanadate-Molybdate reagent, and 50 ml of Milli-Q water were added together and left to react for 10 min;

absorption was then measured at 430 nm (Tallon et al., 2003).

2.2.6. Determination of molecular mass

The apparent molecular weight of microbial EPS was determined using the Zetasizer Nano ZS (Malvern Instruments, UK) at 25 °C; toluene was used for calibration at $\lambda = 632.8$ nm with a scattering angle of $\theta = 90°$. Molecular weight was evaluated using the following equations:

$$\frac{Kc}{R\theta} = \frac{1}{Mw} + 2A_2 c \tag{1}$$

$$K = \left(\frac{2\pi^2}{\lambda0^4 NA}\right)(1 + \cos^2\theta)n^2\left(\frac{dn}{dc}\right)^2 \tag{2}$$

$$R_\theta = R_{\theta,solution} - R_{\theta,solvent} \tag{3}$$

where c is concentration, K is the calibration constant that was obtained with the use of toluene, λ_0 represents the wavelength of the incident beam (632.8 nm), NA is Avogadro's number, $R_{\theta,\ solvent}$ is (1.352×10^{-5} cm^{-1} at 632.8 nm), θ denotes the angle of scattering (=90°), n refers to the solvent's refractive index, and dn/dc denotes the increment in specific refractive index. MW (The weight-average molecular weight) and A_2 (the second virial coefficient) were acquired using the slope of the Debye plot (Kc/Rθ vs. c) and the reciprocal of its intersect, respectively. According to the scattering of static light, molecular weight was obtained at room temperature (Razmkhah, Mohammadifar, Razavi, & Ale, 2016).

2.2.7. Monosaccharide composition analysis of EPS by HPLC analysis

EPSs (1 mg) of *L. acidophilus* isolated from RBS were subjected to hydrolysis for 2 h at 120 °C using 1 ml of 2 M trifluoroacetic acid (TFA). Next, derivatization was performed using 1-phenyl-3-methyl-5-pyrazolone (PMP) and anthranilic acid (AA). HPLC (Waters, Milford, MA, USA) was then used for monosaccharide composition analysis. The reverse phase column was a RP C-18 with 250 mm × 4.6 mm ID and 5 μm particle size. The mobile phase consisted of sodium phosphate (50 mM, pH 7.0)/acetonitrile (82:18 v/v) at a flow rate of 1.0 ml min^{-1}, and the volume injected was 20 μl (Lv et al., 2009).

2.2.8. FT-IR characterization of EPS

An FT-IR spectrum range of 4000 to 400 cm^{-1} (1 cm^{-1} resolution) was used to identify the EPS's functional groups. To prepare the sample pellets, 1 mg of the freeze-dried EPS was mixed with KBr (100 mg) before being analyzed by FT-IR spectrophotometry (Thermo Scientific Nicolet 6700, Avatar 370 FT-IR, USA). Data was evaluated using the essential FTIR 3.5 program (Davis & Mauer, 2010).

2.2.9. NMR spectroscopy of EPS

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy were performed with an FT-NMR spectrometer (300 MHz, Bruker AVANCE III model, Germany) in Iran Bu Ali Research Institute (Mashhad, Iran). For NMR assessment, pure EPS was lyophilized and dissolved in D_2O. The ^{1}H and ^{13}C NMR spectra were obtained at 25 °C (Paulo et al., 2012).

2.2.10. Scanning electron microscopy (SEM) of EPS

Microstructural analysis of the EPS was performed according to the method of Paulo et al. (2012) with slight modifications. Microbial EPS was frozen in liquid nitrogen and freeze-dried using a freeze dryer (Operon, FDO-8606, Korea). Then, the samples were mounted and sputter coated for 2 min with 2 mbar of gold, before being observed at 10 kV with a vacuum of 2×10^{-5} torr. Image Pro Plus 6.0 (Media Cybernetics Inc., USA) was utilized for SEM image analysis.

2.2.11. DPPH radical scavenging activity

The method described by Liu et al. (2010) was utilized to assess the free radical scavenging capacity of the EPS samples with the aid of 1-1-Dipheny1-2 picryl-hydrazyl (DPPH). A 0.1 mM solution of DPPH ethanol (2 ml) and 2 ml samples of EPS (various concentrations) were added together in water. Dark reaction was allowed for 30 min, after which the absorbance (A) was read at 517 nm against blank; the positive control employed was ascorbic acid. The EPS's antioxidant activity was recorded as the inhibition percentage of DPPH radicals, calculated using the following equation:

$$\text{Scavenging ability (\%)} = [(A_{blank} - A_{sample}) / A_{blank}] \times 100$$

2.2.12. Statistical analysis

RBS preparation and all experimental procedures were conducted thrice. Statistical analysis of the data was done according to a completely randomized design. Analysis of variance (ANOVA) was used to compare the means in SAS (9.1) software.

3. Results and discussion

3.1. Physicochemical characteristics of rice bran

Standard methods of the AACC were utilized for chemical analysis of rice bran. The results were as follows: 9.27 ± 0.68% moisture content (NO: 44-15), 15.04 ± 0.32% crude protein (NO: 46-13, N × 5.70), 8.05 ± 0.01% ash content (08-01), 4.25 ± 0.26% lipid (NO: 30-10), 4.1 ± 0.1% acidity, and dough yield (DY) = 360.

3.2. Evaluation of TTA, pH and trend of log (CFU. g^{-1}) of RBS

The results of the prepared RBS's total CFU.g^{-1}, pH and TTA over ten consecutive days of back-slopping and spontaneous fermentation are shown in Fig. 1. As clearly demonstrated, the TTA and pH of RBS produced during spontaneous fermentation at 37 °C over 24 h showed significant differences compared with the control in a confidence interval of 5%. ANOVA and means comparison demonstrated that by increasing the number of back-sloppings/refreshments up until a certain extent, the TTA increased and the pH decreased. These results match those of Katina et al. (2012) and Vogelmann & Hertel, (2011). The acid production of sourdough spontaneous fermentation depends on the specific microbiota as well as factors including fermentation time, temperature and DY (Clarke, Schober, Dockery, O'Sullivan, & Arendt, 2004). Relative to the control (a sample for which the back-slopping process was not performed), a regular trend was not demonstrated by the total log CFU.g^{-1} after 48 h of growth on MRS agar at 37 °C, and no significant differences were seen between each day ($P > 0.05$). These results are consistent with those of Abedfar et al. (2018). The maximum and minimum TTA in sourdough spontaneous fermentation were related to samples 10 and 1, respectively (number of back-sloppings/refreshment), both featuring significant differences relative to the control sample ($P < 0.05$) (Vrancken, Rimaux, Weckx, Leroy, & De Vuyst, 2011).

3.3. Identification of the dominant LAB in RBS

The dominant LAB isolates of sourdough were separated after ten days of repeating the RBS back-slopping process in the way that has previously been described (Abedfar et al., 2018; Vrancken et al., 2011). The results of microscopic analysis (based on Bergey's Manual of Determinative Bacteriology) showed that the dominant isolate, *Lactobacillus,* was Gram positive, catalase, oxidase and triple sugar iron agar test negative, non-motile, positive for carbohydrate fermentation (glucose, arabinose and galactose test), and morphologically had a rod shape (Holt, Krieg, Sneath, Staley, & Williams, 1994; Imran et al., 2016). The results of gel electrophoresis of the PCR products for the specific detection of dominant LAB isolates showed that only the

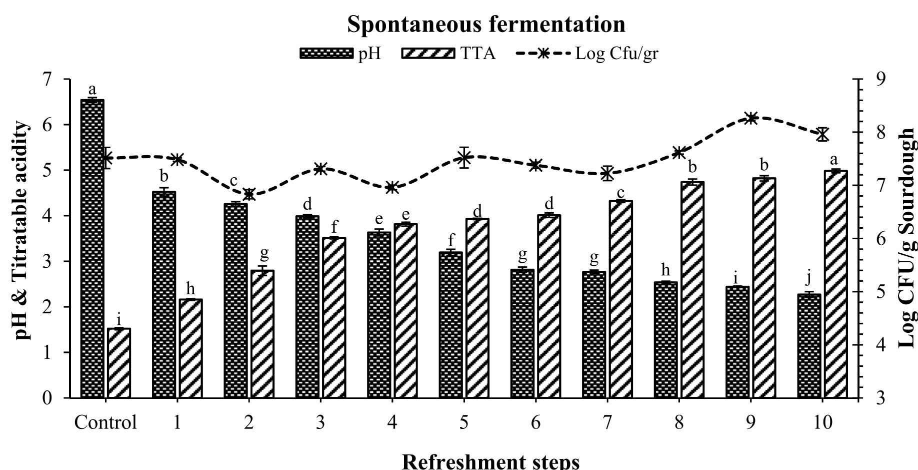


Fig. 1. Evaluation of TTA, pH and CFU. g^{-1} of LAB in spontaneous fermentation of rice bran sourdough (columns sharing the same letter are not significantly different at confidence level of 95%).

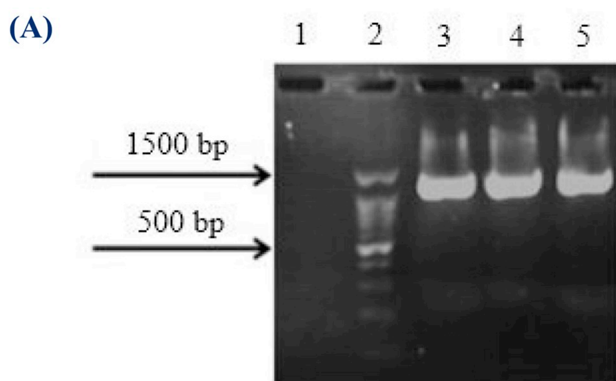


Fig. 2A. Agarose gel electrophoresis of PCR products obtained under optimized conditions for identification of dominant LAB isolates (1500 bp). 100 bp DNA ladder (lane 2), extracted DNA from cultured cells of *Lactobacillus spp.* in MRS broth as positive control (lane 4 and 5), DNA extracted from single colonies of sourdough cultures (lane 3) and no DNA as negative control (lane 1).

expected 1500 bp PCR products were observed after gel electrophoresis, with nonspecific products (primer/dimers) not being visible (Fig. 2A). A BLASTn search was used to confirm the identity of the amplicon; by sequencing the products of PCR, *L. acidophilus* (99% identity percentage, accession number LC_155899.1) was determined as the dominant LAB isolated from RBS. Fig. 2B represents the MEGA7 cluster alignments of the 16S rRNA genes of one distinct strain in RBS (*L. acidophilus*), showing their phylogenetic relationships. Identification of dominant LAB isolated from foods that are spontaneously fermented (e.g., sourdough) using a similar process (to that of the present study) has been done by Manini et al. (2016), Abedfar et al. (2018) and Sadeghi et al. (2019).

3.4. The composition and amount of purified EPS of RBS

The polysaccharide content of the EPS produced by the LAB isolate during the stationary phase of growth was determined per liter of the culture medium by measuring absorbance at 485 nm, with a value of 1.56 ± 0.03 being obtained. After incubation of *L. acidophilus* at 37 °C in MRS agar having 10% glucose, characteristics of the microbial EPS were as follows: microbial growth of 8.14 ± 0.08 log CFU (approximately 1.5 × 10^8 CFU/mL), an EPS concentration of 267.71 ± 10.02 mg/L, and a microbial EPS yield of 32.86 ± 0.25 ($mg.L^{-1}$/log CFU). The chemical composition of purified EPS was recorded as the quantity of polysaccharides formed at an optical density (OD) of 600 nm (Seo, Bajpai, Rather, & Park, 2015; Imran et al., 2016). The biological significance and application of purified EPS due to their various structural and compositional features, EPSs have useful properties for use in the food industry, particularly as thickeners (Wang et al., 2010). The maximum optical density and carbohydrate content obtained at the middle of stationary phase (part of the idiophase) are shown in Table 1. The protein content of the obtained EPS had a direct relationship with its level of purity (Table 1). EPS mainly composed of proteins and polysaccharides, are microbial metabolic products. Considering that metabolite produced is of microbial source and extracted from the bacterial cell wall and body. Also, the augmented protein content in microbial EPS probably depends on the type of substrate and conditions of fermentation (Tsuda & Miyamoto, 2010; Tallon et al., 2003). The total phosphate content of the EPS obtained at the stationary middle phase is shown in Table 1. The phosphate may work as an energy reserve as well as metabolism regulator. Besides, for some microorganisms, it carries out the task of a high energy phosphorous compound which substitutes ATP (Harold, 1966). The molecular mass (w/w %) of EPS isolated from *L. acidophilus* of RBS was 2.04 × 10^6 Da (Table 1). As a basic property of all polysaccharides, molecular weight must be determined given its influence on a number of physical characteristics such as stability, fluid behavior, and gelling and thickening capacity (Wang, Zhao, Tian, Yang, & Yang, 2015).

3.5. Identification of EPS monosaccharide composition by HPLC

The monosaccharide composition of the EPS of *L. acidophilus* was analyzed by HPLC; independent peaks at a retention time of 47.01 ± 0.03 min confirmed the presence of maltose (Fig. 3). Maltose biological significance as one of the functional groups including hydroxyl, aldehyde (-CHO) and carbonyl groups with DPPH scavenging activity. Results related to the other monosaccharides of the EPS are shown in Table 1, with the findings being in agreement with those of previous studies (Ai et al., 2016; Lv et al., 2009). The study of Tallon et al. (2003) showed that glucose, galactose and xylose were the major components of the EPS produced by *L. plantarum* EP56. Wang et al. (2010) reported that the monosaccharide composition of the microbial EPS extracted from *L. plantarum* KF5 comprised mannose, glucose and galactose (1:4.99:6.90% w/w).

3.6. FT-IR characterization

To understand the cell structure and surface composition of the bacteria, FT-IR methods were utilized (Levine, Stevenson, & Kabler, 1953). FT-IR analysis revealed that the EPS was a complex

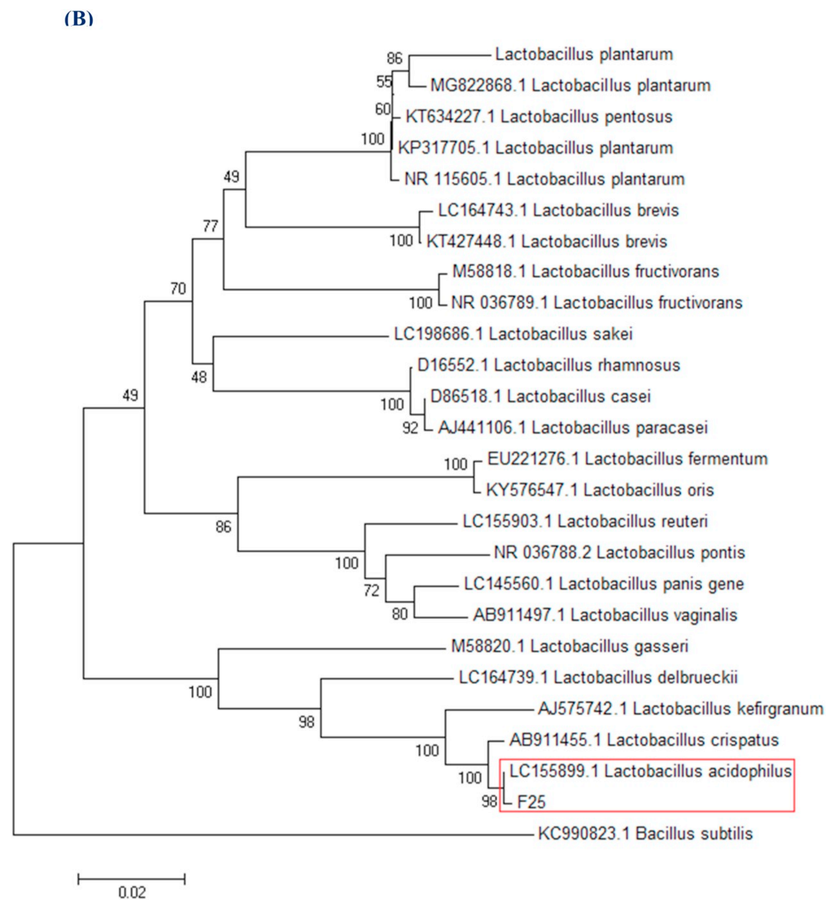


Fig. 2B. Dendrogram showing multiple sequence alignment of 16S rRNA gene sequences of rice bran sourdough of the strain.

polysaccharide with stretching vibrations related to alcoholic, carboxylic acid and aromatic groups. The region of 1200 to 500 cm^{-1} is a fingerprint zone within which various polysaccharides can be identified. FT-IR analysis conveyed strong peaks in the fingerprint zone at wave numbers 1152 cm^{-1}, 1040 cm^{-1}, 710 cm^{-1}, 550 cm^{-1} and 437 cm^{-1}, which correspond with the P=O bond of phosphine oxide, the S=O bond of sulfoxide, RCO-OH bending, 1,2,4,5-tetrasub stretch (aromatics group) and the S–S bond of disulfide, respectively (Fig. 4) http://journal.frontiersin.org/article/10.3389/fmicb.2016.00330/full-F4. Group ranges stretching in the region of 3313 cm^{-1} and 2926 cm^{-1} were related to RCH_2-OH and Ar–H. Also, the intense absorption peaks at around 2349 cm^{-1} (P–H bond of phosphine), 1628 cm^{-1} (Ar-CH=CH-R group with alkene structure) and 1316 cm^{-1} (R-COO-R group with ester structure) were typical of EPSs. These were observed for *L. acidophilus*, representing the stretching vibration of the EPS's alcoholic carboxylic acid, ester groups and aromatic groups. The EPS's water solubility is accounted for by the EPS rings' total characteristic absorption band. Also, their ability in situ is of particular practical importance in fermented foods. These results confirm those of Wang et al. (2010) and Shen, Shi, and Xu (2013).

3.7. NMR characterization of EPS

Nuclear magnetic resonance (NMR) involving ^{1}H and ^{13}C was utilized for further characterization of the EPS. NMR spectroscopy is extensively used for gaining information on organic compounds (Boffo, Tavares, Ferreira, & Ferrera, 2009). The ^{1}H NMR spectrum of the microbial EPS isolated from *L. acidophilus* of RBS (Fig. 5A) demonstrated hydrogen resonances related to the glucosyl residue – a unit repeated within the biopolymer. Carbonyl hydrogens of D-glucopyranose (H-1) were seen as a triplet (t) in δ 3.61 ppm, with a constant coupling (J) of 9.4 Hz; (H-2) was observed in δ 3.84 ppm as a broad doublet (brd;

Table 1
Chemical composition, molecular weight and HPLC analysis of the monosaccharide composition of EPS isolated from traditional rice bran sourdough.

Microbial EPS	Carbohydrate content (0.1mg/10 mL)			Protein content (mg/100 mL)		Phosphate content (W/W %)	Molecular weight (Da)		
L. acidophilus	25.49 ± 0.10			9.55 ± 0.12		0.36 ± 0.02	(2.04×10^{6}) ± 0.06		
	Monosaccharide								
	Galactose			**Glucose**			**Maltose**		
	RT (min)	**Area %**	**C (ppm)**	**RT (min)**	**Area %**	**C (ppm)**	**RT (min)**	**Area %**	**C (ppm)**
	40.04	15.34	19.64	44.47	7.28	12.25	47.01	46.68	122.02

RT: Retention time (min), Area: Area under the peak (%), C: Concentration (ppm).

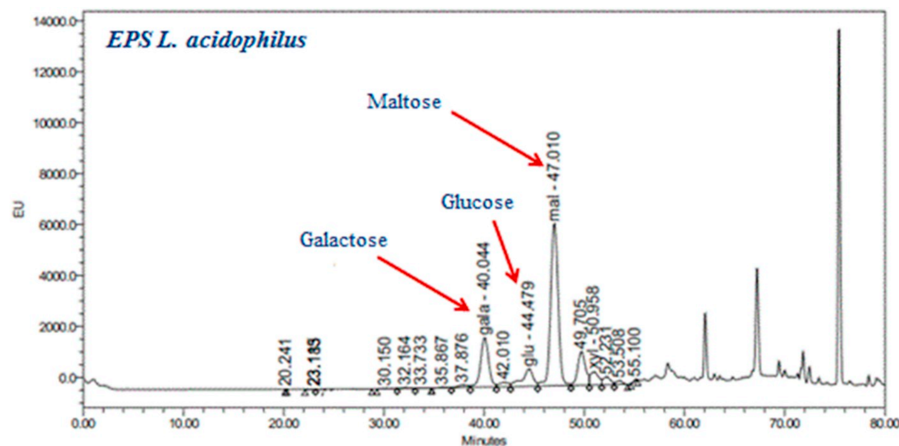


Fig. 3. The HPLC chromatograms of component monosaccharides released from EPS isolated from *L. acidophilus*.

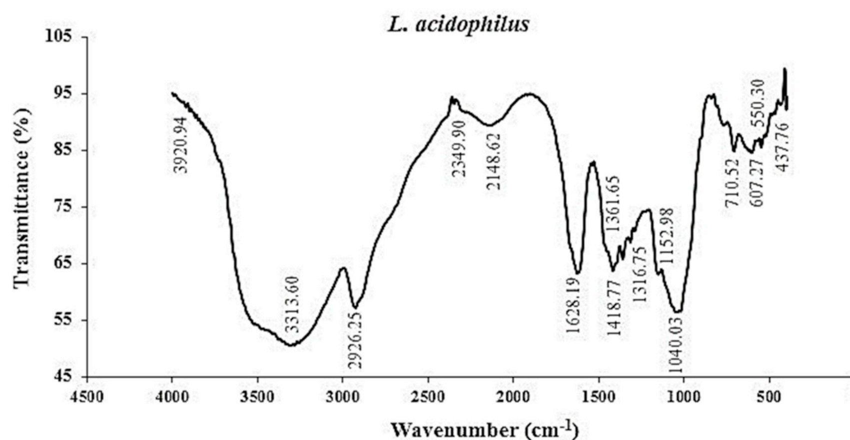


Fig. 4. FT-IR analysis of EPS isolated from *L. acidophilus*.

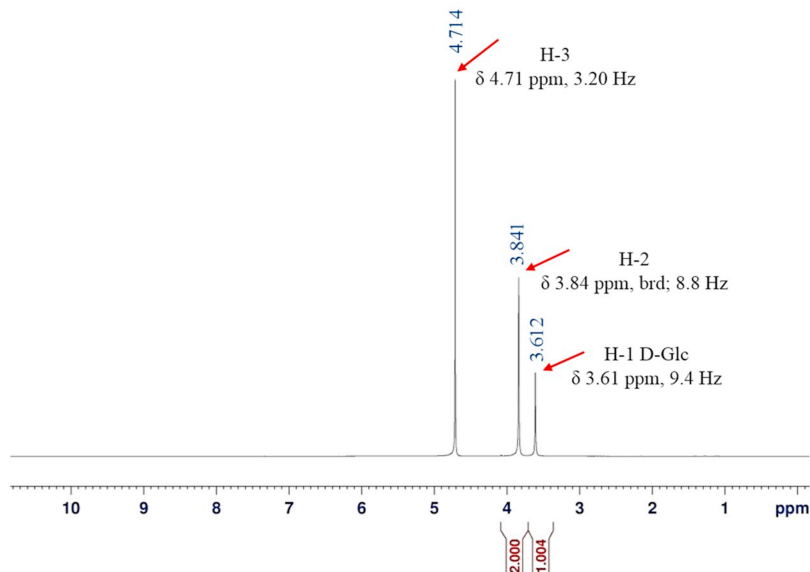
J = 8.8 Hz), and (H-3) was seen as a doublet in δ 4.71 ppm (J = 3.20 Hz) due to anomeric hydrogen. The coupling constant that was seen is typical of D-glucopyranose in its alpha conformation (α). These results match those of Paulo et al. (2012). The ^{13}C NMR spectrum of the EPS extracted from *L. acidophilus* of RBS is shown in Fig. 5B. C-1 was observed at δ 170.7 ppm; C-2 at δ 58.1 ppm and C-3 at δ 51.6 ppm. Branched linages were not indicated since there were no extra peaks between δ 65–100 ppm, meaning that the EPS of *L. acidophilus* is highly linear with α(1 → 6) glycosidic bonds (Uzochukwu, Balogh, Loefler, & Ngoddy, 2002). Also, absorption bands that characterize the EPS's α (1 → 6) bond were found at 1152 cm^{-1} (C–O–C vibrations) and 710 cm^{-1}, indicating an alpha (α) conformation. As explained by Shingel (2002), the highly flexible nature of the polysaccharide chain is represented by the peak at 1040 cm^{-1}. Our result is in consistency with other findings and reconfirms the particular chemical configuration of purified microbial EPS displaying high linearity with long chains and small branching (Yang et al., 2018). The industrial applications of EPS in food industry demonstrated that extremely linear chain of purified EPS from *L. acidophilus* may be applied as a food modulator for thickening, viscosifying as well as a potential soluble fiber acting as a prebiotic. These results are in line with those of Jin et al., (2019).

3.8. Microstructure study by electron microscopy (SEM)

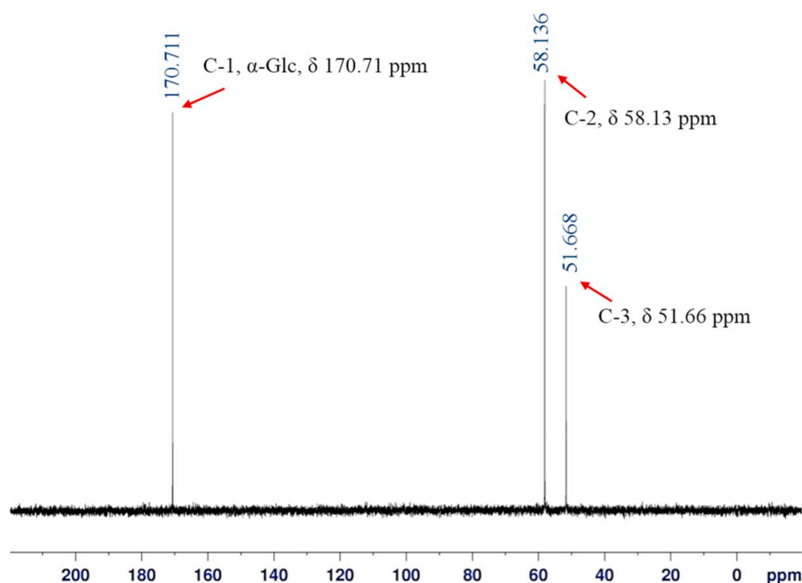
The microscopic structure of the microbial EPS isolated from *L. acidophilus* of RBS (Fig. 6) was highly compact. High molecular weight EPSs result in the formation of highly viscous solutions, with films being formed once the solvents are evaporated (Misaki, Torii, Sawai, & Goldstein, 1980). The EPS extracted from *L. acidophilus* of RBS was dried then crushed before water was used to dissolve it at a low concentration, where it characteristically took the shape of a gel. Also, the presence of this EPS increases product elasticity while facilitating the formation of hydrogels with better 3D networks. The industrial applications of EPS in food industry proved that through addition of the EPS concentration in the RBS, higher link between hydrocolloids and starch could be obtained. Besides, the empty space in the dough system was reduced and, due to that, the water accessibility was diminished. This structure may prepare an appropriate texture in bread baking and decrease the staling time following its production. These results are consistent with those of Salmenkallio-Marttila, Katina, & Autio, (2001). These hydrogels have high hydrophilicity because of their –OH, –COOH, -CONH2 and SO3H groups. These results confirm those of Aouada et al. (2008).

3.9. DPPH scavenging activity of EPS in vitro

Evaluation of DPPH scavenging activity is extensively used for determining antioxidant activity (Hartwig, Brumovsky, Fretes, & Sanchez Boado, 2012) as it involves a low-cost and simple technique that does not require sophisticated equipment. The percentage inhibition of DPPH of the extracted EPS from *L. acidophilus* of RBS was boosted with an increase in the concentration of DPPH radicals relative to the standard ascorbic acid compound. The maximum scavenging capacity was observed at 450 μg/ml of the EPS (81.36%), while the lowest



^{1}H NMR spectrum of purified EPS from *L. acidophilus*, recorded 500-MHz in D_2O.



^{13}C NMR spectrum of purified EPS from *L. acidophilus*, recorded 100-MHz in D_2O.

Fig. 5. H NMR spectrum of EPS produced by *L. acidophilus* (A) and ^{13}C NMR spectrum of EPS produced by *L. acidophilus* (B). (^{1}H NMR: 500-MHz in D_2O; ^{13}C NMR: 100-MHz in D_2O).

scavenging activity was observed at 50 μg/ml of the EPS (17.21%) (Fig. 7). Our results demonstrate a linear correlation between DPPH radical scavenging and EPS concentration when compared to the standard compound. These results correspond with those of Seo et al., 2015. The donation of electrons by the functional groups of the EPSs, particularly the hydroxyl groups, may be the mechanism behind this inhibition (Shen et al., 2013). Abedfar et al. (2018) found that EPS extracted from *L. plantarum* of WBS had dose-dependent antioxidant activity greater than that of ascorbic acid.

4. Conclusion

An important source of LAB is provided by spontaneously fermented RBS. In this research, isolation and screening 16S rDNA showed that the dominant strain which extracted from RBS is *L. acidophilus*. The compositional analysis of the EPS in its partially purified state confirmed that native bacterial strain isolated of RBS is a significant producer of EPS. HPLC analysis revealed that maltose had the greatest retention time among the monosaccharide components of the EPS extracted from

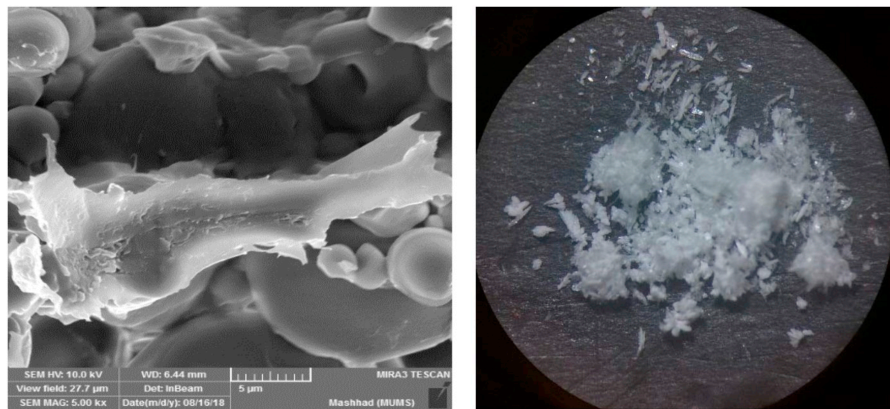


Fig. 6. Microscopic structure of the EPS produced by *L. acidophilus*.

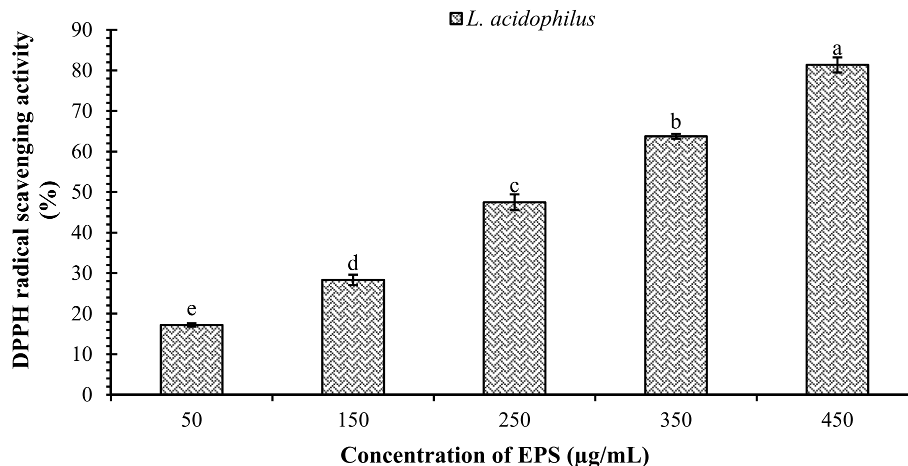


Fig. 7. Antioxidant scavenging capacity by DPPH for *L. acidophilus* (columns sharing the same letter are not significantly different at confidence level of 95%).

L. acidophilus, with glucose and galactose comprising its other major monosaccharide components. FT-IR analysis showed that vibrations in close proximity representative of the functional groups present. Using ^{1}H and ^{13}C NMR spectra, the EPS extracted from *L. acidophilus* was found to be a polymer with an α (1 → 6) straight chain. Finally, remarkable antioxidant characteristics were observed in the isolated EPS.

CRediT authorship contribution statement

Abbas Abedfar: Writing - original draft. **Sepideh Abbaszadeh:** Writing - original draft. **Marzieh Hosseininezhad:** Formal analysis. **Maryam Taghdir:** Formal analysis.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

This study was financially supported by Research Institute of Food Science and Technology (RIFST), Iran. The authors would like to thank Dr. A. Sadeghi for providing the microbial strain and helping in isolation and screening 16S rDNA identification of the dominant LAB.

References

AACC International (2010). *Approved methods of the American association of cereal chemists.* 11th, the St Paul.

Abedfar, A., Hosseininezhad, M., Sadeghi, A., Raeisi, M., & Feizy, J. (2018). Investigation on "spontaneous fermentation" and the productivity of microbial exopolysaccharides by *Lactobacillus plantarum* and *Pediococcus pentosaceus* isolated from wheat bran sourdough. *LWT-Food Science and Technology, 96*, 686–693.

Ai, L., Guo, Q., Ding, H., Guo, B., Chen, W., & Cui, S. W. (2016). Structure characterization of exopolysaccharides from Lactobacillus casei LC2W from skim milk. *Food Hydrocolloids, 56*, 134–143.

Aouada, F. A., Moura, M. R. D., Girotto, E. M., Rubira, A. F., Muniz, E. C., & Campese, G. M. (2008). Caracterização de hidrogéis condutores constituídos por PAAm e PEDOT/PSS por meio de planejamento fatorial. *Polímeros, 18*, 126–131.

Aplevicz, K. S., Ogliari, P. J., & Sant'Anna, E. S. (2013). Influence of fermentation time on characteristics of sourdough bread. *Brazilian Journal of Pharmaceutical Sciences, 49*, 233–239.

Bajpai, V. K., Rather, I. A., Majumder, R., Shukla, S., Aeron, A., Kim, K., et al. (2015). Exopolysaccharide and lactic acid bacteria: Perception, functionality and prospects. *Bangladesh Journal of Pharmacology, 11*, 1–23.

Boffo, E. F., Tavares, L. A., Ferreira, M. M., & Ferreira, A. G. (2009). Classification of Brazilian vinegars according to their 1H NMR spectra by pattern recognition analysis. *LWT-Food Science and Technology, 42*, 1455–1460.

Boza, Y., Neto, L. P., Costa, F. A., & Scamparini, A. R. (2004). Exopolysaccharide production by encapsulated Beijerinckia cultures. *Process Biochemistry, 39*, 1201–1209.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*, 248–254.

Clarke, C. I., Schober, T. J., Dockery, P., O'Sullivan, K., & Arendt, E. K. (2004). Wheat sourdough fermentation: Effects of time and acidification on fundamental rheological properties. *Cereal Chemistry, 81*, 409–417.

Dal Bello, F., Clarke, C. I., Ryan, L. A. M., Ulmer, H., Schober, T. J., Ström, K., et al. (2007). Improvement of the quality and shelf life of wheat bread by fermentation with the antifungal strain *Lactobacillus plantarum* FST 1.7. *Journal of Cereal Science, 45*, 309–318.

Davis, R., & Mauer, L. J. (2010). Fourier transform infrared (FT-IR) spectroscopy: A rapid tool for detection and analysis of food borne pathogenic bacteria. *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, 2*, 1582–1594.

De Vuyst, L., Van Kerrebroeck, S., Harth, H., Huys, G., Daniel, H. M., & Weckx, S. (2014). Microbial ecology of sourdough fermentations: Diverse or uniform? *Food Microbiology, 37*, 11–29.

Di Cagno, R., Pontonio, E., Buchin, S., De Angelis, M., Lattanzi, A., Valerio, F., et al.

(2014). Diversity of the lactic acid bacteria and yeast microbiota switching from firm to liquid sourdough fermentation. *Applied and Environmental Microbiology, 80*, 3161–3172.
Galle, S., & Arendt, E. K. (2014). Exopolysaccharides from sourdough lactic acid bacteria. *Critical Reviews in Food Science and Nutrition, 54*(7), 891–901.
Gobbetti, M., Rizzello, C. G., Di Cagno, R., & De Angelis, M. (2014). How the sourdough may affect the functional features of leavened baked goods. *Food Microbiology, 37*, 30–40.
Goh, K. K., Haisman, D. R., Archer, R. H., & Singh, H. (2005). Evaluation and modification of existing methods for the quantification of exopolysaccharides in milk-based media. *Food Research International, 38*, 605–613.
Harold, F. M. (1966). Inorganic polyphosphates in biology: Structure, metabolism, and function. *Bacteriological Reviews, 30*(4), 772–794.
Hartwig, V. G., Brumovsky, L. A., Fretes, R. M., & Boado, L. S. (2012). A novel procedure to measure the antioxidant capacity of yerba maté extracts. *Food Science and Technology (Campinas), 32*, 126–133.
Holt, G., Krieg, N., Sneath, P., Staley, J., & Williams, S. (1994). *Bergey's manual of determinative of Bacteriology* (9th ed.). USA: Williams and Wilkins.
Imran, M. Y. M., Reehana, N., Jayaraj, K. A., Ahamed, A. A. P., Dhanasekaran, D., Thajuddin, N., et al. (2016). Statistical optimization of exopolysaccharide production by *Lactobacillus plantarum* NTMI05 and NTMI20. *International Journal of Biological Macromolecules, 93*, 731–745.
Katina, K., Heiniö, R. L., Autio, K., & Poutanen, K. (2006). Optimization of sourdough process for improved sensory profile and texture of wheat bread. *LWT-Food Science and Technology, 39*, 1189–1202.
Katina, K., Juvonen, R., Laitila, A., Flander, L., Nordlund, E., Kariluoto, S., et al. (2012). Fermented wheat bran as a functional ingredient in baking. *Cereal Chemistry, 89*, 126–134.
Leite, A. M., Miguel, M. A., Peixoto, R. S., Ruas-Madiedo, P., Paschoalin, V. M., Mayo, B., et al. (2015). Probiotic potential of selected lactic acid bacteria strains isolated from Brazilian kefir grains. *Journal of Dairy Science, 98*, 3622–3632.
Levine, S., Stevenson, H. J., & Kabler, P. W. (1953). Qualitative studies of pneumococcal polysaccharides by infrared spectrophotometry. *Archives of Biochemistry and Biophysics, 45*, 65–73.
Lhomme, E., Lattanzi, A., Dousset, X., Minervini, F., De Angelis, M., Lacaze, G., et al. (2015). Lactic acid bacterium and yeast microbiotas of sixteen French traditional sourdoughs. *International Journal of Food Microbiology, 215*, 161–170.
Li, J. Y., Jin, M. M., Meng, J., Gao, S. M., & Lu, R. R. (2013). Exopolysaccharide from *Lactobacillus planterum* LP6: Antioxidation and the effect on oxidative stress. *Carbohydrate Polymers, 98*, 1147–1152.
Liu, X., Zhou, B., Lin, R., Jia, L., Deng, P., Fan, K., et al. (2010). Extraction and antioxidant activities of intracellular polysaccharide from Pleurotus sp. mycelium. *International Journal of Biological Macromolecules, 47*, 116–119.
Lv, Y., Yang, X., Zhao, Y., Ruan, Y., Yang, Y., & Wang, Z. (2009). Separation and quantification of component monosaccharides of the tea polysaccharides from Gynostemma pentaphyllum by HPLC with indirect UV detection. *Food Chemistry, 112*, 742–746.
Lynch, K. M., Coffey, A., & Arendt, E. K. (2018). Exopolysaccharide producing lactic acid bacteria: Their techno-functional role and potential application in gluten-free bread products. *Food Research International, 110*, 52–61.
Manini, F., Casiraghi, M. C., Poutanen, K., Brasca, M., Erba, D., & Plumed-Ferrer, C. (2016). Characterization of lactic acid bacteria isolated from wheat bran sourdough. *LWT-Food Science and Technology, 66*, 275–283.
Misaki, A., Torii, M., Sawai, T., & Goldstein, I. J. (1980). Structure of the dextran of *Leuconostoc mesenteroides* B-1355. *Carbohydrate Research, 84*, 273–285.
Paulo, E. M., Boffo, E. F., Branco, A., Valente, Â. M., Melo, I. S., Ferreira, A. G., et al. (2012). Production, extraction and characterization of exopolysaccharides produced by the native *Leuconostoc pseudomesenteroides* R2 strain. *Anais da Academia Brasileira de Ciências, 84*, 495–508.
Razmkhah, S., Mohammadifar, M. A., Razavi, S. M. A., & Ale, M. T. (2016). Purification of cress seed (*Lepidium sativum*) gum: Physicochemical characterization and functional properties. *Carbohydrate Polymers, 141*, 166–174.
Sadeghi, A., Ebrahimi, M., Mortazavi, S. A., & Abedfar, A. (2019). Application of the selected antifungal LAB isolate as a protective starter culture in pan whole-wheat sourdough bread. *Food Control, 95*, 298–307.
Salmenkallio-Marttila, M., Katina, K., & Autio, K. (2001). Effects of bran fermentation on quality and microstructure of high-fiber wheat bread. *Cereal Chemistry, 78*(4), 429–435.
Seo, B. J., Bajpai, V. K., Rather, I. A., & Park, Y. H. (2015). Partially purified exopolysaccharide from *Lactobacillus plantarum* YML009 with total phenolic content, antioxidant and free radical scavenging efficacy. *Indian Journal of Pharmaceutical Education and Research, 49*, 282–292.
Shen, J. W., Shi, C. W., & Xu, C. P. (2013). Exopolysaccharides from *Pleurotus pulmonarius*: Fermentation optimization, characterization and antioxidant activity. *Food Technology and Biotechnology, 51*, 520–527.
Shingel, K. I. (2002). Determination of structural peculiarities of dexran, pullulan and γ-irradiated pullulan by Fourier-transform IR spectroscopy. *Carbohydrate Research, 337*, 1445–1451.
Tallon, R., Bressollier, P., & Urdaci, M. C. (2003). Isolation and characterization of two exopolysaccharides produced by *Lactobacillus plantarum* EP56. *Research in Microbiology, 154*, 705–712.
Tamani, R. J., Goh, K. K. T., & Brennan, C. S. (2013). Physico-chemical properties of sourdough bread production using selected *lactobacilli* starter cultures. *Journal of Food Quality, 36*, 245–252.
Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution, 30*, 2725–2729.
Tieking, M., Korakli, M., Ehrmann, M. A., Gänzle, M. G., & Vogel, R. F. (2003). In situ production of exopolysaccharides during sourdough fermentation by cereal and intestinal isolates of lactic acid bacteria. *Applied and Environmental Microbiology, 69*(2), 945–952.
Tsuda, H., & Miyamoto, T. (2010). Production of exopolysaccharide by *Lactobacillus plantarum* and the prebiotic activity of the exopolysaccharide. *Food Science and Technology Research, 16*, 87–92.
Uzochukwu, S., Balogh, E., Loefler, R. T., & Ngoddy, P. O. (2002). Structural analysis by 13C-nuclear magnetic resonance spectroscopy of glucan extracted from natural palm wine. *Food Chemistry, 76*, 287–291.
Venturi, M., Guerrini, S., Granchi, L., & Vincenzini, M. (2012). Typing of *Lactobacillus sanfranciscensis* isolates from traditional sourdoughs by combining conventional and multiplex RAPD–PCR profiles. *International Journal of Food Microbiology, 156*, 122–126.
Vogelmann, S. A., & Hertel, C. (2011). Impact of ecological factors on the stability of microbial associations in sourdough fermentation. *Food Microbiology, 28*, 583–589.
Vrancken, G., Rimaux, T., Weckx, S., Leroy, F., & De Vuyst, L. (2011). Influence of temperature and backslopping time on the microbiota of a type I propagated laboratory wheat sourdough fermentation. *Applied and Environmental Microbiology, 77*, 2716–2726.
Vu, B., Chen, M., Crawford, R. J., & Ivanova, E. P. (2009). Bacterial extracellular polysaccharides involved in biofilm formation. *Molecules, 14*, 2535–2554.
Wang, Y., Li, C., Liu, P., Ahmed, Z., Xiao, P., & Bai, X. (2010). Physical characterization of exopolysaccharide produced by *Lactobacillus plantarum* KF5 isolated from Tibet Kefir. *Carbohydrate Polymers, 82*, 895–903.
Wang, J., Zhao, X., Tian, Z., Yang, Y., & Yang, Z. (2015). Characterization of an exopolysaccharide produced by *Lactobacillus plantarum* YW11 isolated from Tibet Kefir. *Carbohydrate Polymers, 125*, 16–25.
Yang, Y., Feng, F., Zhou, Q., Zhao, F., Du, R., Zhou, Z., et al. (2018). Isolation, purification and characterization of exopolysaccharide produced by *Leuconostoc pseudomesenteroides* YF32 from soybean paste. *International Journal of Biological Macromolecules, 114*, 529–535.
Zhou, X., Hong, T., Yu, Q., Nie, S., Gong, D., Xiong, T., et al. (2017). Exopolysaccharides from *Lactobacillus plantarum* NCU116 induce c-Jun dependent Fas/Fasl-mediated apoptosis via TLR2 in mouse intestinal epithelial cancer cells. *Scientific Reports, 7*, 1–13.

View publication stats