LWT - Food Science and Technology 71 (2016) 116–124

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Characterisation of lactic acid bacteria from Turkish sourdough and determination of their exopolysaccharide (EPS) production characteristics

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ARTICLE INFO

Article history:
Received 13 November 2015
Received in revised form
20 January 2016
Accepted 16 March 2016
Available online 19 March 2016

Keywords:
Sourdough
Lactic acid bacteria (LAB)
Microbial biodiversity
Exopolysaccharides (EPS)

ABSTRACT

A total of 249 Lactic Acid Bacteria (LAB) isolates were found in traditional Turkish wheat sourdoughs from Eastern Black Sea region of Turkey. Genotypic characterization of these isolates revealed the presence of 47 distinct LAB strains belonging to 11 different species: *Lactobacillus plantarum*, *Lactobacillus paraplantarum*, *Lactobacillus curvatus*, *Lactobacillus rossiae*, *Lactobacillus sanfranciscensis*, *Lactobacillus brevis*, *Lactobacillus paralimentarius*, *Weissella paramesenteroides*, *Leuconostoc mesenteroides*, *Leuconostoc pseudomesenteroides* and *Weissella cibaria*. The sourdough LAB microbiota differed depending on the sample origin and the collection period and heterofermentative LAB were dominant. The number of different species within a sourdough varied from 3 to 6 with the association of different hetero- and homofermentative LAB species. Exopolysaccharide (EPS) production characteristics of the isolates were determined and strain specific properties appeared to be important for the final EPS yields. Genes required for homopolysaccharide (*gtf* and *lev*) and heteropolysaccharide (*epsA*, *epsB*, *p-gtf*) production were PCR detected and several distribution patterns were observed. Results of this study confirmed the biodiversity of LAB species in traditional Turkish sourdough and highlighted the importance of EPS production in sourdough LAB strains.

1. Introduction

There is an increasing demand for sourdough based bakery products due to several advantages of sourdough in comparison to use of baker's yeast in cereal fermentations (Robert, Gabriel, & Fontagne-Faucher, 2009). Preparation of bread dough with sourdough improves technological properties of dough, enhances the nutritional and sensory properties of bread and increases the keeping properties of bread by retarding the staling process and preventing bacterial and mould spoilage (Arendt, Ryan, & Dal Bello, 2007; Hammes & Gänzle, 1997). Sourdough is a mixture of flour and water that is fermented with lactic acid bacteria (LAB) and yeasts (De Vuyst & Neysens, 2005; Gobbetti, 1998). The sourdough microflora contains metabolically active LAB and yeasts that form this intermediate product for dough preparation. The positive effects of sourdough generally rely on the metabolism of the LAB in sourdough that can be originated from flour and other dough ingredients (De Vuyst & Neysens, 2005). The main metabolic activities of sourdough LAB determining importance of sourdough are their proteolytic activity (Gobbetti et al., 1995), formation of volatile, antibacterial and antimould compounds (Corsetti & Settanni, 2007) as well as their exopolysaccharide (EPS) production characteristics (Galle & Arendt, 2014).

Several types of traditional sourdoughs having cultural and geographical identities exist all over the world in which different types of flours and other ingredients as well as fermentation methodologies are used (De Vuyst & Neysens, 2005; Robert et al., 2009). These differences in sourdough production process determine the sourdough LAB microflora. In addition to the LAB in sourdoughs, natural yeasts also play important roles on fermentation process and in general the LAB: yeast ratio in sourdough is 100:1. Both homo- and hetero-fermentative LAB species are

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http://dx.doi.org/10.1016/j.lwt.2016.03.030
0023-6438/

present in sourdoughs although unlike to the other food fermentations heterofermentative species generally dominate sourdoughs. Sourdough has a rich LAB microflora in which *Lactobacillus* strains are present more frequently than *Leuconostoc*, *Pediococcus* and *Weissella* species (De Vuyst & Neysens, 2005). The variety of sourdough LAB depends mainly on fermentation kinetics which determines the final characteristics of the bread. The most frequent *Lactobacillus* species in sourdoughs appeared to be *Lactobacillus sanfranciscensis, Lactobacillus brevis* and *Lactobacillus plantarum* although more than 30 *Lactobacillus* species have been isolated from traditional sourdoughs. Additionally several studies also showed the importance of *Leuconostoc* and *Weissella* as less dominant species in sourdoughs (Wolter et al., 2014).

Recent studies showed that one of the important properties of sourdough LAB is their EPS production characteristics (Galle & Arendt, 2014). EPS are natural biopolymers produced by several LAB species that can either encapsulate bacteria or be secreted in their environment (Dertli et al., 2013). EPS play critical roles in stress resistance at single cell level (Dertli, Mayer, & Narbad, 2015) and they have unique physicochemical effects improving the technological properties of sourdough and bread (Tieking, Korakli, Ehrmann, Gänzle, & Vogel, 2003). Structurally, EPS are divided into two groups as homopolysaccharides and heteropolysaccharides which are composed of only one type of sugar monomer and two or more types of sugar monomers, respectively. For the production of homopolymeric one, only one gene determined as *gtf* or *ftf* is required whereas for the heteropolymeric EPS production a complex *eps* gene cluster harbouring several genes is required (Dertli et al., 2013). Identification of the EPS production characteristics of sourdough LAB is crucial in order to reflect their overall role during fermentation process.

Up to date, only few reports appeared on identification of LAB microflora of Turkish sourdoughs despite the great importance of sourdough technology in Turkish bakery industry and the presence of different *Lactobacillus* species as well as some *Pediococcus* species were shown in Turkish sourdoughs (Gül, Özçelik, Sağdıç, & Certel, 2005; Şimşek, Çon, & Tulumoğlu, 2006). In this study sourdough samples were collected from Eastern Black sea region of Turkey where famous 'Vakfıkebir bread' is produced with sourdough technology in order to identify the LAB microflora of traditional Turkish sourdoughs. Our results showed the presence of 11 different species of LAB in sourdoughs in which collection period determined the variety of the presented LAB species. Both homo- and heterofermentative LAB species were present although the later ones were dominant. PCR detection of the *eps* genes revealed that all selected strains were positive for genes required for the homopolysaccharide and heteropolysaccharide production although the level of EPS production varied among the tested strains. This study shows the wide diversity of LAB species present in sourdoughs collected from Vakfıkebir region and reveals the domination of the EPS producing LAB strains in sourdough samples.

2. Materials and methods

2.1. Sample collection

In total 12 sourdough samples (A-L) were collected aseptically from small bakeries in Vakfıkebir, Trabzon in order to isolate and identify the LAB strains from traditional sourdoughs. Samples A-C, D-F and G-L were collected at one month intervals representing the three collection periods. All sourdough samples were produced from wheat flour with regular propagation by backslopping at 20–30 °C to keep microorganisms in an active state and all sourdoughs were at the final stage of fermentation stage before the inoculation to final dough for the bread production.

2.2. Characterization of sourdough samples and microbiological analyses

The pH value of sourdough samples was determined by a pH metre (WTW 720) with a suitable penetration probe. Total LAB and yeast counts of sourdough samples were determined by plating to corresponding agar plates. For the isolation of LAB from sourdough samples, serial dilutions were conducted up to 10^{-5} dilution factor with PBS and plated onto MRS5 agar containing 10 g of maltose, 5 g of fructose, 5 g of glucose, 10 g of tryptone, 5 g of meat extract, 5 g of yeast extract, 5 g of $C_2H_3NaO_2 \cdot 3H_2O$, 3 g of ammonium chloride, 2.6 g of $K_2HPO_4 \cdot 3H_2O$, 4 g of KH_2PO_4, 0.1 g of $MgSO_4 \cdot 7H_2O$, 0.05 g of $MnSO_4 \cdot 4H_2O$, 0.5 g of cysteine-HCl, 1 ml of Tween 80, and 1 ml of a vitamin mixture (pH 5.8) per litre and 0.1 g of cycloheximide for the inhibition of yeast growth (Meroth, Walter, Hertel, Brandt, & Hammes, 2003) and plates were incubated under anaerobic conditions at 30 °C for 2 days. At the end of incubation period colonies with potential different morphologies and slimy characteristics were picked randomly from agar plates of all samples and propagated into MRS5 medium and incubated at 30 or 37 °C for 2 days and then tested for Gram stain, cell morphology and catalase reaction.

2.3. Bacterial growth conditions

In total, 249 LAB isolates were selected for further analysis. All isolates were grown in MRS5 medium at 30 °C anaerobically and stock solutions of isolates were prepared in 20% (v/v) glycerol and stored at −80 °C. For the isolation of genomic DNA from bacterial cultures all strains were grown overnight at 30 °C in MRS5 broth. For the isolation of EPS from sourdough LAB, selected strains were grown in 100 ml MRS5 culture at 30 °C and 37 °C for 2 d under anaerobic conditions.

2.4. Genotypic characterization by rep-PCR, Box-PCR and RAPD-PCR analysis

For the discrimination LAB strains isolated from sourdough at species level firstly rep-PCR analysis was performed as described elsewhere (Sagdic, Ozturk, Yapar, & Yetim, 2014). For the isolation of genomic DNA a commercial isolation kit was used and extractions were performed according to manufacturer's protocol (Qiagen, Turkey). For the repetitive sequence based-PCR (rep-PCR) analysis primer $(GTG)_5$ (5′-GTGGTGGTGGTGG-3′) was used and PCR reactions were prepared containing 1 μl DNA template, 10 μl 5 × PCR buffer for Taq polymerase (Promega), 0.4 μl dNTPs (Bioline), 2 μl 20 mM primer $(GTG)_5$, 0.25 μl 5 U Taq polymerase and up to 50 μl of sterile H_2O. PCR was performed using a thermal cycler (Benchmark, TC9639) with the following program: Initial denaturation for 10 min at 95 °C, 35 cycles at 94 °C for 60 s, 40 °C for 60 s, and 65 °C for 8 min; and followed by a final elongation step of 65 °C for 16 min. The rep-PCR products were separated on a 1% (wt/vol) agarose gel and visualised by ethidium bromide staining and photographed under UV illumination.

The BOX-PCR repetitive element analysis as a second molecular identification method was performed with primer BOX A1R (5′-CTACGGCAAGGCGACGCTGACG-3′) using the following program: initial denaturation for 7 min at 95 °C, 35 cycles at 94 °C for 60 s, 53 °C for 60 s, and 65 °C for 8 min; and followed by a final elongation step of 65 °C for 16 min and PCR products were separated using previously described methodology (Sagdic et al., 2014).

In the final step of strain differentiation of all 249 sourdough isolates, RAPD-PCR analysis was conducted with primer M13.