

Diversity of *Lactobacillus* Strains in Iranian Traditional Wheat Sourdough

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Abstract

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Background: Lactic acid bacteria play a key role in sourdough fermentation. *Lactobacilli* are the most important group of lactic acid bacteria in sourdough. Therefore, the main objective of this study was to identify *Lactobacillus* strains in Iranian traditional wheat sourdoughs as a basis for further studies on the technological applications in the production of typical breads.

Methods: Fourteen traditional sourdough samples were collected from the Southern regions in East-Azarbaijan province of Iran. In all sourdough samples, pH and Total Titratable Acidity (TTA) values were determined, and then isolation and identification of *Lactobacillus* isolates were carried out.

Results: Most sourdough samples had the pH value of 3.64 to 3.90 and the TTA varied from 16.8 to 19.2 ml of 0.1 N NaOH/10 g sourdough. Isolates were divided into two main groups (group A and group B) using phenotypic characteristics, group B subdivided into eight groups. Overall, 38% of the isolates, which clustered together in group A, were closely related to *Lactobacillus paralimentarius*, while 27% and 20% of the isolates were closely related to *Lactobacillus plantarum* and *Lactobacillus curvatus*, respectively. A few isolates were also identified as *Lactobacillus agilis* and *Lactobacillus hilgardii*.

Conclusion: *Lactobacillus paralimentarius*, *Lactobacillus plantarum*, *Lactobacillus curvatus*, and the rare presence of *Lactobacillus agilis* and *Lactobacillus hilgardii* were identified in Iranian traditional sourdough. These *lactobacilli* may have potential to be used as starter cultures in the manufacture of sourdough breads with perfect and good quality.

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Introduction

Sourdough is a complex microbial ecosystem, basically represented by lactic acid bacteria and yeasts (Clarke and Arendt, 2005; Corsetti and Settanni, 2007). Many researchers have studied the lactic microflora of sourdoughs (Corsetti et al., 2001; Rocha and Malcata, 1999). Presence of lactic acid bacteria in sourdough is well known and the most of these bacteria belong to the genus *Lactobacillus*

(Clarke and Arendt, 2005; Gobbetti, 1998; Gül et al., 2005). Traditional sourdoughs, which are produced by continuous propagation at ambient temperature, are dominated by *Lactobacillus sanfranciscensis*, *Lactobacillus pontis*, *Lactobacillus brevis*, *Lactobacillus plantarum*, *Lactobacillus paralimentarius*, and *Lactobacillus rossiae* (De Vuyst et al., 2009; Ricciardi et al., 2005). However, lactic acid bacteria especially *Lactobacilli* play an important role in the sourdough breads making process, they also improve wheat bread quality (Crowley et al., 2002; Dal Bello et al., 2007).

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Sourdough breads are traditional products which are still produced in a few rural regions of Iran. These breads are produced by traditional techniques and differ according to their flour, sourdough, other ingredients and baking method. Nowadays, there is an increasing attention towards wheat breads production with good quality in the country; thereby using sourdough can gain popularity as a means to improve the quality and flavor of the breads. Although many studies have been carried out on the physicochemical and microbiological characteristics of sourdoughs from different countries (Iacumin et al., 2009; Okada et al., 1992; Reale et al., 2005; Rosenquist and Hansen, 2000; Şimşek et al., 2006), but there is no information about the properties of Iranian wheat sourdoughs which are produced in rural regions.

The main objective of this study was to identify *Lactobacillus* strains in Iranian traditional wheat sourdoughs as a basis for further studies on the technological applications in the production of typical breads.

Materials and methods

Sourdough sampling areas

A total of 14 samples of sourdough were collected from household bakeries in villages belonged to areas of Bostanabad, Hashtrud, Charoimagh and Mianeh in East-Azərbayjan province, Iran. This province, with a cool dry climate, is located in Northwest of Iran.

Chemical analyses of sourdough samples

Total Titratable Acidity (TTA) and pH values of sourdough samples were determined by a method described by Paramithiotis et al. (2006). The acidity was determined by titrating the samples with 0.1 N NaOH to final pH of 8.5 and expressed in 0.1 N NaOH.

Isolation and identification of *Lactobacillus* strains

Appropriate dilutions of sourdough were plated in MRS agar and the plates were incubated under anaerobic conditions at 30 °C for 48-72 h. Following incubation, pure colonies were isolated as explained by Ricciardi et al. (2005).

All isolates were initially subjected to Gram staining and the catalase test. Presumptive *Lactobacillus* isolates (Gram-positive and catalase-negative bacilli) were selected for further identification. For the isolates, growth was also tested at 15 °C and 45 °C in MRS broth for 7 days and 48 h, respectively and in the presence of 2, 4 and 6.5% NaCl. The fermentation of carbohydrates was determined in MRS broth containing phenol red as a pH indicator, and supplemented with 1% of the following carbohydrates: glucose, lactose, melibiose, arabinose, fructose, cellobiose, sucrose, trehalose,

melezitose, rhamnose, raffinose, ribose, mannose, mannitol, xylose, galactose, and salicin (Corsetti et al., 2001).

Statistical analysis

The relationships among the isolated strains were determined by cluster analysis. Phenotypic characteristics were used for grouping the isolates. The results of the phenotypic tests were coded as 0, negative or 1, positive and then their related trees were drawn. Analysis was performed using the Numerical Taxonomy and Multivariate Analysis System (NTSYS) package version 2.02.

Results

The results of the pH and TTA measurements of the sourdough samples are presented in Fig. 1. As shown in the mentioned figure, two different groups were detected. The sourdough samples of group A (11 samples) were characterized by higher degree of acidification with pH values of 3.64 to 4.04 and TTA between 13.8 and 19.2. Three samples (group B) had a lower degree of acidification, with pH values of 4.67 to 5.02 and TTA between 5.6 and 10.

Forty five presumptive *Lactobacilli* were isolated from 14 sourdoughs and subjected to biochemical and physiological tests. All isolates were Gram-positive and catalase-negative rods which could grow at 15 °C. Twenty three of 45 strains grew at 45 °C. The strains belonging to groups B2, B4, B7, and B8 were not able to grow at 45 °C. All isolates yielded positive results in MRS broth medium containing 2, 4, and 6.5% NaCl (Table 1).

The phenotypic characteristics were used to cluster the isolated strains, and are shown as a dendrogram (Fig. 2). Based on the biochemical tests, the isolates were clustered into two major groups (group A and group B). Group A contained 28 isolates (B1a, B1b, B2a, B2b, B2c, B3a, B3d, CH1c, CH2a, CH2b, H2b, M1b, M3c, B1c, H1a, H2a, B3b, H3a, M2b, B2d, B3e, M2c, M2a, CH1d, CH2d, M3a, M3b, M4a). Group B, which contained the remaining 17 isolates, could be subdivided into eight subgroups (B1 to B8): subgroup B1: B3c, CH1b, CH3a, H3c, CH3b, H3b, M1c, B4c, M4c, and H1b; subgroup B2: B4a; subgroup B3: M1a; subgroup B4: CH2c; subgroup B5: B4b; subgroup B6: CH1a; subgroup B7: M4b and subgroup B8: H1c.

There were differences in sugar fermentation profiles among the strains from groups A and B (Table 2). About 38% of the isolates, which were in group A, had close resemblance to *Lactobacillus paralimentarius*. A group of twelve isolates (27%) and nine isolates (20%) were identified as *Lactobacillus plantarum* and *Lactobacillus curvatus*, respectively with regard to their phenotypic properties. Two isolates (B3b, H3a) were closely related to *Lactobacillus agilis* by comparing with the species descriptions. The only

isolate in group B4 (CH2c) seemed to be *Lactobacillus hilgardii*. The strains in groups B6, B7, and B8 were pheno-

typically close to each other but their identification was not possible because they did not cluster with reference strains.

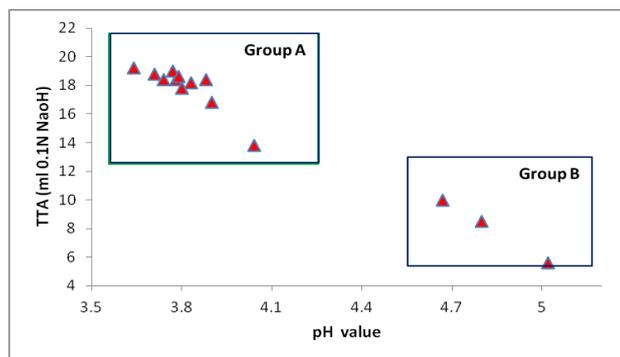


Fig. 1: TTA and pH of the sourdoughs from different regions of East-Azərbayjan province, Iran

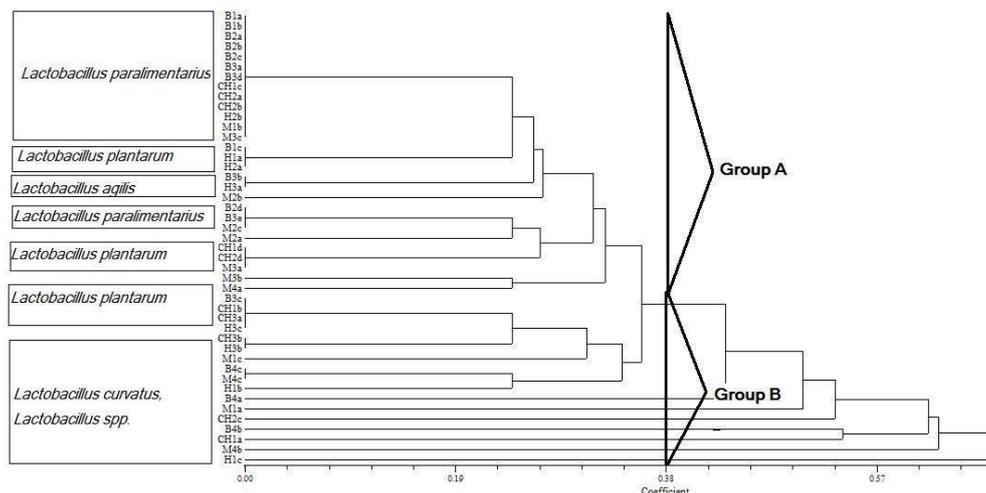


Fig. 2: Dendrogram showing the clustering of the isolated *Lactobacillus* strains based on their phenotypic characteristics

Table 1: Characteristics of presumptive *Lactobacillus* isolates based on some physiological tests

	Group A		Group B (1-8)							
			B1	B2	B3	B4	B5	B6	B7	B8
Number of isolates	28		10	1	1	1	1	1	1	1
Gram staining	+		+	+	+	+	+	+	+	+
Catalase test	-		-	-	-	-	-	-	-	-
Growth at 15 °C	+		+	+	+	+	+	+	+	+
Growth at 45 °C	+(14)*		+(4)	-	+	-	+	+	-	-
Growth at 2% NaCl	+		+	+	+	+	+	+	+	+
Growth at 4% NaCl	+		+	+	+	+	+	+	+	+
Growth at 6.5% NaCl	+		+	+	+	+	+	+	+	+

*Numbers in parentheses indicate the number of strains showing a negative reaction

Table 2: Fermentative characteristics of presumptive *Lactobacillus* strains isolated from traditional sourdoughs*

	Group A		Group B (1-8)						
	A	B1	B2	B3	B4	B5	B6	B7	B8
Number of isolates	28	10	1	1	1	1	1	1	1
Glucose	+	+	+	+	+	+	+	+	+
Lactose	26+, 2W	9+, 1W	+	+	+	-	-	+	-
Melibios	25+, 3-	+	W	+	+	-	-	+	-
Arabinose	19+, 2-, 7W	7+, 3W	-	+	+	-	+	+	-
Fructose	+	+	+	+	+	W	+	-	+
Cellobios	+	+	+	+	+	-	+	+	W
Sucrose	+	+	+	-	+	-	-	-	W
Trehalose	+	9+, 1-	+	-	-	+	+	+	-
Melezitose	26+, 1-, 1W	+	+	-	+	+	-	+	-
Salicine	+	+	+	+	+	+	+	-	-
Rhamnose	5+, 23-	7-, 3W	W	+	-	-	-	-	-
Raffinose	15+, 13W	-	-	+	-	W	-	-	-
Ribose	+	+	+	+	W	+	+	-	-
Mannose	+	+	+	+	-	+	+	+	-
Manitol	+	9+, 1-	+	+	-	+	-	+	-
Xylose	3+, 22-, 3W	2+, 8-	+	-	+	W	W	-	-
Galactose	27+, 1-	9+, 1W	+	+	W	+	+	+	-

*+: Positive reaction; -: Negative reaction; W: Weak reaction

Discussion

The results of pH and TTA of sourdough samples in the present study fall within the ranges reported in the literature. The pH scale of sourdoughs varies with the kind of starter culture, flour ash content, and nature of the process used but for wheat sourdough it ranges from 3.5 to 4.3 (Clarke and Arendt, 2005). In spontaneous sourdough fermentation, the initial pH usually is within the range of 5 to 6.2 and during the fermentation, it reaches to approximately 3.5 to 4.2 (Corsetti et al., 2001). The higher pH in three sourdough samples of this research might be due to incomplete fermentation of the sourdoughs by lactic acid bacteria; since these sourdoughs had been fermented for a short time and then stored in freezers.

All isolates were found to be *Lactobacillus*. It was reported that *Lactobacilli* comprised the greatest group of lactic acid bacteria in sourdough (Savic et al., 2006), and so, this finding confirmed the previous published records about the dominance of *Lactobacillus* genus in wheat sourdough (Corsetti et al., 2001; Ricciardi et al., 2005). One characteristic of the sourdough samples of the East-Azerbaijan region was the large presence of *Lactobacillus paralimentarius*. *Lactobacillus agilis* and *Lactobacillus hilgardii* were also identified in these sourdoughs. However, to confirm the proposed identification and classify unidentified *Lactobacillus* strains, molecular genetics techniques should be applied. These strains will be subjected to 16S rRNA analyses in further investigations. *Lactobacillus*

brevis, *Lactobacillus reuteri*, *Lactobacillus panis*, *Lactobacillus pontis*, and *Lactobacillus hilgardii* were known as obligatory hetero-fermentative lactic acid bacteria which generally associated with sourdough fermentation (Corsetti and Settanni, 2007). Although *Lactobacillus plantarum* is more adapted to plant materials (Rocha and Malcata, 1999), but it has been also isolated from dairy products and their environment, human mouth, and intestinal tract. *Lactobacillus plantarum* was the species which isolated from Russian rye sourdough and Finnish rye sourdough as the predominant microorganism (Rosenquist and Hansen, 2000). Ricciardi et al. (2005) isolated *Lactobacillus plantarum*, *Lactobacillus paracasei*, and *Lactobacillus casei* from the sourdoughs used for traditional durum wheat bread in high percentages (88% of the isolates). *Lactobacillus plantarum* was also isolated from several Italian sourdoughs but in lower percentages comparing to the other strains (Corsetti et al., 2001).

Recent studies (Catzeddu et al., 2006; Ferchichi et al., 2007; Randazzo et al., 2005) showed that members of the genera *Lactobacillus* (*Lactobacillus sanfranciscensis*, *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus pentosus*, and *Lactobacillus plantarum*), *Leuconostoc* and *Weissella* were the most important sourdough microflora. However, the knowledge about sourdough microflora is useful for safeguard of typical local production and also it helps us to select strains which can be better exploited in technological processes.

Conclusion

The sourdough samples of this study had different degree of acidification. Our results revealed the presence of *Lactobacillus paralimentarius*, *Lactobacillus plantarum*, *Lactobacillus curvatus*, and the rare presence of *Lactobacillus agilis* and *Lactobacillus hilgardii* in Iranian sourdough samples. However, flour type, other ingredients, fermentation and process conditions, and also bakery environment may have influence on the composition of sourdough microflora. The isolated *lactobacilli* may be used as starter cultures in the production of sourdough bread. More investigations are needed to identify the *lactobacilli* by molecular methods and to test their technological characteristics in order to select them as a starter culture.

Conflicts of interest

The authors declare that they have no conflict of interest.

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