Avicenna Journal of Pharmaceutical Research
2020 July;1(1):1-4 http://ajpr.umsha.ac.ir

Research Article

doi:10.34172/ajpr.2020.01

Identification, Characterization, and Investigation of the Antimicrobial Activities of Autochthonous Lactic Acid Bacteria Isolated from Iranian Traditional Sourdoughs

Maryam Chandal[1], Reza Mahjub[2*]

[1]Department of Drug and Food Control, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran.
[2]Department of Pharmaceutics, School of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran.

***Corresponding author:**
Reza Mahjub, Department of Pharmaceutics, School of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran
Email: r.mahjoub@umsha.ac.ir

Open Access
Scan to access more free content

Abstract

Background: The microflora of Iranian traditional sourdoughs was investigated by microscopic and biochemical tests. All of the isolated lactic acid bacteria (LAB) belonged to the genus *Lactobacillus* and most of them belonged to *L. plantarum* species.
Methods: LAB of Iranian traditional sourdoughs were selected for antimicrobial activity by measuring diameters of the inhibition zones.
Results: The best result belonged to *Lactobacillus casei*. The supernatant of the specified species was added to specific culture media of each pathogenic organism, and then the optical density before and after adding the supernatant was measured at 600 nm by UV spectrophotometer every 2 hours.
Conclusion: The results showed that *L. casei* had suitable bacteriostatic and fungistatic effects and can be used as a potent biopreservative agent in preparing various baked products such as all kinds of bread.
Keywords: Antibacterial properties, Biopreservative, Lactic acid bacteria, Sourdough

Received 21 August 2019, **Accepted**: 22 December 2019, **ePublished**: 28 May 2020

Introduction

Sourdoughs are considered extremely complex ecosystems where lactic acid bacteria (LAB) represent the prevailing microflora. Typical sourdough LAB, responsible for the acidification of dough, are lactobacilli. They consist of obligately and facultatively heterofermentative and obligately homofermentative species (1).

Sourdoughs have been traditionally used for improving flavor, texture, and microbiological shelf life of bread for thousands of years and are generally regarded as safe (2). Sourdough is an important modern fermentation of cereal flours versus a spontaneous process (3). In addition, there has been much interest in the potential application of LAB as a means of biopreservation (4).

A common way for sourdough fermentation by means of sourdough is the unique symbiosis of certain hetero and homo fermentative LAB with certain yeasts. The interaction of yeasts with lactobacilli is necessary for the metabolic activity of sourdough.

Although several yeasts are found in sourdough, *Saccharomyces cerevisiae* is considered the dominant organism for leavening bread.

The most dominant bacteria isolated from sourdough belong to the genus *Lactobacillus*. The activity of these microorganisms has a great effect on the rheology, flavor, nutritional, and functional properties of sourdough-based baked products (5,6).

The ratio of LAB to yeast in sourdough is generally 100:1. Heterofermentative LAB are dominant in sourdough, especially when traditionally prepared.

The identification of LAB by means of tests that are based on physiological and biochemical characteristics is intrinsically ambiguous and somehow unreliable (7).

Some varieties have been identified from sourdough that consist of *L. acidophilus, L. delbrueckii, L. farciminus, L. casei, L. homohiochii, L. plantarum, L. brevis, L. buchneri, L. fermentum, L. hilgardii, L. sanfrancisco*, and *L. viridescens* (8).

Fungal growth is the major cause of spoilage in baked products. Besides the great economic losses associated with spoilage, another concern is the possibility that mycotoxins can cause public health problems. *Penicillium*, *Aspergillus*, *Monillia*, *Mucor*, *Endomyces*, *Cladosporium*, *Fusarium*, and *Rhizopus* are the most common spoiling fungi isolated from bakery products (9).

LAB are associated with the production of safer foods via inhibition of the growth of pathogenic organisms or via elimination of chemicals or toxic contaminants (10). Acetic acid is one of the main short chain fatty acids that is responsible for the fungistatic effects of LAB (9).

© 2020 The Author(s); Published by Hamadan University of Medical Sciences. This is an open-access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

[Downloaded from ajpr.umsha.ac.ir on 2026-10-05]

[DOI: 10.34172/ajpr.2020.01]

The aims of this study were as follows: 1) Isolation, identification, and characterization of LAB from Iranian traditional sourdoughs; 2) Investigation of the antimicrobial activities of autochthonous LAB isolated from Iranian traditional sourdoughs against common pathogenic organisms and finally, recommendation of the use of Iranian traditional sourdoughs as the native biostarter and biopreservative in the preparation of fermentative products.

Materials and Methods

Collection and Transportation of the Samples

Several kinds of traditional Iranian sourdoughs were collected from Tabriz (northwest of Iran).

These samples were transferred to Probiotic Research Center of Faculty of Pharmacy of Tehran University of Medical Sciences in an aseptic condition.

Identification, Characterization, and Isolation of LAB

Ten grams of each sample was homogenized with 90 mL of sterile 0.85% (w/v) NaCl solution. Serial dilutions were spread-plated on MRS agar and incubated under anaerobic conditions in the presence of CO2 (48 hours, 37°C).

After enumeration, randomly selected colonies from plates were isolated, transferred in MRS broth and after incubation (48 hours, 37°C), triply purified by streaking on MRS agar. Then, they were checked for morphology. Moreover, Gram staining and catalase test were conducted.

All Gram-positive, catalase negative, hydrogen sulfide production negative, indole negative, acid/acid and non-spore forming bacillus without producing gas and without motility were transferred and maintained in phosphate buffer containing glycerol in a -80°C refrigerator for further examinations.

After genus identification, the carbohydrate fermentation test was performed for the determination of species. All samples were transferred to MRS broth without glucose and meat extract and in the presence of 1% of desirable carbohydrates (i.e., xylose, trehalose, sucrose, sorbitol, salicin, ribose, rhamnose, raffinose, melibiose, cellobiose, maltose, lactose, gluconate, glucose, galactose, fructose, esculin, arabinose, amygdalin, mannose, and mannitol) as the base culture media. The identification of species was performed according to Bergey's manual of systematic bacteriology (Data were not shown). After the incubation period (3-5 days), the color of each medium was determined. The results were considered positive if the color changed from red to yellow.

Anti-pathogenic Activity of Isolated LAB

Four pathogenic microorganisms including *E. coli, S. aureus, Penicillium*, and *A. flavus* were used in this study. The isolated LAB were used for agar spot test and inhibition zones were measured. A total of 16 species were identified that were coded by the authors (T1 to T16). Some species were excluded because of the lack of proper anti-pathogenic activity.

Among T1 to T16, T7 (*L. casei*) showed the maximum inhibition zone and was selected for further experiments: measurement of optical density of pathogens in presence of *L. casei* supernatant for plotting death kinetic curves. After *L. casei* have been grown in MRS broth for 48 hours, the broth medium was centrifuged and the supernatant was separated from biomass.

Afterwards, 20 mL of filter-sterilized supernatant of *L. casei* (T7) was added to 100 mL of SCDB (specific culture media for *E. coli* and *S. aureus*) and to SDB (specific culture media for *Penicillium* and *A. flavus*) in the same manner. Then, 1 mL of pathogenic microbial suspension was added to SCDB and SDB and incubated at 37°C and 20-25°C, respectively.

Every 2 hours, the optical density (OD) was measured with UV-visible spectrophotometer (Shimadzu/Japan) at 600 nm against the distilled water as blank.

Pour plate and serial dilution methods were used for measuring and reporting the total count of pathogens before and 8 hours after adding 20 mL of the supernatant of *L. casei*.

After the incubation period (24 hours), total counts of 0 and 8 hour cultures were determined and reported in the results.

Statistical Analysis

In this study, all the experiments were done in triplicate and the data were reported as mean ± SD. While the independent samples *t* test was used for comparing data in two groups, one-way analysis of variance (ANOVA) and appropriate post hoc tests were used for the comparison of data in more than two groups. A *P* value of less than 0.05 was considered statistically significant.

Results

Identification and Characterization

In this research, the isolated bacteria were coded (T1-T16). After performing Gram staining, microscopic and biochemical tests (such as carbohydrates fermentation) on the isolated bacteria, one genus and three different species were identified. All of them belonged to the genus *Lactobacillus* and *L. plantarum*, *L. casei*, and *L. alimentarius* species.

The pattern of carbohydrate fermentation was investigated and the results were compared to Bergey's manual of systematic bacteriology and then the species of isolated bacteria were identified (Table 1). Table 1 shows the coding, genus, and species of bacteria isolated from Iranian traditional sourdoughs based on microscopic and biochemical tests such as carbohydrate fermentation test.

Anti-pathogenic Activity of Isolated LAB

After performing the identification tests, the inhibition zones of each pathogen in the presence of isolated *Lactobacillus* were measured accurately. Tables 2, 3, and 4

[Downloaded from ajpr.umsha.ac.ir on 2026-10-05]

[DOI: 10.34172/ajpr.2020.01]

Table 1. Coding, Genus, and Species of Isolated Bacteria from Iranian Traditional Sourdoughs

Coding of Isolated Bacteria	Genus and Species
T1	*Lactobacillus plantarum*
T3	*Lactobacillus alimentarius*
T7	*Lactobacillus casei*
T10	*Lactobacillus plantarum*
T11	*Lactobacillus plantarum*
T12	*Lactobacillus plantarum*
T14	*Lactobacillus plantarum*
T16	*Lactobacillus plantarum*

Table 2. The Diameter (mm) of Inhibition Zones of *S. aureus* in the Presence of Isolated LAB (n = 3)

Isolated *Lactobacillus*	Diameter of Inhibition Zone (mm)
T1	37.5±2.63
T3	37±5.42
T7	42±8.34
T10	30±6.56
T11	25±3.75
T12	35±2.1
T14	35±3.76
T16	25±3.21

Table 3. The Diameter (mm) of Inhibition Zones of *E. coli* in the Presence of Isolated LAB (n = 3)

Isolated *Lactobacillus*	Diameter of Inhibition Zone (mm)
T1	45±5.43
T3	64±6.72
T7	65±3.86
T10	45±5.67
T11	30±2.76
T12	41±4.58
T14	64±3.21
T16	35±2.76

show the related results.

The inhibition zones of *Penicillium* in the presence of isolated LAB were not measurable because of the rapid growth of this special fungus.

According to these results, *Lactobacillus casei* (T7) was selected as superior LAB among the isolated bacteria for carrying out future experiments using the supernatant of this bacterium as a probable anti-pathogenic agent.

Total Count and Optical Density Investigation

The optical density of culture media of the pathogens against distilled water (as blank) was measured by UV-visible spectrophotometer at 600 nm every 2 hours. The results are presented in Tables 5 and 6.

The total count of each pathogenic bacterium was estimated using serial dilution and pour plate methods just before and 8 hours after adding the supernatant of

Table 4. The Diameter (mm) of Inhibition Zones of *A. flavus* in the Presence of Isolated LAB (n = 3)

Isolated *Lactobacillus*	Diameter of Inhibition Zone (mm)
T1	14±2.68
T3	18±3.57
T7	22±4.28
T10	18±5.31
T11	14±3.78
T12	18±2.65
T14	19±4.37
T16	14±3.37

Table 5. Optical Density of *A. flavus* and *Penicillium* Culture Media in the Presence of 20 mL of Supernatant of *Lactobacillus* T7 (*L. casei*)

Time	OD of *Penicillium* Culture Media	OD of *A. flavus* Culture Media
2 hours	0.026	0.000
4 hours	0.024	0.002
6 hours	0.025	0.003
8 hours	0.025	0.003
After 24 hours	0.035	0.011

Table 5. Optical Density of *E. coli* and *S. aureus* Culture Media in the Presence of 20 mL of Supernatant of *Lactobacillus* T7 (*L. casei*)

Time	OD of *S. aureus* Culture Media	OD of *E. coli* Culture Media
2 hours	0.015	0.014
4 hours	0.014	0.016
6 hours	0.016	0.021
8 hours	0.018	0.017
After 24 hours	0.045	0.038

L. casei (T7) to culture media (SCDB). The results are presented in Tables 7 and 8. According to these data, *L. casei* (T7) showed a desirable static effect on the growth of pathogenic organisms such as *E. coli, S. aureus, Penicillium*, and *A. flavus.*

Discussion

The genus *Lactobacillus* is the main microflora of wheat sourdough and it accounts for one-third of the LAB community (10).

LAB greatly influence the organoleptic, nutritional, and microbiological shelf-life characteristics of sourdough baked goods, especially breads.

A previous study reported the anti-pathogenic effects of LAB isolated from sourdoughs against molds and fungi (11). In addition, the isolation, identification, and characterization of different species of LAB from sourdoughs have been reported by previous studies (12, 13).

It should be noted that all isolated LAB in this research belonged to the genus *Lactobacillus*. Although other different species such as *L. casei* and *L. alimentarius* were

[Downloaded from ajpr.umsha.ac.ir on 2026-10-05]

[DOI: 10.34172/ajpr.2020.01]

Table 7. Total Count of *E. coli* Before and 8 Hours after Adding the Supernatant of T7

Total Count, cfu/mL	Time
4.9*E+8	Before addition of the supernatant
4*E+8	8 hours after addition of the supernatant

Table 8. Total Count of *S. aureus* Before and 8 Hours after Adding the Supernatant of T7

Total Count, cfu/mL	Time
4.6*E+8	Before addition of the supernatant
3.7*E+8	8 hours after addition of the supernatant

isolated, most of them belonged to *L. plantarum* species.

Based on the results of various anti-pathogenic tests, *L. casei* (T7) was the most powerful strain for exerting static effect on the growth of pathogens such as *E. coli, S. aureus, Penicillium*, and *A. flavus*.

In previous reports, the fungal contamination of baked goods such as bread, the production of different types of mycotoxins by pathogenic molds and fungi and the relationship of this phenomenon to public health were focused, in this study, in addition to the investigation of the antifungal activity of T7, the anti-bacterial activity of this strain against the most common microbial agents of contamination of baked products such as *E. coli* and *S. aureus* was accurately investigated.

The results have shown that *L. casei* (T7) has a desirable static effect on the growth of these pathogenic organisms. Although other different types of isolated bacteria from Iranian traditional sourdoughs showed some potential inhibitory effect on the growth of these pathogens in the preliminary studies, we preferred to follow up the most powerful one.

Among the pathogenic organisms that have been used for carrying out this study, *Penicillium* was considered the most resistant genus against the anti-pathogenic activity of T7.

We hypothesized that this resistance may be related to heredity of *Penicillium* or the insufficiency of the amount of supernatant that has been used for experimentations (i.e., 20 mL).

According to the results, adding some Iranian traditional sourdoughs as the biostarter to the other ingredients for production of baked goods may exert beneficial effects such as natural, safe and reliable preservation via the biopreservative action against the most common pathogenic organisms. These effects include increasing nutritional value, detoxification of baked products from potential microbial and fungal toxic substances, preventing early bread staling and early bread spoilage, lengthening microbiological shelf-life of bread, and preventing great economic losses.

In the end, it is probable that using sourdoughs may cause improvement in texture and flavor of baked products.

Conflict of Interests

The authors declare no conflict of interests.

Acknowledgement

This study was supported by financial grant from Islamic Azad University, Pharmaceutical Sciences Branch, Tehran, Iran.

References

1. Corsetti A, Settanni L, van Sinderen D, Felis GE, Dellaglio F, Gobbetti M. *Lactobacillus rossii* sp. *nov*., isolated from wheat sourdough. Int J Syst Evol Microbiol. 2005;55(Pt 1):35-40. doi: 10.1099/ijs.0.63075-0.
2. Sadeghi A, Shahidi F, Mortazavi SA, Koocheki A, Mohebbi M. Evaluation of sourdough effect on Iranian barbari bread staling. World Appl Sci J. 2007;2(5):490-8.
3. Ur-Rehman S, Nawaz H, Hussain S, Ahmad MM, Murtaza M, Ahmad M. Effect of sourdough bacteria on the quality and shelf life of bread. Pak J Nutr. 2007;6(6):562-5. doi: 10.3923/pjn.2007.562.565.
4. Clarke CI, Arendt EK. A review of the application of sourdough technology to wheat breads. Adv Food Nutr Res. 2005;49:137-61. doi: 10.1016/s1043-4526(05)49004-x.
5. De Vuyst L, Neysens P. The sourdough microflora: biodiversity and metabolic interactions. Trends Food Sci Technol. 2005;16(1):43-56. doi: 10.1016/j.tifs.2004.02.012.
6. De Vuyst L, Vancanneyt M. Biodiversity and identification of sourdough lactic acid bacteria. Food Microbiol. 2007;24(2):120-7. doi: 10.1016/j.fm.2006.07.005.
7. Settanni L, van Sinderen D, Rossi J, Corsetti A. Rapid differentiation and in situ detection of 16 sourdough *lactobacillus* species by multiplex PCR. Appl Environ Microbiol. 2005;71(6):3049-59. doi: 10.1128/aem.71.6.3049-3059.2005.
8. Wiese BG, Strohmar W, Rainey FA, Diekmann H. *Lactobacillus panis* sp. *nov*., from sourdough with a long fermentation period. Int J Syst Bacteriol. 1996;46(2):449-53. doi: 10.1099/00207713-46-2-449.
9. Lavermicocca P, Valerio F, Evidente A, Lazzaroni S, Corsetti A, Gobbetti M. Purification and characterization of novel antifungal compounds from the sourdough *Lactobacillus plantarum* strain 21B. Appl Environ Microbiol. 2000;66(9):4084-90. doi: 10.1128/aem.66.9.4084-4090.2000.
10. Di Cagno R, De Angelis M, Lavermicocca P, De Vincenzi M, Giovannini C, Faccia M, et al. Proteolysis by sourdough lactic acid bacteria: effects on wheat flour protein fractions and gliadin peptides involved in human cereal intolerance. Appl Environ Microbiol. 2002;68(2):623-33. doi: 10.1128/aem.68.2.623-633.2002.
11. Savić D, Savić T, Škrinjar M, Joković N. Profile of lactic acid bacteria in rye flour and sourdough. J Cult Collect. 2006;5(1):38-45.
12. Vogel RF, Böcker G, Stolz P, Ehrmann M, Fanta D, Ludwig W, et al. Identification of lactobacilli from sourdough and description of *Lactobacillus pontis* sp. *nov*. Int J Syst Bacteriol. 1994;44(2):223-9. doi: 10.1099/00207713-44-2-223.
13. Valcheva R, Korakli M, Onno B, Prévost H, Ivanova I, Ehrmann MA, et al. *Lactobacillus hammesii* sp. *nov*., isolated from French sourdough. Int J Syst Evol Microbiol. 2005;55(Pt 2):763-7. doi: 10.1099/ijs.0.63311-0.

[Downloaded from ajpr.umsha.ac.ir on 2026-10-05]

[DOI: 10.34172/ajpr.2020.01]