

Isolation and identification of probiotic *Lactobacillus* from local dairy and evaluating their antagonistic effect on pathogens

Sahar Karami, Mohammad Roayaei, Hosna Hamzavi, Mahmoud Bahmani¹, Hassan Hassanzad-Azar², Mahmoodnia Leila³, Mahmoud Rafeiean-Kopaei⁴

Department of Biology, Faculty of Science, University of Chamran, Ahwaz, ¹Biotechnology and Medicinal Plants Research Center, Ilam University of Medical Sciences, Ilam, ²Department of Food Safety and Hygiene, School of Paramedical and Health, Zanjan University of Medical Sciences, Zanjan, ³Department of Internal Medicine, Shahrekord University of Medical Sciences, ⁴Medical Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran

Abstract

Objective: Probiotics such as lactobacilli prevent the development of a wide range of human and animal's pathogens. The aim of this study was evaluation of antagonistic effect of isolated lactobacilli from local dairy products against three standard strains of *Staphylococcus aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*.

Materials and Methods: Twenty samples of local dairy products including cow milk, buffalo milk, cheese, and yogurt were collected from different areas of Ahwaz city. Antimicrobial disc diffusion method was applied on *S. aureus* (ATCC-6538), *B. subtilis* (ATCC-12711), and *P. aeruginosa* (ATCC-27853). Antimicrobial effects of isolates were evaluated by disc diffusion test on Mueller-Hinton agar medium plated with three pathogens.

Results: Obtained results showed that only three strains of isolated lactobacilli of local dairy samples had inhibitory effects on understudy pathogens including *Lactobacillus alimentarius*, *Lactobacillus sake*, and *Lactobacillus collinoides*. All three isolates showed moderate activity (inhibition zone <15 mm) except of *L. collinoides* and *L. alimentarius* that had relatively strong activity (inhibition zone ≥15 mm) against *P. aeruginosa* and *B. subtilis*, respectively.

Conclusion: These bacteria can be raised for the production of various kinds of food, pharmaceutical products, and functional foods.

Keywords: Bacteria, Iran, *Lactobacillus*, probiotics

Address for correspondence: Prof. Mahmoud Rafeiean-Kopaei, Medical Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran. E-mail: rafeiean@yahoo.com

INTRODUCTION

Probiotics are living organisms that are used as food additives with beneficial effects on the healthy body by setting microbial balance in gastrointestinal tract.^[1,2] Lactic acid bacteria (LAB) as protective cultures are common probiotic organisms that are considered safe

due to having specific characteristics. Main genera of LAB are *Leuconostoc*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Bifidobacterium*, *Pediococcus*, and *Streptococcus*.^[3] These bacteria cause reduction of gastrointestinal diseases by increasing benefit microorganisms' growth and reducing

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Karami S, Roayaei M, Hamzavi H, Bahmani M, Hassanzad-Azar H, Leila M, *et al.* Isolation and identification of probiotic *Lactobacillus* from local dairy and evaluating their antagonistic effect on pathogens. *Int J Pharma Investig* 2017;7:137-41.

Access this article online	
Quick Response Code:	Website: www.jpionline.org
	DOI: 10.4103/jphi.JPHI_8_17

pathogens' population mechanisms.^[4] LAB are widely distributed in the environment that can prevent the growth of pathogenic microorganisms by producing particular substances.^[4] According to scientific reports, antiallergic and anticancer effects, increasing fat loss and immune response of the host, improvement symptoms of irritable bowel syndrome, intestinal inflammation, and antibiotic-induced diarrhea are other useful effects of probiotics.^[5,6]

Nowadays, probiotics are used not only as a driver of growth but also as a stimulator of the immune system and prevention of many diseases.^[7] Food probiotic products because of their nutritional value and health sector over the past parallel to the therapeutic effects are taken into consideration.^[8,9] Lactobacilli are Gram-positive and catalase-negative bacteria that are known as most important probiotics and desirable gut microflora.^[6] They are normal flora of mouth, intestine, and female genital tract with important role in the control of undesirable microorganisms that can be considered as natural biopreservatives.^[4]

Lactobacilli have an important role in controlling undesirable microflora in the gut and are able to prevent the rise of pathogenic bacteria by producing antimicrobial metabolites. They can be used as biological preservatives and are raised naturally in foods.^[10]

Antimicrobial activity, bile salts, and acid tolerance are three important features for screening probiotic potential of bacteria that can be used as medical.^[2,11] LAB produce organic acids, hydrogen peroxide, diacetyl, bacteriocins, and antifungal compounds such as fatty acids during lactic fermentation. Bacteriocins are protein compounds with growth inhibition ability of sensitive pathogenic bacteria and different degradation system in digestive system compared with antibiotics.^[4,11] LAB are resistant to lysozyme, gastric acid, gastrointestinal juice, and bile salts. Antimicrobial compounds are also prepared from them to compete and inhibit pathogenic microorganisms.^[4] Such compounds may affect metabolism or toxins of pathogenic bacteria.^[12,13]

LAB have protective effects in dairy products against harmful bacteria. Several studies have been done on antibacterial effects of probiotics against Gram-negative and positive bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.^[14,15] The aim of this study was evaluating antagonistic effect of isolated lactobacilli from local dairy products against three standard strains of *S. aureus*, *Bacillus subtilis*, and *P. aeruginosa*.

MATERIALS AND METHODS

Twenty samples of local dairy products including cow milk, buffalo milk, cheese, and yogurt were collected from different areas of Ahwaz city, center of Khuzestan province of Iran. Eight strains of *Lactobacillus* cultures were isolated and identified by biochemical tests.

Biochemical tests such as Gram stain, catalase, oxidase, indole production and motion study, growth at different temperatures (15–37–45), and various fermentation of sugars (sucrose, lactose, maltose, trehalose, galactose, arabinose, mannitol, fructose, and salicin) were studied.^[16]

To isolate and proliferate *Lactobacillus* cultures, MRS Broth (Merck, Germany), MRS Agar (Merck, Germany), and Mueller-Hinton agar (MHA) media (Merck, Germany) were used.

Isolation and Identification of *Lactobacillus* (lactic acid bacteria)

Two grams of each sample was transferred in a flask containing MRS Broth as enrichment media and added distilled water to 100 ml and incubated in 37°C. After 24 h, 100 µl of enriched samples was spread on MRS agar and incubated at 37°C and anaerobic condition for 48 h. Bacterial colonies were purified by subsequent subcultures.

Final identification was done using classic microbiology tests including Gram-staining for detecting morphology, catalase and oxidase tests, motility, indole producing, growth at 15°C, and carbohydrates fermentation (arabinose, fructose, galactose, lactose, mannitol, salicin, sucrose, and trehalose) test.^[17]

All Gram-positive and catalase-negative bacilli were selected for the assessment of antimicrobial ability. Antimicrobial effect of isolates was evaluated by disc diffusion test on MHA medium plated with three pathogens. For this purpose, fresh culture of isolates was centrifuged (8000 rpm, 15 min) and supernatants were removed. Blank discs were inoculated with 40 µl supernatant of each isolate and were placed on separate MHA medium inoculated with *S. aureus* (ATCC-6538), *B. subtilis* (ATCC-12711), and *P. aeruginosa* (ATCC-27853) strains. Growth inhibition zones of pathogens and isolated lactobacilli inhibitory ability were assessed after incubation of all agar media at 37°C for 24 h.^[18]

RESULTS

Obtained results showed that only three strains of isolated lactobacilli of local dairy samples had inhibitory effects on

Table 1: Biochemical characteristics of three isolated *Lactobacillus* strains

Isolates	Test											
	Catalase	SIM	Indole	Oxidase	Growth at 15°C	Arabinose	Galactose	Lactose	Manitol	Salicin	Sucrose	Trehalose
<i>Lactobacillus collinoides</i>	-	-	-	-	+	+	+	d	-	d	-	-
<i>Lactobacillus sake</i>	-	-	-	-	+	+	+	+	-	+	+	+
<i>Lactobacillus alimentarius</i>	-	-	-	-	+	d	+	-	-	+	+	+

d: The 11%-89% of the species positive, SIM: (Sulfide, Indole, Motility), +: Positive, -: Negative

understudy pathogens including *L. alimentarius*, *L. sake*, and *L. collinoides*. Biochemical characteristics of these strains are shown in Table 1.

Seventy percent of isolated lactobacilli showed antimicrobial effects on selected pathogens, but inhibitory effects of three strains were more considerable than others [Figure 1]. Growth inhibition zone of three *Lactobacillus* strains including *L. alimentarius*, *L. sake*, and *L. collinoides* is shown in Table 2.

DISCUSSION

It is a long time that scientists are trying to substitute synthetic drugs with natural products.^[19-22] Nowadays, various natural materials and methods are used to prevent or treat diseases.^[22-28] The use of probiotics is one of these methods. Lactobacilli and bifidobacteria are normal intestinal flora which by preventing intestinal infection, lowering cholesterol, stimulating the immune system, and reducing the risk of colon cancer play an important role in human health. Probiotic bacteria produce lactic acid and organic acids, reduce the pH environment, and try to prevent the growth of many bacteria. These bacteria produce antimicrobial compounds such as bacteriocin which can be used as natural preservatives.^[29]

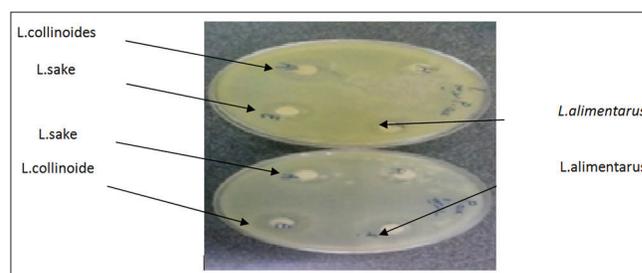
In this study, it was found that the metabolites produced by these bacteria, which were isolated by centrifugation, were able to prevent the growth of pathogenic bacteria.

The study of Boris *et al.* showed that lactobacilli strains isolated from dairy products were able to inhibit the growth of *P. aeruginosa*, *E. coli*, *Salmonella typhimurium*, and *S. aureus*, the latter was in the highest inhibitory effect.^[30]

In the study of Ogunbanwo *et al.*, the microbial activity and bacteriocin production of probiotic strains of *Lactococcus plantarum* and *Lactobacillus brevis* were searched on multiple pathogens in which the highest inhibitory effect was observed on *Bacillus cereus*.^[31] Coconnier *et al.* reported that consumption of supernatant (culture supernatant) bacteria *Lactobacillus fermentum*, *Lactobacillus casei*, *Lactobacillus acidophilus*, and *Lactococcus lactis* had inhibitory effects on a wide range of disease-causing bacteria.^[32] Based on the

Table 2: Growth inhibition zone diameter (mm) of pathogens with three *Lactobacillus* strains

Isolates	Pathogen		
	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>
<i>Lactobacillus alimentarius</i>	15	11	10
<i>Lactobacillus sakei</i>	10	11	10
<i>Lactobacillus collinoides</i>	10	10	15

**Figure 1: Inhibition zone of three *Lactobacillus* strains on the *Pseudomonas aeruginosa* and *Bacillus subtilis***

results of the present study, *L. alimentarius* had the most effect on *L. collinoides* the highest inhibitory effect on *P. aeruginosa* and *S. aureus* showed the greatest effect on *L. sake*.

Antagonistic activity of three selected isolated lactobacilli of some dairy products was evaluated in this study. The obtained results [Table 2] showed that all three isolates had moderate activity (inhibition zone <15 mm) except *L. collinoides* and *L. alimentarius* that had relatively strong activity (inhibition zone ≥15 mm) against *P. aeruginosa* and *B. subtilis*, respectively. Antimicrobial effect of lactobacilli against pathogens is mainly due to the production of organic acids and pH reduction in coculture with pathogenic bacteria although they can produce some other substances.^[33]

For many years, dairy products have been recognized as valuable products to human health.^[34] In recent years, many scientists have isolated and identified LAB and lactobacilli from traditional products worldwide and have evaluated their antagonistic effects against various pathogens.^[35] Microorganisms such as lactobacilli and many other bacteria can eliminate pathogens through multiple mechanisms including competitive elimination that results in food safety.^[36]

CONCLUSION

Given the results of this study, antagonistic effects of produced substances by the bacteria on a wide range of microorganisms have an important role in food preservation and human health. These bacteria can be raised for the production of various kinds of food and pharmaceutical products. They can also be used for the production of new functional foods. Therefore, increasing use of dairy products containing probiotics, identification and production of foods containing highest and most effective lactobacilli are recommended in daily diet.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. FAO/WHO. Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria: Report of a Joint FAO/WHO Expert Consultation 2006; 25 November, 2008.
2. Hassanzadazar H, Ehsani A, Mardani K, Hesari J. Investigation of antibacterial, acid and bile tolerance properties of lactobacilli isolated from Koozeh cheese. *Vet Res Forum* 2012;3:181-5.
3. Tafvizi F, Tajabadi Ebrahimi M, Khajareh L. Study genotypic and phylogenetic bacteriocin-producing lactobacilli isolated from dairy product to local and traditional food. *J Fasa Univ Med Sci* 2012;2:84.
4. Hawaz E. Isolation and identification of probiotic lactic acid bacteria from curd and *in vitro* evaluation of its growth inhibition activities against pathogenic bacteria. *Afr J Microbiol Res* 2014;8:1919-425.
5. Shokryazdan P, Sieo CC, Kalavathy R, Liang JB, Alitheen NB, Faseleh Jahromi M, et al. Probiotic potential of *Lactobacillus* strains with antimicrobial activity against some human pathogenic strains. *Biomed Res Int* 2014;2014:927268.
6. Nsofor CA, Sarah U, Chinyere U. Isolation and characterization of lactic acid bacteria from ogi sold in Elele Nigeria. *J Biol Food Sci Res* 2014;3:19-22.
7. Del Piano, M, Ballare, M, Montino, F, Orsello, M, Garelo, E, Sforza, F. Clinical experience with probiotics in the elderly on total enteral nutrition. *J Clin Gastroenterol.* 2004;38:S111–S4.
8. Smid EJ, van Enkevort FJ, Wegkamp A, Boekhorst J, Molenaar D, Hugenholtz J, et al. Metabolic models for rational improvement of lactic acid bacteria as cell factories. *J Appl Microbiol* 2005;98:1326-31.
9. Islam T, Sabrin F, Islam E, Billah M, Islam Didarul KM. Analysis of antimicrobial activity of *Lactobacillus paracasei* ssp *paracasei*-1 isolated from regional yogurt. *J Microbiol Biotechnol Food Sci* 2012;7:80-9.
10. Oyetyo VO. Phenotypic characterization and assessment of the inhibitory potential of lactobacillus isolates from different sources. *Afr J Biotechnol* 2004;3:355-7.
11. Salehi M. Antagonistic effect of lactobacilli isolated from native food. *J Food Sci Technol Innov* 2012;5:1.
12. Rushdy Abeer A, Gomaa Zakaria E. antimicrobial compounds produced by probiotic *Lactobacillus brevis* isolated dairy products. *J Am Microbiol* 2013;63:81-90.
13. Osuntoki A, korie I. Antiooxidant activity of whey from milk fermented with *Lactobacillus* species isolated from Nigerian fermented foods. *J Food Technol Biotechnol* 2010;48:505-11.
14. Sharafi H, Maleki H, Ahmadian G, Shahbani Zahiri H, Sajedinejad N, Houshmand B, et al. Antibacterial activity and probiotic potential of *Lactobacillus plantarum* HKN01: A new insight into the morphological changes of antibacterial compound-treated *Escherichia coli* by electron microscopy. *J Microbiol Biotechnol* 2013;23:225-36.
15. Smaoui S, Elleuch L, Bejar W, Karray-Rebai I, Ayadi I, Jaouadi B, et al. Inhibition of fungi and Gram-negative bacteria by bacteriocin BacTN635 produced by *Lactobacillus plantarum* sp. TN635. *Appl Biochem Biotechnol* 2010;162:1132-46.
16. Salaj R, Stofilová J, Soltessová A, Hertelyová Z, Hijová E, Bertková I, et al. The effects of two *Lactobacillus plantarum* strains on rat lipid metabolism receiving a high fat diet. *ScientificWorldJournal* 2013;2013:135142.
17. Saavedra L, Taranto MP, Sesma F, de Valdez GF. Homemade traditional cheeses for the isolation of probiotic *Enterococcus faecium* strains. *Int J Food Microbiol* 2003;88:241-5.
18. Kumar A, Saini P, Shrivastava JN. Production of peptide antifungal antibiotic and biocontrol activity of *Bacillus subtilis*. *Indian J Exp Biol* 2009;47:57-62.
19. Sewell RD, Rafeian-Kopaei M. The history and ups and downs of herbal medicine usage. *J Herbmed Pharmacol* 2014;3:1-3.
20. Nasri H, Baradaran A, Shirzad H, Rafeian-Kopaei M. New concepts in nutraceuticals as alternative for pharmaceuticals. *Int J Prev Med* 2014;5:1487-99.
21. Bahmani M, Shirzad H, Rafeian S, Rafeian-Kopaei M. *Silybum marianum*: Beyond hepatoprotection. *J Evid Based Complementary Altern Med* 2015;20:292-301.
22. Ebrahimie M, Bahmani M, Shirzad H, Rafeian-Kopaei M, Saki K. A review study on the effect of Iranian herbal medicines on opioid withdrawal syndrome. *J Evid Based Complementary Altern Med* 2015;20:302-9.
23. Asadi-Samani M, Rafeian-Kopaei M, Azimi N. Gundelia: A systematic review of medicinal and molecular perspective. *Pak J Biol Sci* 2013;16:1238-47.
24. Bahmani M, Banihabib E, Rafeian-Kopaei M, Gholami-Ahangaran M. Comparison of disinfection activities of nicotine with copper sulphate in water containing *Limnatis nilotica*. *Kafkas Univ Vet Fak Derg* 2015;21:9-11.
25. Nasri H, Behradmanesh S, Ahmadi A, Rafeian-Kopaei M. Impact of oral Vitamin D (cholecalciferol) replacement therapy on blood pressure in type 2 diabetes patients; a randomized, double-blind, placebo controlled clinical trial. *J Nephropathol* 2014;3:29-33.
26. Amini FG, Rafeian-Kopaei M, Nematbakhsh M, Baradaran A, Nasri H. Ameliorative effects of metformin on renal histologic and biochemical alterations of gentamicin-induced renal toxicity in Wistar rats. *J Res Med Sci* 2012;17:621-5.
27. Nasri H, Mortazavi M, Ghorbani A, Shahbazian H, Kheiri S, Baradaran A, et al. Oxford-MEST classification in IgA nephropathy patients: A report from Iran. *J Nephropathol* 2012;1:31-42.
28. Rafeian-Kopaei M, Asgary S, Adelnia A, Setorki M, Khazaei M, Kazemi S, et al. The effects of cornelian cherry on atherosclerosis and atherogenic factors in hypercholesterolemic rabbits. *J Med Plants Res* 2011;5:2670-6.
29. Aroutcheva AA, Simoes JA, Faro S. Antimicrobial protein produced by vaginal *Lactobacillus acidophilus* that inhibits *Gardnerella vaginalis*. *Infect Dis Obstet Gynecol* 2001;9:33-9.
30. Boris S, Jiménez-Díaz R, Caso JL, Barbés C. Partial characterization of a bacteriocin produced by *Lactobacillus delbrueckii* subsp. *lactis* UO004, an intestinal isolate with probiotic potential. *J Appl Microbiol* 2001;91:328-33.
31. Ogunbanwo ST, Sanni AI, Onilude AA. Characterization of bacteriocin produced by *Lactobacillus plantarum* F1 and *Lactobacillus brevis* OG1. *Afr J Biotechnol* 2003;2:219-27.
32. Coconnier MH, Lievin V, Hemery E, Servin AL. Antagonistic activity against *Helicobacter* infection *in vitro* and *in vivo* by the human *Lactobacillus*

- acidophilus* strain LB. Appl Environ Microbiol 1998;64:4573-80.
33. Millette M, Luquet FM, Lacroix M. *In vitro* growth control of selected pathogens by *Lactobacillus acidophilus*- and *Lactobacillus casei*-fermented milk. Lett Appl Microbiol 2007;44:314-9.
 34. Chassarard C, Grattepanche F, Lacroix C. Probiotics and health claim: Challenges for tailoring their efficacy. In: Kneifel W, Salminen S, editors. Probiotics and Health Claims. Wiley-Blackwell: John Wiley & Sons; 2011. p. 49-74.
 35. Rossetti L, Fornasari ME, Gatti M, Lazzi C, Neviani E, Giraffa G. Grana Padano cheese whey starters: Microbial composition and strain distribution. Int J Food Microbiol 2008;127:168-71.
 36. Schillinger U, Geisen R, Holzapfel WH. Potential of antagonistic microorganisms and bacteriocins for the biological preservation of foods. Trends Food Sci Technol 1996;7:158-64.