

Investigating genes encoding pln A and pln EF bacteriocins from lactobacilli isolated from traditional dairy products.

Sina Dabir¹, Zahra Hojjati Bonab^{*1}, Hossein Soltanzadeh^{2,1}

¹Department of Microbiology, Bon.C., Islamic Azad University, Bonab, Iran.

²Medical Plants Research Center, Maragheh University of Medical Sciences, Maragheh, Iran

***Corresponding Author:** Zahra Hojjati Bonab, Department of Microbiology, Bonab Branch, Islamic Azad University, Bonab, Iran.
Email: 1688884221@iau.ir, Phone: +989143225096

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Abstract

Introduction: Lactobacilli are long, gram-positive bacilli. The food industry widely uses lactic acid bacteria (LAB) as natural preservatives due to their ability to produce antibacterial compounds such as bacteriocins. The purpose of this study is to investigate the genes and beneficial lactobacilli from traditional yogurt and cheese of northwestern Iran in 1401.

Methods: For this purpose, 29 samples of traditional dairy products, including 18 samples of cheese and 11 samples of yogurt, were collected under sterile conditions and inoculated in MRS culture medium. Additional tests were performed to identify them and molecular PCR tests were performed to detect the presence of plnA, plnEF genes in the isolates. In this research, after isolating *Lactobacillus* from traditional yogurt and cheese samples, the presence of plnEF bacteriocin gene was confirmed in 7 *Lactobacillus* isolates and the absence of plnA gene in the isolates.

Results: In previous studies that succeeded in isolating *Lactobacillus* strains from traditional dairy products, plantaricin EF is present in all pln positions. In this research, after *Lactobacillus* was isolated from samples, two genes encoding bacteriocin plnA and plnEF were investigated in *Lactobacillus* isolates, plnEF was present in all of them and plnA was not present in any of the isolates. Bacteriocins induce beneficial health properties in probiotic products.

Conclusion: we succeeded in isolating and identifying lactobacilli from the traditional yogurt and cheese of the northwestern region of the country by the method of culture and biochemical identification and isolation based on PCR.

Keywords: probiotic, bacteriocin, plnA, plnEF.

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Introduction

In recent years, probiotics have become a popular approach to managing gastrointestinal health and immunity and are often recommended as effective therapeutic interventions by medical professionals. Introduced in the early 20th century, the science of probiotics has made significant progress, especially in the last two decades. Probiotics are live microorganisms that, when administered in sufficient amounts, are beneficial to the host. In the global market, probiotics are mainly found in three main categories: foods, nutritional supplements, and drugs¹. The existing belief about the beneficial effects of probiotics is based on the fact that the microbial flora of the intestine shows a protective role against various

diseases; the main effect of probiotics is determined by stabilization of intestinal flora. Probiotic products as a natural treatment method and a suitable alternative to antibiotics have received much attention in recent years².

The importance of these microorganisms and their health-giving properties have led to the increase of new beneficial products, including probiotic products. Probiotic is also used as an attribute of food containing these bacteria. The main mechanisms by which probiotics lead to improved host health are not known. It seems that certain strains of probiotics have a positive effect on the host's health and this effect cannot be generalized to other strains³.

Probiotics have been used in fermented dairy products for centuries. In recent years, the interest in food and agricultural applications of probiotics has increased, the selection of new probiotic strains and the development of new applications have become very important. Although dairy products are the first known sources of probiotic foods⁴.

Probiotics are food supplements of live microorganisms that, when present in appropriate amounts in the digestive system, have beneficial effects on the host. Among the bacteria, lactic acid bacteria are the most common type of bacteria that have been introduced as probiotics. Due to the increasing expansion of industrial dairy products instead of traditional products, there is a possibility of losing many probiotic bacteria, so it is necessary to isolate and identify these bacteria from traditional sources and use them in the production of dairy products.

Bacteriocins

Protein metabolites, usually with a molecular weight below 10 kilodaltons, are synthesized by ribosomes and show their antimicrobial activity against strains close to the bacteriocin producer or distant strains. Bacteriocins are natural non-toxic antimicrobial substances⁵.

Studies have shown that there are three main mechanisms by which bacteriocins modulate host health: by protecting probiotics in the gut, competitively inhibiting harmful bacteria, and influencing the host's immune system⁶. The difference between bacteriocins and common antibiotics is in its limited lethality spectrum. Bacteriocin is synthesized by ribosomes. Another characteristic of bacteriocins is their high inhibitory power, which makes them preferable to antibiotics⁷.

Lactobacilli

Lactobacilli are gram-positive bacteria, immobile, without spores, catalase negative, nitrate reduction negative, cytochrome oxidase negative, which are not able to dissolve gelatin and produce indole. Currently, more than a hundred species of this genus have been identified⁸. Lactic acid bacteria play an important role in food fermentation. They mostly produce antimicrobial compounds such as lactic acid, acetic acid, hydrogen peroxide, aldehyde and bacteriocins⁹.

Bacteriocins produced by lactic acid bacteria can be defined as ribosomally synthesized compounds that inhibit the growth of other bacteria. Consequently, the ability to produce bacteriocins gives producers a great advantage in competing with other bacteria for the same nutrients. In addition, bacteriocins may also play a protective role, acting to prevent other strains or species

from invading an occupied niche or limiting the settlement of neighboring cells. Some lactobacilli of enological origin producing bacteriocin have been introduced¹⁰. LAB bacteriocins show inhibitory spectra against important pathogens and food-borne bacteria and therefore have a high potential as second-generation antibiotics. Lactobacilli produce four class IIb bacteriocins including EF, JK, NC8 and J51 plantaricins and a plantaricin A pheromone peptide with antimicrobial activity. One of these bacteriocins, plantaricin EF, is present in all pln loci while the remaining plantaricins are found in different strains-specific combinations. At can replace or be used in combination with traditional antibiotics.

Lactobacillus bacteriocin in food industry

Chemical preservatives are traditionally used during the production of processed products. However, the ever-growing consumer interest in fresh and natural products necessitates the search for alternative ingredients. In this context, the food industry widely uses lactic acid bacteria as natural preservatives due to their ability to produce antibacterial compounds such as bacteriocins.

Studies show that cheese and yogurt produced in the traditional way are better carriers for bacteria with probiotic potential. Therefore, adding this type of bacteria as a starter to cheese and industrial yogurts makes it possible to market dairy products with favorable characteristics. At the same time, consumers are also allowed to find a new way to benefit from health, because the consumption of these bacteria can improve the health of the digestive system and strengthen the immune system.

Material and methods

Sample collection

For the purpose of this study, 29 samples of traditional dairy products, including 18 samples of cheese and 11 samples of yogurt, were collected from East Azarbaijan and West Azarbaijan provinces under sterile conditions and transferred to the laboratory in sterile falcons. The samples were collected during a period of 4 months from October to February 1401.

Cultivation of samples

Lactobacillus MRS agar culture medium is used for the cultivation of Lactobacillus species isolated from food sources, feces and other samples. This culture medium causes the proper growth of Lactobacillus. For this reason, it is useful for strains that grow poorly in other culture environments. 1 mL of each of the yogurt and 1 g of each of the cheese samples was inoculated in 10 mL of

MRS liquid culture medium and mixed completely and incubated at 37 °C under anaerobic conditions. Then it was transferred to MRS agar medium.

Isolation of lactobacilli based on morphology and biochemical tests

The grown samples of lactobacillus were isolated by gram staining and observing under the microscope and performing the catalase test.

DNA extraction from isolated lactobacilli

DNA extraction from *Lactobacillus* isolates was performed according to the protocol of Pouya Gene Azma DNA extraction kit:

- 3-5 mL of liquid bacterial culture medium was collected in a microtube and centrifuged for 1 minute, then the sediment at the bottom of the microtube was dissolved in 100 µL of buffer I.
- First, 200 µL of buffer II was added to the microtube, then 150 µL of buffer III was added and it was slowly stirred, then it was placed at 37 °C for 45 minutes.
- 180 µL of buffer X was added to the microtube and microcentrifuged for 10 minutes. The supernatant solution was transferred to a new microtube. Cold ethanol was added to the supernatant and microcentrifuged for 5 minutes.
- Ethanol was discarded by gently inverting the microtube and the sediment was kept. 700 µL of cold ethanol was added to the sediment at the bottom of the microtube and microcentrifuged for one minute.
- Ethanol was completely discarded and the sediment at the bottom of the microtube was dried for 2-3 minutes at room temperature, then according to the sediment created, 20-50 µL of solvent buffer was added to the microtube.

Table 1. PCR schedule.

| | Temperature | Time | cycle |
|----------------------------|-------------|-------|-------|
| Initial denaturatio | 94°C | 5min | 1 |
| Denaturation | 94°C | 30s | 35 |
| Annealing | 55°C | 30s | 35 |
| Extension | 72°C | 30s | 35 |
| Final extension | 72°C | 10min | 1 |

For the extracted DNAs, PCR was performed according to schedule 1.

Sequence of primers

Primers are oligonucleotide fragments that are usually between 18 and 24 nucleotides in size and are one of the most important components of the PCR reaction for amplifying the desired fragment. In this research, the primers were taken from the article of Tai et al. [11](#).

Table 2 shows the information related to the primers used in this research. Primers were ordered from Sina Clone and were prepared according to the company's brochure before use.

Table 2. Sequence of PlnA and PlnEF gene primers.

| Target gene | Primer Sequence | Annealing Temperature | Amplified product |
|--------------|--|-----------------------|-------------------|
| PlnEF | F: GGCATAGTTAAAATTCCTCC R: CAGGTTGCCGCAAAAAAAG | 55°C | 428 bp |
| plnA | F:CAAATTAAGGTATGAAGCAACT R: TTCTTACCTGTTTAATTGCAG | 55°C | 450 bp |

Checking the quality of the extracted DNA and revealing the PCR products

Agarose gel electrophoresis method was used to check the quality of extracted DNA sample and PCR products. The process of electrophoresis is actually the movement of particles in the gel bed under the influence of an electric field. Using this method, the size of DNA fragments can be determined.

Data analysis

The obtained data were analyzed with SPSS version 27 statistical software, and the corresponding graphs were drawn, and one-way variance test was performed on the data, and P Value < 0.005 was considered significant.

Results

Results of staining and biochemical tests in *Lactobacillus* bacteria

The results of gram staining and observation of the samples with a microscope according to Figure 1 and Table 3 showed that all the samples took on a pink color with gram staining and were seen as rods under the light microscope and were catalase negative.

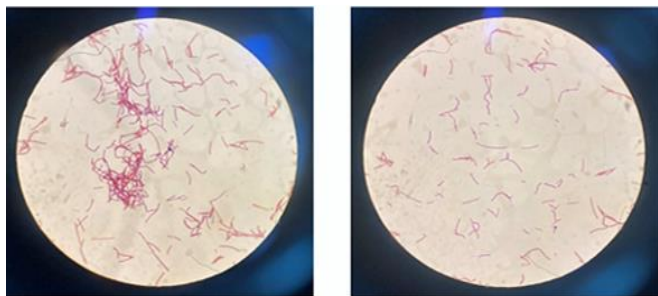


Figure 1. Gram staining of Lactobacillus isolates.

Table 3. Results of diagnostic tests for Lactobacillus bacteria.

| | shape | Catalase test | Catalase test |
|--|-------|---------------|---------------|
| Characteristics of isolated lactobacilli | rod | positive | negative |

Bacterial species investigated

Among the 29 samples, 6 yogurt samples and 1 cheese sample contained Lactobacillus bacteria, which are shown in Figure 2 and Table 4

Table 4. Frequency of the examined bacterial species.

| Sample type | Number of samples | Lactobacillus sample |
|-------------|-------------------|----------------------|
| Yogurt | 11 | 6 |
| Cheese | 18 | 1 |

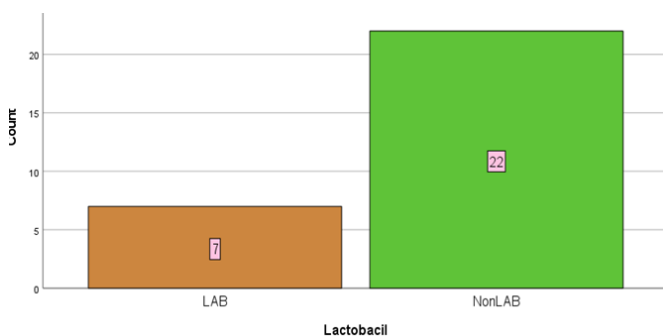


Figure 2. Frequency diagram of the examined bacterial species

The results of confirming the identification of lactobacilli by PCR method

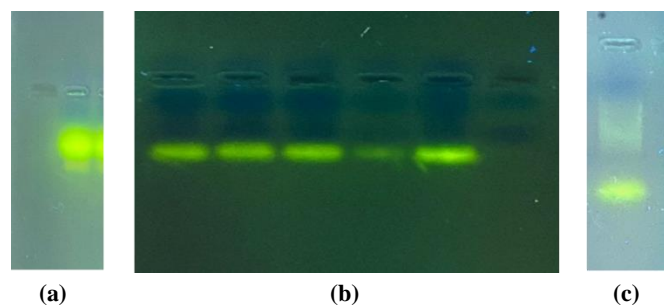


Figure 3. (a): DNA quality extracted from Lactobacillus acidophilus as a standard sample with code PTCC1643. (b): Checking the quality of DNA extracted from lactobacilli isolated from yogurt. (c): Checking the quality of DNA extracted from lactobacilli isolated from cheese

Results of identification of pln A and pln EF genes in Lactobacillus bacteria samples

Lactobacillus bacteria samples were checked by PCR method in the samples. Therefore, pln EF gene frequency in positive bacteria samples is 100% and pln A gene frequency is 0%. This can be seen in figure 4 and 5.

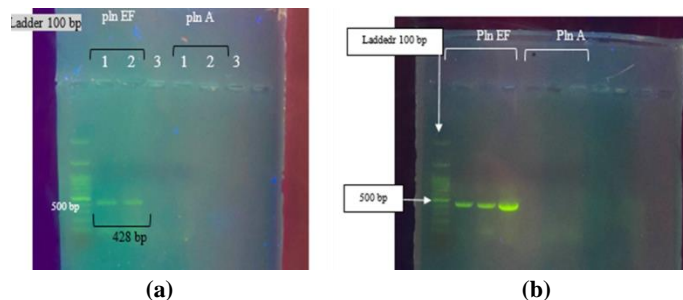


Figure 4. (a) Lactobacillus PCR products of positive control, cheese sample and negative control in order of number for PlnEF and PlnA genes. (b) Observation of PCR products caused by lactobacilli isolated from yogurt samples.

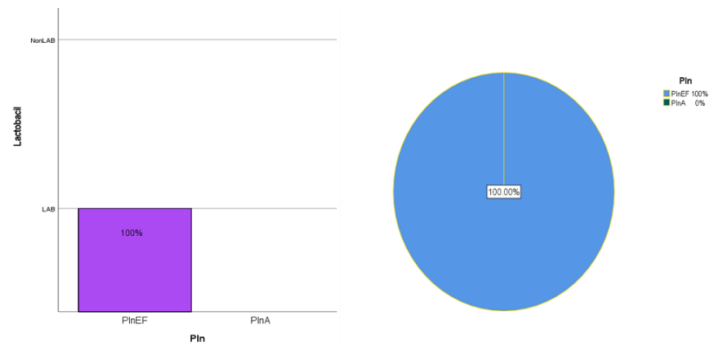


Figure 5. Bar and circle graph of abundance of plnA, plnEF genes in samples.

Discussion

In the present study, diagnostic tests and PCR analysis of traditional yogurt and cheese isolates showed that 7 out of 29 cultured samples contained *Lactobacillus*, and the presence of plnA and plnEF genes as bacteriocin coding genes was investigated in them. Therefore, pln EF gene frequency in bacteria positive samples is 100% and pln A gene frequency is 0%.

In previous studies, five different plantaricin (pln) loci have been identified from different *Lactobacillus* strains. The conserved portion, common to all loci, contains a bacteriocin operon (plnEFI) and a transfer operon. Plantaricin EF is present at all pln loci while the remaining plantaricins, including plantaricin A, are found in different strains-specific combinations.

Kumar et al. (2022) in their previous research on curd bacteria prepared using boiled milk and raw milk from dairy farms in Assam, India, isolated a potential probiotic bacterium and classified it as *Lactobacillus plantarum* strain JBC5. (LPJBC5) was determined. In this research, it was concluded that LPJBC5 promotes longevity and various aspects of healthy aging, including delayed physical functions associated with aging, reducing fat accumulation, improving resistance to abiotic and biotic stress, intestinal integrity, cognitive functions, mitochondrial function and Decreased apoptosis. This study shows that the bacterium LPJBC5 affects diverse features of healthy aging, making it an ideal candidate for promotion as next-generation probiotics. In this study, we succeeded in isolating *Lactobacillus* from the traditional yogurt and cheese of northwestern Iran, which have potential probiotic properties. Investigating the actual property of these probiotics and its impact on the general health of the body and healthy aging requires more studies in this area¹². Yolanda Sáenz et al. (2009) 33 strains of *Lactobacillus plantarum* obtained from mice and grape wines during alcoholic and malolactic fermentations by PCR analysis with primers specific to 27 plantaricin locus (pln) genes previously identified for strains *L. plantarum* was introduced, they investigated. The number of genes that were identified varied by strain, and cluster analysis yielded seven groups of results. Common features for most strains include plnABCD regulatory system, two-peptide bacteriocin plnEF genes, and genes for a membrane-bound ABC transporter system, pln locus among ecological strains, as well as being highly flexible. Attention and variable regions are related to the regulation and production of bacteriocin¹³.

In this study, we investigated two plantaricin coding genes pln sites. The two investigated genes were plnA and plnEF, and the results of PCR indicated the presence of the plnEF gene in 100% of the investigated isolates and the presence of the plnA gene in 0% of the investigated

isolates. These genes are responsible for the regulation and production of bacteriocin.

Mohammad Ali Shabkhiz et al. (1401) with the aim of isolating and identifying lactobacilli from the flora in traditional Maragheh cheese and determining their technological characteristics, lipolytic and proteolytic activity, isolated lactic bacteria by conventional methods of culture and identification based on biochemical properties. Then additional tests were performed in order to fully identify the available isolates, including gram staining, oxidase, catalase and PCR tests. The results showed that acidity increased and pH decreased with time in all samples. Therefore, traditional Maragheh cheese is a valuable source of *Lactobacillus* bacteria, which allows consumers to find a new way to benefit from health¹⁴.

Conclusion

In this study, we succeeded in isolating and identifying lactobacilli from the traditional yogurt and cheese of the northwestern region of the country by the method of culture and biochemical identification and isolation based on PCR.

Highlights

What Is Already Known?

In previous studies that succeeded in isolating *Lactobacillus* strains from traditional dairy products, plantaricin EF is present in all pln positions.

What Does This Study Add?

In this research, after *Lactobacillus* was isolated from samples, two genes encoding bacteriocin plnA and plnEF were investigated in *Lactobacillus* isolates.

Authors' Contributions

Z.H was involved in planning and supervised the work and proof outline S.D, Z.H and H.S drafted the manuscript and designed the figures. Z.H was responsible for the substantive review of the article and the project. All authors discussed the results and commented on the manuscript.

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The authors conflict of interests.

Consent For Publication

The authors declare their consent for publication.

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The extent of AI use

AI is not used.

References:

- Allen SJ, Wareham K, Wang D et al. Lactobacilli and bifidobacteria in the prevention of antibiotic-associated diarrhoea and *Clostridium difficile* diarrhoea in older inpatients (PLACIDE): a randomised, double-blind, placebo-controlled, multicentre trial. [Doi:10.1016/S0140-6736\(13\)61218-0](https://doi.org/10.1016/S0140-6736(13)61218-0).
- Salminen S, Bouley C, Bourtron-Ruault MC, et al. Functional food science and gastrointestinal physiology and function. *Br J Nutr* 1998; 80: 147-71. [Doi: 10.1079/bjn19980108](https://doi.org/10.1079/bjn19980108).
- Mal-Ganji Sh, Eivani MJ, Sohrabvandi S, Mortazavian AM. Health related aspects of probiotics. *Iranian Journal of Nutrition Sciences & Food Technology*. 2013; 5(7):579-590.
- Nouri S, Nazeri S, Hosseyni P. Biochemical and molecular identification of probiotic bacteria, "Lactobacillus plantarum", isolated from apple vinegar. *Cellular and Molecular Research (Iranian Journal*
- Mirzaei M, Mirdamadi S, Ehsani MR, Aminlari M, Hosseini E. Purification and identification of antioxidant and ACEinhibitory peptide from *Saccharomyces cerevisiae* protein hydrolysate. *J Funct Foods* 2015; 19: 259-68. [Doi:10.1016/j.jff.2015.09.031](https://doi.org/10.1016/j.jff.2015.09.031).
- Xiaoxiang Gao, Jianxin Zhao, Hao Zhang, Wei Chen, Qixiao Zhai, Modulation of gut health using probiotics: the role of probiotic effector molecules, 2022, *Journal of Future Foods*, Volume 2, Issue 1. [Doi:10.1016/j.jfutfo.2022.03.011](https://doi.org/10.1016/j.jfutfo.2022.03.011).
- Balciunas EM, Martinez FAC, Todorov SD, de Melo Franco BDG, Converti A, de Souza Oliveira RP. Novel biotechnological applications of bacteriocins: a review. *Food Control*. 2013; 32(1): 134-142. [Doi:10.1016/j.foodcont.2012.11.025](https://doi.org/10.1016/j.foodcont.2012.11.025).
- Sanders ME, Klaenhammer TR. Invited Review: The scientific basis of *Lactobacillus acidophilus* NCFM functionality as a probiotic. *JDS*. 2001. [Doi:10.3168/jds.S0022-0302\(01\)74481-5](https://doi.org/10.3168/jds.S0022-0302(01)74481-5).
- Pakdin A, Nasr SH. Isolation, identification and description of lactic acid bacteria from traditional dairy products of Mazandaran province Paper presented at: The 2nd International Congress and the 25th National Congress of Food Science and Technology; April 25, 2018. Sari
- Knoll, Caroline, Benoit Divol, and Maret Du Toit. "Genetic screening of lactic acid bacteria of oenological origin for bacteriocin-encoding genes." *Food microbiology* 25.8 (2008). [Doi:10.1016/j.fm.2008.06.010](https://doi.org/10.1016/j.fm.2008.06.010).
- Tai, H. F., Foo, H. L., Abdul Rahim, R., Loh, T. C., Abdullah, M. P., & Yoshinobu, K. (2015). Molecular characterisation of new organisation of *plnEF* and *plw* loci of bacteriocin genes harbour concomitantly in *Lactobacillus plantarum* I-UL4. *Microbial Cell Factories*, 14, 1-13. [Doi: 10.1186/s12934-015-0280-y](https://doi.org/10.1186/s12934-015-0280-y).
- Kumar, V., Naik, B., Kumar, A., Khanduri, N., Rustagi, S., & Kumar, S. (2022). Probiotics media: Significance, challenges, and future perspective-a mini review. *Food Production, Processing* [Doi:10.1186/s43014-022-00098-w](https://doi.org/10.1186/s43014-022-00098-w).
- Yolanda Sáenz, Beatriz Rojo-Bezares, Laura Navarro, Lorena Díez, Sergio Somalo, Myriam Zarazaga, Fernanda Ruiz-Larrea, Carmen Torres, Genetic diversity of the *pln* locus among oenological *Lactobacillus plantarum* strains, *International Journal of Food Microbiology*, (2009) Volume 134, Issue 3, Pages 176-183. [Doi:10.1016/j.ijfoodmicro.2009.06.004](https://doi.org/10.1016/j.ijfoodmicro.2009.06.004).
- Mirzaai tash O, Shabkhiz MA. Isolation and determination of technological characteristics of lactobacillus of traditional Maragheh cheese. *Journal of Food Microbiology*. 2022;9(4):44 55. [Doi:10.30495/JFM.2022.1831690.1442](https://doi.org/10.30495/JFM.2022.1831690.1442).