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A Review of Isolation Sources of *Lactiplantibacillus Plantarum* in Iran and Other Countries from Food Sources and with Food Applications

Davood Zare[1]*, Hadis Aryaee [1], Saeed Mirdamadi[1], Faezeh Shirkhan[2]

[1] Department of Biotechnology, Iranian Research Organization for Science & Technology (IROST), Tehran, Iran
[2] Department of Food Science and Technology, Faculty of Pharmacy, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran

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Abstract

Lactiplantibacillus plantarum (Lb. plantarum) is one of the safest probiotic strains. *Lb. plantarum* is mainly isolated from dairy products, fermented and non-fermented products, and the mucous and digestive systems of humans and animals. In addition to its many uses and benefits in industrial and clinical fields, this bacterium can be used by people of different ages and health statuses because it is one of the main members of the gastrointestinal microflora. Investigations have shown that *Lb. plantarum* has many strains, and while it is found in all regions, certain strains are more abundant in the native products of that region, which may cause differences in the microbiota of people, resulting in differences in health and behavioral characteristics. Therefore, studying the native species and strains of bacteria and changing or regulating the microbiota of people with appropriate probiotics can overcome many health and behavioral problems. This study shows that Iran has a high potential for many different types of probiotics, including *Lb. plantarum*, due to the variety of food and dairy products.

1. Introduction

Fermented foods are popular for their beneficial effects on human health (Shah et al., 2023). Fermented food products can be generally classified into five groups: meat, grain, dairy, vegetable, bread, and fermented drinks (Samappito et al., 2011). In the preparation and production of fermented foods, a wide range of lactic acid bacteria are added either as a starter culture or as the natural flora of the primary raw materials. Lactic acid bacteria (LAB) are gram-positive, catalase-negative, and spore-free bacteria, the main product of which is lactic acid, and they are known As Recognized Generally Safe (GRAS) (Mirdamadi & Tangestani, 2011). These bacteria produce various antimicrobial compounds such as organic acids, acetone, diacetyl, hydrogen peroxide, fatty acids, peptides, and bacteriocins. They are of great interest in discussing pharmaceutical products and food preservation because they control pathogenic and spoilage microorganisms, which can increase the shelf life

*Corresponding author. Davood Zare. Department of Biotechnology, Iranian Research Organization for Science & Technology (IROST), Tehran, Iran, E-mail address: Zare@irost.ir
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of foods, reduce food contamination, and improve the flavor and nutritional value of foods. The use of LABs and their metabolites as "Bio preservatives" has increased in recent years (Mirdamadi & Tangestani, 2011; Motahari et al., 2016).

Lactiplantibacillus plantarum (*Lb. plantarum*) is a highly useful *LAB isolated* from traditional fermented foods. It is a safe, useful, and resistant LAB abundantly found in most environments, including the digestive systems of humans and animals (Esmaeili et al., 2012). *Lb. plantarum* has also been reported as a pervasive microorganism in natural fermented dairy products. Probiotics are generally isolated from human sources and considered non-pathogenic bacteria. In addition to the abundance of a probiotic bacterium like *Lb. plantarum* in different sources such as distinct habitats, foods, and microbiota, *Lb. plantarum*'s stability in the digestive system makes it an ideal probiotic bacterium, attracting the attention of the food and supplement industries (Filannino et al., 2018). It should also be mentioned that Iran has four seasons and much biological diversity due to its different ethnic groups. This diversity gives rise to distinct cultures and customs with diverse traditional food products, making the country unique in terms of food habits. Therefore, in this review, we have investigated the origins, uses, and identification methods of food products containing *Lb. plantarum*. Knowing the different sources of bacterial strains is an effective step in introducing new microbial strains with potential as starter cultures, creating a native microbial bank, producing healthy food products for human, livestock and poultry nutrition, and developing further research therapeutic purposes.

2. Isolation sources of *Lb. plantarum*

Lb. plantarum isolates from greatly diverse sources, and this bacterium has been found in a wide range of fermented foods worldwide, especially dairy and meat products, probably because *Lb. plantarum* is a dairy starter (Bringel et al., 2005). Dairy and meat products provide a better platform for the growth and reproduction of *Lb. plantarum*. Some of the food products in which *Lb. plantarum* has been found are presented in (Table 1).

Table 1: *Food Products in which Lb. Plantarum is Found*

Grouping	Products	Country	References
Dairy products	Local cheeses	Argentina	(Zago et al., 2011)
	Soft cheese	Belgium	(Burns et al., 2011)
	Camel milk cheese	India	(Nanda et al., 2011)
	Ewe cheese	New Zealand	(Zhou et al., 2005)
	Fermented Milk	Zimbabwe	(Todorov et al., 2007)
	Flemish artisan gouda-type cheese	Belgium	(Van Hoorde et al., 2008)
	Traditional Greek Graviera cheese	Greece	(Samelis et al., 2011)
	Local cheese	Iran	(Ershadian, 2015)
	Kumis	Bulgaria	(Koleva et al., 2009)
	Mozzarella cheese	Italy	(De Angelis et al., 2008)
	Raw milk cheese	Germany	(Feld et al., 2009)
	Regional ovine cheese	Spain	(Nespolo CR & A., 2010)
	Manchego cheese	Spain	(Nieto-Arribas et al., 2009)
	Local cheese	Tenerife (Spain)	(Hernández et al., 2005)
	Qula cheese	Tibet	(Duan et al., 2008)
	Traditional dairy (Kumis)	China	(Xie et al., 2011)
	Mexican cheeses	Mexico	(Morales et al., 2011)
	Traditional kefir	Tiber (Italy)	(Wang et al., 2010)
Meat and meat products	Dry-fermented sausages	Argentina	(Müller et al., 2009)
	Salt meat	Tunisia	(Essid et al., 2009)
	Raw turkey meat	Germany	(Cho et al., 2010)

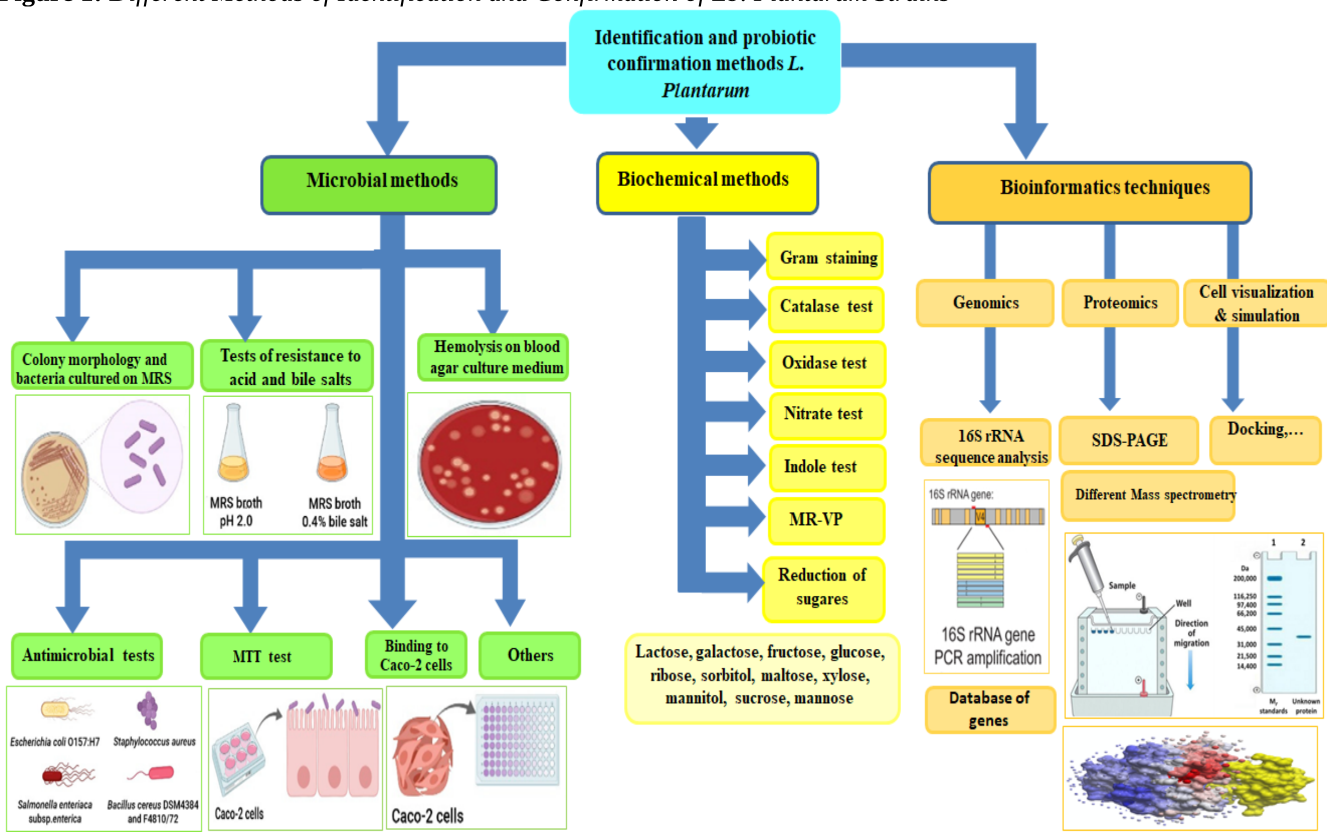
Table 1 continued:

Grouping	**Products**	**Country**	**References**
	Traditional fermented meat (Ajinarezuski)	Japan	(Kuda et al., 2010)
	pork Meat)(Chouriço & Beloura	Portugal	(Todorov et al., 2010)
	Spanish traditional pork sausage	Spanish pork sausage	(Fontán et al., 2007)
	Thai traditional meat sausage	Thai traditional meat sausage	(Samappito et al., 2011)
	Traditional salted meat	Tunisia	(Essid et al., 2009)
	Dry Fermented Sausage (Sucuk)	Turkey	(Kaban et al., 2009)
Fermented and alcoholic beverages	Barley beer	South of Africa	(Todorov et al., 2004)
	Apple vinegar	Beijing (China)	(Yin et al., 2008)
	A type of grape wine	Spain	(Rojo-Bezares et al., 2006)
	Olives in saltwater	South of Africa	(Todorov & LMT., 2006)
	Traditional alcoholic drink (Mezcal)	Mexico	(Escalante-Minakata P et al., 2008)
	Fermented drink (Pulque)	Mexico	(Escalante-Minakata et al., 2008)
Fermented and non-fermented grains and vegetables	Ayurvedic medicine (Kutajarista) (Fermented herbal medicine)	India	(Kumar et al., 2011)
	Cocoa beans	Ghana	(Camu et al., 2007)
	Sauerkraut	Ireland	(Crowly et al., 2012)
Other fermented and non-fermented foods	Corn fodder	France	(Tallon et al., 2007)
	Fermented vegetables	Finland	(Mäkimattila et al., 2010)
	Fermented sourdough	Italy	(Pepe et al., 2004)
	Traditional fermented foods	Japan	(Kawashima et al., 2011)
	Fermented Korean red ginseng (Panax ginseng)	Philippines	(Kim et al., 2010)
	Traditional food (Fu-Tsan)	Taiwan	(Liu et al., 2011)
	Thai fermented fruits and vegetables	Thailand	(Tanganurat et al., 2009)
	Traditional fermented food based on sorghum (corn on the ear)	Kalaburagi	(Rao et al., 2015)
	Honey Stomach of Honeybee	Malaysia	(Tajabadi et al., 2013)

3. Identification and confirmation of *Lb. plantarum*

Lb. plantarum, proposed by Orla-Jensen in 1919 as *Streptobacterium plantarum*, is a species widely distributed in most animal and vegetable fermented products, in both controlled and uncontrolled fermentation (Melgar-Lalanne et al., 2012). The *Lb. plantarum* subspecies *plantarum* was also introduced by Bringel et al. (2005). Its genome size is 3.45 Mbp, and its G+C DNA mole is 44.2%. This species has been isolated from several sources such as dairy products and dairy environments, leftover grass, sauerkraut, pickled vegetables, sourdough, cow dung, human mouth, intestinal tract and feces, and from sewage (Zheng et al., 2020). Another *Lb. plantarum* subspecies, *argentoratensis*, was also introduced by (Bringel et al., 2005). The strains of this species differ from the *Lb. plantarum* subspecies by the absence of maltose fermentation. Its genome size is 3.20 Mbp, and its G+C DNA mole content is 45%. This species has been isolated from starchy food, fermented food of plant origin, timothy, garden grass, elephant grass, fermented uttapam batter, and fermented idli batter (Zheng et al., 2020). Thus, *Lb. plantarum* is divided into subspecies *Lb. plantarum subsp. Plantarum* and *Lb. plantarum subsp. Argentoratensis*'s chromosomal DNA was identified using recA gene sequencing and hybridization with pyr probe on BglI digestion (Corsetti & Valmorri., 2011; Guidone et al., 2014). Probiotics, being living microbes, may impact the health of their hosts when appropriate quantities are swallowed. The main criteria for choosing probiotics are their acid and bile salt tolerance, safety, capacity to adhere to and colonize the digestive tract, and host health benefits. Different methods and tests used to identify *Lb. plantarum* and confirm its probiotic status (Esmaeili et al., 2012; Isa & Razavi, 2017) are briefly shown in (Fig 1).

Figure 1: *Different Methods of Identification and Confirmation of Lb. Plantarum Strains*



Identification and confirmation of *Lb. plantarum* strains can be classified into (i) microbial methods, (ii) biochemical methods, and (iii) bioinformatics techniques (Figure 1). The traditional approach to bacterial identification, including biochemical and morphological methods, cannot be used alone to identify strains accurately. Nowadays, new methods of identifying bacteria are used in addition to biochemical methods. Researchers have used many different methods to identify different types of bacteria globally, including lactic acid-producing bacteria. Since these bacteria (also known as probiotics) play an essential role in the food industries of countries, accurate strain identification and genetic change control are of great importance (Amor et al., 2007).

In this field, omics techniques help identify strains and understand the roles and mechanisms of the bacteria's functional characteristics (Echegaray et al., 2023). Several studies have utilized omics techniques (such as genomics, metabolomics, transcriptomics, and proteomics) to better understand functional activity and identify LAB strains (O'Donnell et al., 2020; Echegaray et al., 2023). Genomics is an omic technique that studies genomes to identify genes and genome functionality (Amor et al., 2007).

Metabolomics primarily encompasses biological metabolites identified through various methods. This approach serves as an efficient tool for characterizing and identifying fermented foods by *Lb. plantarum* (Echegaray et al., 2023). A review of these studies reveals that some studies have explored the relationship between the functional properties of *Lb. plantarum* and metabolic changes, while others have evaluated the application of metabolomics techniques in food groups fermented by this bacterium (Echegaray et al., 2023). According to certain studies, certain strains of *Lb. plantarum* exhibits functional properties such as antioxidant and antimicrobial activities and the ability to synthesize vitamin B, making it a promising candidate for food preservation (Yilmaz et al., 2022). Consequently, there have been reports of using transcription techniques to enhance antifungal and antioxidant properties, folate synthesis during fermentation, hyperglycemic reduction, and amino acid biosynthesis to control inflammation and immune response (Echegaray et al., 2023).

Proteomics is a technique used to study the identification and characterization of protein-related properties and reconstruct metabolic and regulatory pathways. In proteomics, various studies have investigated specific changes in *Lb. plantarum* proteins using proteomic techniques. These studies have reported that identifying cellular components is crucial for probiotic activity (Hamon et al., 2011). Furthermore, some studies have employed proteomics to determine stress responses to various factors (such as acids and bile), aiming to investigate the functional properties and survival of *Lb. plantarum* in the gut (Echegaray et al., 2023). In conclusion, the literature review indicates that omics techniques can successfully help characterize and evaluate the effectiveness of the probiotic features of *Lb. plantarum*. Therefore, it appears that these capabilities can be utilized for practical biological and food applications.

Cell visualization and simulation is another new assay used to study and model cell behavior using different methods (Bansal, 2005; Yeoh & Cheah, 2020). One of the most accurate methods for identifying bacteria is identification using a specific 16s rRNA primer with polymerase chain reaction (PCR). Multiple molecular methods with different objectives have been designed to identify lactic acid-producing bacteria, including PCR, restriction fragment length polymorphism (RFLP), relative afferent pupillary defect (RAPD), pulse field gel electrophoresis (PFGE), probing, denaturing gradient gel electrophoresis (DGGE), amplified fragment length polymorphism (AFLP), fluorescence in-situ hybridization (FISH) ribotyping, and complex methods. These techniques can identify the differences between bacteria in terms of gender, species, and even strain (Amor et al., 2007).

4. Isolation of *Lb. plantarum* from different regions of Iran

4.1. Recent studies on the isolation of *Lb. plantarum* in Iran

In recent years, many studies have been conducted on the isolation of native probiotics, especially *Lb. plantarum*, see (Table 2). The major contribution of *Lb. plantarum* isolation in Iran has been related to fermented and non-fermented dairy products, probably due to the fact that the majority of traditional products in Iran are dairy products, especially cheese and fermented dairy products. One reason for this could be this bacterium's tolerance to salt and acid, its central role in the cheese ripening process, and its role in the fermentation of these products. Since there is a wide range of traditional dairy products in the different regions of Iran, it is not unexpected that the contribution of *Lb. plantarum* isolated from these products is greater than other products (Edalatian et al., 2012).

4.2. Aims and application of native Iranian products-isolated probiotics

Isolations are carried out for various purposes, such as improving the quality of food products, producing functional food, anti-pathogen effects, therapeutic purposes, and helping to maintain

Table 2: *Species and Strains of Lb. plantarum Isolated from Different Foods in the Provinces and Cities of Iran*

Grouping	Name of species/strain	Isolation city/district	Products	References
Fermented and non-fermented dairy products	*Lb. plantarum gp 57* *Lb. plantarum gp 46* *Lb. plantarum KLDS 610.1*	Gorgan	Jug cheese	(Samappito et al., 2011)
	Lb. plantarum gp106	Gorgan	Camel milk	(Samappito et al., 2011)
	Lb. plantarum	East Azerbaijan	Liqvan cheese	(Abdi et al., 2006)
	Lb. plantarum	Lorestan, Kermanshah, Hamadan and Ilam	Pasteurized and local milk and cheese (unpasteurized)	(Bahadori et al., 2010)
	Lb. plantarum	Nadushan of Yazd	Local dairy products	(Pourabdi Sarabi et al., 2020)
	Lb. plantarum ktbs2	Semnan	Traditional cheese	(Mosallami et al., 2020)
	Lb. plantarum	Rural areas of Rafsanjan	Traditional yogurts	(Farahbakhsh et al., 2013)
	Lb. plantarum	Sabzevar	Traditional yogurt	(Ershadian et al., 2015)
	Lb. plantarum CJLP55 *Lb. plantarum Lb3*	North of Iran	Siyahmuzgi cheese (a traditional cheese from the north of Iran)	(Zamani, 2016)
	Lb. plantarum	Gilan	Gilan Seyahmezgi Cheese	(Partovi et al., 2017)
	Lb. plantarum TW29-1	Zabul	Yellow curd	(Saboktakin-Rizi M et al., 2021)
	Lb. plantarum	Iran	10 types of traditional Iranian cheese	(Afshari et al., 2022)
	Lb. plantarum	Northeast of Iran (Sahara Turkmen)	Chal (Iranian traditional fermented camel milk)	(Soleymanzadeh et al., 2017)
	Lb. plantarum	Kleiber, Harris and Varezghan	Traditional milk, yogurt and buttermilk	(Narimani et al., 2012)
	Lb. plantarum	Different regions of East Azerbaijan	Local milk and yogurt products	(Pourabdi Sarabi et al., 2020)
Fermented and non-fermented non-dairy products	*Lb. plantarum*	Tehran	pickled cucumber	(Rajabloo et al., 2012)
	Lb. plantarum	Tarem city, Zanjan province	Different types of olive products	(Esmaeili et al., 2012)
	Lb. plantarum subsp. plantarum W2 *Lb. plantarum CSCWL 7-3* *Lb. plantarum partial 16S rRNA gene* *Lb. plantarum CSI 7* *Lb. plantarum NBRC 15891*	Gorgan	Fermented olives	(Samappito et al., 2011)
	Lb. plantarum	Thirteen provinces of Iran (Tehran, Mazandaran, etc.	Honey	(Lashani et al., 2018)
	Lb. plantarum CIP 103151	Gorgan	Sourdough	(Samappito et al., 2011)
	Lb. plantarum	Esfahan	Apple vinegar	(Nouri et al., 2018)

health status. For example, it has been shown that strengthening and increasing the number of *Lb. plantarum* strains isolated from Iranian native olives during the de-bittering and fermentation stages increase the olives' probiotic value (Esmaeili et al., 2012). In a study conducted by DehghanKhalili and Erjaee (2020) *Lb. plantarum* and *Lb. reuteri* were utilized in the preparation of sourdough and were shown to be effective in increasing the quality and sensory properties of barley bread. Additionally, they evaluated the effect of *Lb. plantarum* strains isolated from different foods (fermented olives, jarred cheese, camel milk, and sourdough) to find their antimicrobial, antioxidant, and cumulative activity. Their results demonstrated that the native strains of *Lb. plantarum* and the produced metabolites can be used as biological preservatives in the food industry and pharmaceutical supplements (DehghanKhalili & Erjaee, 2020). In terms of the inhibitory effect of this bacteria, two separate studies were carried out. The results of one study showed that Iranian honey samples contain *Lb. plantarum* species, which have been shown to have acceptable inhibitory effects on pathogenic bacteria, such as *Staphylococcus aureus* (Lashani, 2018). The effect of *Lb. plantarum* strain Ktbs2 probiotic isolated from Semnan traditional cheese on the total serum oxidant and antioxidant capacity, oxidative stress index, and some biochemical parameters in diabetic rats, and the results showed that *Lb. plantarum strain ktbs2* isolated from Semnan traditional cheese reduced hyperglycemia, dyslipidemia, and oxidative stress in diabetic rats. In another study designed by Farahbakhsh et al. (2013), probiotic lactobacilli were isolated from traditional yogurts in rural areas of Rafsanjan to investigate their antimicrobial effects. In this study, 40 local yogurt samples from four rural areas were screened, and 33 bacterial strains were isolated in the first stage; results showed that all the probiotic strains were able to destroy pathogenic bacteria, with the most antibacterial effect being observed from *Lb. plantarum*. They also stated that the presence of probiotic bacteria with antibacterial activity against some pathogenic bacteria in traditionally prepared yogurts could be used in the production of industrial dairy products (Farahbakhsh et al., 2013). There are similar studies on the isolation of *Lb. plantarum*. For instance, Partovi et al. (2017) isolated *Lb. plantarum* from Gilan siah-mazgi cheese and assessed different microbial, biochemical, and molecular identification methods, such as antimicrobial activity. Saboktakin-Rizi et al., (2021) isolated *Lb. plantarum* TW29-1 from the fermented product of Zabul yellow curd, showing it to have significant tolerance to acidic pH, bile salts, and simulated digestive juices, as well as strong antimicrobial properties against pathogenic bacteria. Consequently, they proposed *Lb. plantarum* TW29-1 be introduced as a new probiotic strain with therapeutic and preservation properties for food and health promotion purposes (Saboktakin-Rizi et al., 2021). On the subject of isolating probiotic bacteria, Afshari et al. (2021) investigated ten types of traditional Iranian cheese to isolate new strains of probiotic bacteria. It was shown that *Lb. plantarum* was the most resistant bacteria in the bile resistance test and the most durable bacteria in digestive conditions, such as in an acidic environment (pH = 2.5) and trypsin. Additionally, the result of this study noted that probiotic strains isolated from local cheeses could be considered suitable bio-preservatives and used as specific starter cultures for producing functional cheeses (Afshari et al., 2022).

Anticancer activities of different *Lb. plantarum* strains isolated from traditional Iranian fermented food have been observed in various types of tumors (Sadeghi-Aliabadi et al., 2014; Gholipour et al., 2023; Adiyoga et al., 2022). Moreover. in vitro cytotoxic effects have been reported on cell lines. For example, in Rouhi et al.'s 2024 study on the cytotoxic properties *of Lb. plantarum* TW57-4 isolated from Zabuli yellow kashk, the cytotoxicity of cell-free supernatant (CFS) of the cultured bacterium was evaluated using the MTT method. Their results revealed that as the concentration of CFS increased, there was an increase in the percentage of cytotoxic influence on Caco-2 cells. The half-maximal inhibitory concentration (IC_{50}) value of the CFS of *Lb. plantarum* was found to be 44.64 mg/mL (Rouhi et al., 2024). In another study, the cytotoxic effect of postbiotic metabolites (PM) produced by six strains of *Lb. plantarum* was further investigated for breast and colon cancer (Chuah LiOon et al., 2019). They found that the PM produced by the six strains exhibited selective cytotoxicity via an anti-proliferative effect and induction of apoptosis against cancer cells in a strain-specific and cancer cell type-specific manner while sparing the normal cells. These results reveal the vast potential of PM from *Lb. plantarum* as a functional supplement and as an adjunctive treatment for cancer (Chuah LiOon et al., 2019). Another study by Samanarad et al. (2023) assessed the synergistic and separate cytotoxicity effects of carboplatin (a chemotherapy medication) and *Lb. plantarum* cell lysate supernatant (CLS) in the SK-OV-3 ovarian cancer cell line and the expression change of apoptotic Bax and anti-apoptotic Bcl-2 genes. The results showed the highest increases of toxicities in the separate and synergistic application of carboplatin and *Lb. Plantarum* CLS after 48-h treatment against cancer cells (Samanarad et al., 2023). According to these reports, *Lb. plantarum* seems to have a cytotoxic effect. Therefore, more attention should be paid to the cytotoxic effect of *Lb. plantarum* isolated from different fermented foods.

5. Conclusion

Lb. plantarum is a safe, widely abundant probiotic that can be isolated from many indigenous sources, and it can be said that the dominant strain is isolated from fermented products. Since this bacterium can be isolated from many different sources, from plants and fruits to meat, dairy, and the human body, many types of isolated strains exist. According to the isolation source, the frequency of strains is different in each region, which emphasizes the need to study and investigate the identification of native strains of *Lb. plantarum* and other lactobacilli. *Lb. plantarum* can live in a wide range of ecological niches, including the mucosa of the human digestive system, making it possible to use as a probiotic that can tolerate different ecological conditions. At the same time, it has many functional properties, including nutritional properties, and can provide clinical benefits for people of different ages. Another benefit of reviewing studies on isolating different strains of each probiotic bacteria from native products and sources is collecting information to form a bank of native probiotics.

Author contribution:

Davood Zare, Hadis Aryaee and Faezeh Shirkhan were responsible of literature collection and writing the manuscript. Figures were prepared by Hadis Aryaee. Faezeh Shirkhan and Hadis Aryaee were prepared tables. Davood Zare and Saeed Mirdamadi commented on and edited subsequent versions. All authors read and approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest

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Ethical approval

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