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Original Article

Molecular Identification of *Lactobacillus acidophilus* Strain ABC240 Isolated from Traditional Yogurt and Assessing Its Stability and Safety as well as Antibacterial and Antioxidant Activities

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ABSTRACT

Background and Objectives: In recent years, research has focused on the isolation of new bacterial strains with diverse and beneficially biological activities. This study investigated stability (under various acid concentrations, various bile-salt concentrations, cholesterol absorption ability and surface hydrophobicity) and antagonistic, antioxidant and immune activities of *Lactobacillus acidophilus* strain ABC240 isolated from traditional yogurts.

Materials and Methods: Strain was identified using polymerase chain reaction technique. Its viability was assessed under acidic conditions (pH values of 3, 4 and 5) and in presence of bile salts (concentrations of 0.3, 0.5 and 0.7%). Additionally, antimicrobial activity was assessed through well diffusion and disc diffusion methods. Antioxidant capacity was assessed using ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) and DPPH (2,2-diphenyl-1-picrylhydrazyl) assays. This study analyzed the microbial cell surface hydrophobicity, cholesterol absorption ability and safety parameters, including antibiotic sensitivity, hemolytic activity, DNase activity and biogenic amine production.

Results: Results demonstrated high stability of this strain against various concentrations of acid (pH values of 3, 4 and 5) and bile salts (0.3, 0.5 and 0.7%). Ability of *Lactobacillus acidophilus* strain ABC240 to absorb cholesterol included 43.60% ±0.66 and its cell surface hydrophobicity was assessed at 50.49% ±0.63. The antimicrobial potential assessments revealed strong activity against several bacterial species. Furthermore, antioxidant activities, assessed using DPPH and ABTS methods, were detected as 44.69% ±0.89 and 49.80% ±0.79, respectively, indicating the bacterial high potential in inhibiting free radicals. Antibiotic sensitivity assay showed a non-growth zone diameter ranging from 13.20 mm ±0.55 (vancomycin) to 24.50 mm ±0.66 (chloramphenicol). *Lactobacillus acidophilus* strain ABC240 showed no hemolytic or DNase activity and did not produce biogenic amines.

Conclusions: In conclusion, *Lactobacillus acidophilus* strain ABC240 can be introduced as a new probiotic strain with biological and functional characteristics appropriate for use in the food industry.

Keywords: Dairy fermentation products, Probiotic characteristics, Antimicrobial activity, Biogenic amines

Highlights

- The high stability of *Lactobacillus acidophilus* strain ABC240 against various concentrations of acid (pH values of 3, 4 and 5) and bile salts (0.3, 0.5 and 0.7%) was demonstrated.
- The ability of *Lactobacillus acidophilus* strain ABC240 to absorb cholesterol was 43.60% ±0.66 and its cell surface hydrophobicity was assessed as 50.49% ±0.63.
- The antioxidant activities of *Lactobacillus acidophilus* strain ABC240, assessed using DPPH and ABTS methods, were detected as 44.69% ±0.89 and 49.80% ±0.79, respectively, indicating the microbial high potential in inhibiting formation of free radicals.
- Antibiotic sensitivity assays showed a non-growth zone diameter ranging from 13.20 mm ±0.55 (vancomycin) to 24.50 mm ±0.66 (chloramphenicol). The *Lactobacillus acidophilus* strain ABC240 showed no hemolytic or DNase activity and did not produce biogenic amines.

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Introduction

According to the United Nations Food and Agriculture Organization (UN FAO), more than 80% of the world population regularly consume dairy products. It is estimated that the global fermented milk market will reach approximately 493.10 billion dollars by 2031, with a compound annual growth rate of 4.9% within 2023–2031. These reports indicate that the consumption of dairy products has significantly increased in recent years due to their health benefits and high nutritional values. Yogurt, one of the most popular fermented dairy products, is consumed worldwide. This fermented product contains proteins with a higher biological value than milk and provides almost all the essential amino acids necessary for preserving human health. Yogurt is addressed as a primary food product that contains probiotics. Continuous consumption of yogurt helps improve lactose intolerance, strengthens the immune system, manages metabolic disorders and prevents digestive diseases. Thus, consumer demand has increased, making yogurt production the fastest-growing segment of dairy products in the global market (1–3).

Due to the high diversity of microbial strains and the complexity of fermentation substrates, it can be challenging to identify the exact composition and molecular structure of fermented dairy products, including yogurts. One reason includes that it is not often possible to cultivate all microorganisms under laboratory conditions. Moreover, existing commercial culture environments do not allow for the simultaneous growth of all strains. Therefore, genetic information of microbial species can be achieved using polymerase chain reaction (PCR) technique (2–5).

Lactic acid bacteria (LAB) are the most abundant microorganisms isolated from fermented dairy products. Common milk-fermenting LAB strains include *Lactobacillus acidophilus*, *Lacticaseibacillus rhamnosus*, *L. casei*, *Streptococcus thermophilus*, *Bifidobacterium breve*, *B. lactis*, *B. longum* and *B. animalis* (2). From these, *Lb. acidophilus* is an important intestinal probiotic strain that is focused by the researchers, especially due to its association to human health. The *Lb. acidophilus* is a prevalent microorganism used in various food products, demonstrating a better resistance to acid and bile salts, compared to other probiotics. This characteristic enables the bacterial survival and reproduction in the challenging environment of the digestive system; thereby, enhancing the efficacy of products containing the strain in the human body (6). Moreover, *Lb. acidophilus* shows strong antioxidant activity and can inhibit linoleic acid. Research studies in 2021 indicated that the supernatant of *Lb. acidophilus* possessed antioxidant characteristics attributable to phenolic and flavonoid compounds (7). Since antimicrobial activity is a defining characteristic of probiotics, demonstrating antimicrobial potential of *Lb. acidophilus* supports its probiotic classification. Previous studies have shown that *Lb. acidophilus* shows strong antimicrobial activity against important pathogens such as *Escherichia coli*, *Staphylococcus aureus* and *Clostridium perfringens* (8).

Isolation and identification of LAB strains are important because these can lead to the discovery of new probiotic strains that may offer unique health benefits, enhancing peoples' understanding of gut microbiota and potentially improving digestive health and immune function. Isolating new probiotic strains is significant because it allows for the development of tailored therapies that can target specific health conditions, thus contributing to further personalized and effective approaches to healthcare. Accordingly, this study aimed to molecularly identify *Lb. acidophilus* strain ABC240 isolated from traditional yogurt and investigate its stability characteristics (tolerance to various acid and bile-salt concentrations, cholesterol absorption ability and surface hydrophobicity), as well as its antagonistic, antioxidant and immune activities.

Materials and Methods

Chemicals and culture media

Chemicals and culture media included genomic DNA isolation kit (Asian Dena-Zist, Iran), PCR kit (Parstous Biotech, Iran), DNase culture media (HiMedia, India), Muller-Hinton agar (Merck, Germany), de Man-Rogosa-Sharpe (MRS) agar (Merck, Germany), MRS broth (Merck, Germany), sheep blood agar (Merck, Germany), bile salt (Sigma-Aldrich, USA) and standard antibiotics (vancomycin, gentamicin, chloramphenicol, nitrofurazone, nalidixic acid, penicillin, imipenem and ciprofloxacin) (Padtan Teb, Iran).

Collection of the samples

Conventional yogurt samples were collected randomly from a market in Behbahan, Iran, and quickly transported to the laboratory under cold conditions. In the laboratory, 5 g from each sample were mixed with peptone water (45 ml) and homogenized.

Isolation and identification of *Lactobacillus acidophilus* strain ABC240 using polymerase chain reaction

To isolate and identify the target strain, samples were homogenized using peptone water and successive dilutions (10^{-1} to 10^{-6}) were prepared. Samples were cultivated on the surface of MRS agar and incubated at 37 °C for 48 h. Heat staining and catalase tests were carried out on the strain. Genomic DNA was extracted using DNA isolation kit. General primers for 16S rRNA gene amplification included 27FYM (5'-AGA GTT

TGATYMTGG CTC AG-3') and 1492R (5'-GGT TAC CTT GTT ACG ACT T-3'). Then, DNA amplification was carried out in a 25.15-µl reaction. The PCR conditions included an initial denaturation cycle, multiple amplification cycles and final elongation. The PCR protocol comprised several key steps as follows:

1. Initial denaturation: Reaction began with an initial denaturation at 94 °C for 5 m to achieve complete melting of the DNA.
2. Amplification cycles: Each cycle included:
   - Denaturation: The first phase of each cycle involved denaturation at 94 °C for 30 s.
   - Annealing: Temperature decreased to a specific annealing temperature, generally 50–65 °C, depending on the primers, for 30 s. This allowed the primers to hybridize to the template DNA.
   - Extension: The final step of each cycle was carried out at 72 °C for 1 m per kb of the target DNA, where DNA polymerase enzyme synthesized new DNA strands.
3. Number of cycles: Typically, amplification included 25–35 cycles, which was assessed by the needed level of DNA amplification.
4. Final elongation: After the amplification cycles, a final elongation step was carried out at 72 °C for 5–10 m to ensure complete extension of all DNA strands.

The PCR products were electrophoresed on 1.5% agarose gels. The isolate, which was catalase-negative and Gram-positive, included a similarity rate of 98% and was identified as *Lb. acidophilus* strain ABC240 (5).

Assessing stability of the microbial strain against various acid concentrations

In this study, *Lb. acidophilus* strain ABC240 was cultured on MRS broth and incubated at 37 °C for 24 h. Microbial cells were separated via refrigerating centrifugation at 9000× g for 5 m. These were washed with sterile phosphate buffer solution and samples were diluted to achieve an absorbance of 0.6 at 600 nm. A sample of 50 µl was mixed with 450 µl of acidic phosphate buffer solutions (pH 3, 4 and 5) and incubated at 37 °C for 3 h. Successive dilutions were prepared and surface cultures were carried out on MRS agar. Plates were incubated and colonies were counted. Viability percentage was calculated against the control (9).

Assessment of the stability of the microbial strain against various concentrations of bile salts

The *Lb. acidophilus* strain ABC240 was cultured on media containing various bile salt concentrations (0.3, 0.5 and 0.7%) and incubated at 37 °C for 24 h. Visual assessments were carried out on the results (9).

Measurement of cholesterol absorption ability

Polyoxyethylene-cholesteryl subcategory and oxgall (3%) were added to MRS broth, resulting in a cholesterol concentration of 100 mg/l. A 1% microbial culture was added to the prepared media and incubated at 37 °C for 24 h. The control sample contained media without the microbial culture (10).

Measurement of surface hydrophobicity

The *Lb. acidophilus* strain ABC240 was isolated via centrifugation (5000× g for 15 min) and washed with phosphate buffer. Phosphate buffer was added to achieve an absorbance of approximately 1 (H1). A suspension of 3 ml was mixed with 0.6 ml of n-hexane and incubated at 25 °C for 1 h. Absorbance of the aqueous phase was recorded at 600 nm (H2). Surface hydrophobicity was calculated using the following formula (11):

Surface hydrophobicity (%) = [(H1 - H2) / H1] × 100

Assessment of antibacterial activity

The antimicrobial activity of *Lb. acidophilus* strain ABC240 against foodborne pathogens such as *Shigella dysenteriae*, *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella enterica* serovar Typhimurium, *E. coli* and *S. aureus* was assessed using disc diffusion and well diffusion methods (12).

Antioxidant activity

DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay: Briefly, 2 ml of methanolic 2,2-Diphenyl-1-picrylhydrazyl (DPPH) solution (0.14 mM) were mixed with 2 ml of microbial suspension and incubated at 37 °C for 30 m. Absorbance was measured at 517 nm (12).

ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) assay: A 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) stock solution was prepared and set in dark for at least 6 h. Solution was diluted to reach an absorbance of approximately 1 at 734 nm. Then, 0.3 ml of cell-free supernatant (CFS) was mixed with 2.7 ml of ABTS solution and incubated and then the absorbance was measured (13).

Microbial strain safety assay

Antibiotic sensitivity: Antibiotic sensitivity of *Lb. acidophilus* strain ABC240 against various antibiotics was assessed on MRS agar, followed by assessing the halo diameter of no growth (5).

DNase activity: Briefly, DNase activity was assessed using DNase culture media and identifying transparency around the bacterial colonies (12).

Hemolytic activity: Strain was assessed on sheep blood agar to investigate the hemolytic activity based on the color changes around the bacterial colonies (10).

Production of biogenic amines: Production of putrescine, tyramine and histamine was assessed by adding amino

acid precursors to MRS agar and observing color changes (12).

Statistical analysis

All experiments were carried out in triplicate. Results were analyzed using SPSS software v.22 (IBM, USA) via one-way analysis of variance (ANOVA), with differences assessed using Duncan's test at 95% confidence levels ($p < 0.05$).

Results

Stability of the microbial strain against various acid concentrations

Findings from this study indicated that the strain showed high stability across various acidic pH levels, with survival rates ranging from 78% at pH 3, 96% at pH 4 and 99% at pH 5 (Figure 1).

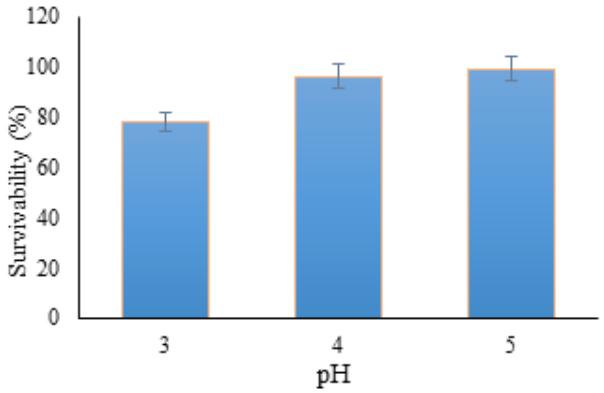

Figure 1. Survivability of *Lactobacillus acidophilus* strain ABC240 under various acidic pH

Stability of the microbial strain against various concentrations of bile salts

In the present study, growth of *Lb. acidophilus* strain ABC240 against various concentrations of bile salts was investigated (Table 1). Results revealed that this strain was stable against various concentrations of bile salts; however, the survival rate decreased with increasing salt concentration.

Table 1. Survivability of *Lactobacillus acidophilus* strain ABC240 under various bile salt concentrations

	0%	0.3%	0.5%	0.7%
Survivability	++++*	+++	++	+

* ++++ Very high survivability, +++ High survivability, ++ Moderate survivability, + Low survivability

Cholesterol absorption ability

Ability of *Lb. acidophilus* strain ABC240 to absorb cholesterol shown in Table 2.

Table 2. Cholesterol assimilation and hydrophobicity ability of *Lactobacillus acidophilus* strain ABC240

Cholesterol assimilation (%)	Hydrophobicity (%)
43.60 ± 0.66	50.49 ± 0.63

Surface hydrophobicity

Results of this study indicated that the surface hydrophobicity of *Lb. acidophilus* strain ABC240 was 50.49% ±0.63 (Table 2). This value suggested a moderate level of hydrophobicity, which is an important attribute for the strain ability to adhere to intestinal cells and contribute to its probiotic effects.

Antibacterial activity

In the current study, antimicrobial activity of *Lb. acidophilus* strain ABC240 against several significant pathogenic bacteria was assessed using two common methods of well diffusion agar and disk diffusion agar (Figure 2). As demonstrated in Figure 2, *Lb. acidophilus* strain ABC240 showed strong antimicrobial activities. In well diffusion agar method, values for non-neutralized cell-free supernatant (nCFS) ranged from 6.20 mm ±0.11 against *S. enterica* serovar Typhimurium to 9.10 mm ±0.23 against *L. monocytogenes*. The neutralized cell-free supernatant (aCFS) demonstrated the lowest inhibition rate of 7.10 mm ±0.14 against *S. enterica* serovar Typhimurium and the highest inhibition rate of 10.09 mm ±0.27 against *L. monocytogenes*.

In disk diffusion agar method, nCFS values ranged from 0 mm (no inhibition against *S. enterica* serovar Typhimurium) to 8.30 mm ±0.27 against *L. monocytogenes*. The aCFS values showed a range from 7.30 mm ±0.12 against *E. coli* to 9.90 mm ±0.14 against *L. monocytogenes*. Overall, a comparison of the two types of CFS indicated that the antimicrobial effect of aCFS of *Lb. acidophilus* strain ABC240 was greater than that of its nCFS (Figure 2). This finding suggested that neutralization of the supernatant improved its antimicrobial characteristics, likely by decreasing effects of organic acids and allowing for further favorable pH for antimicrobial compounds to exert their effects.

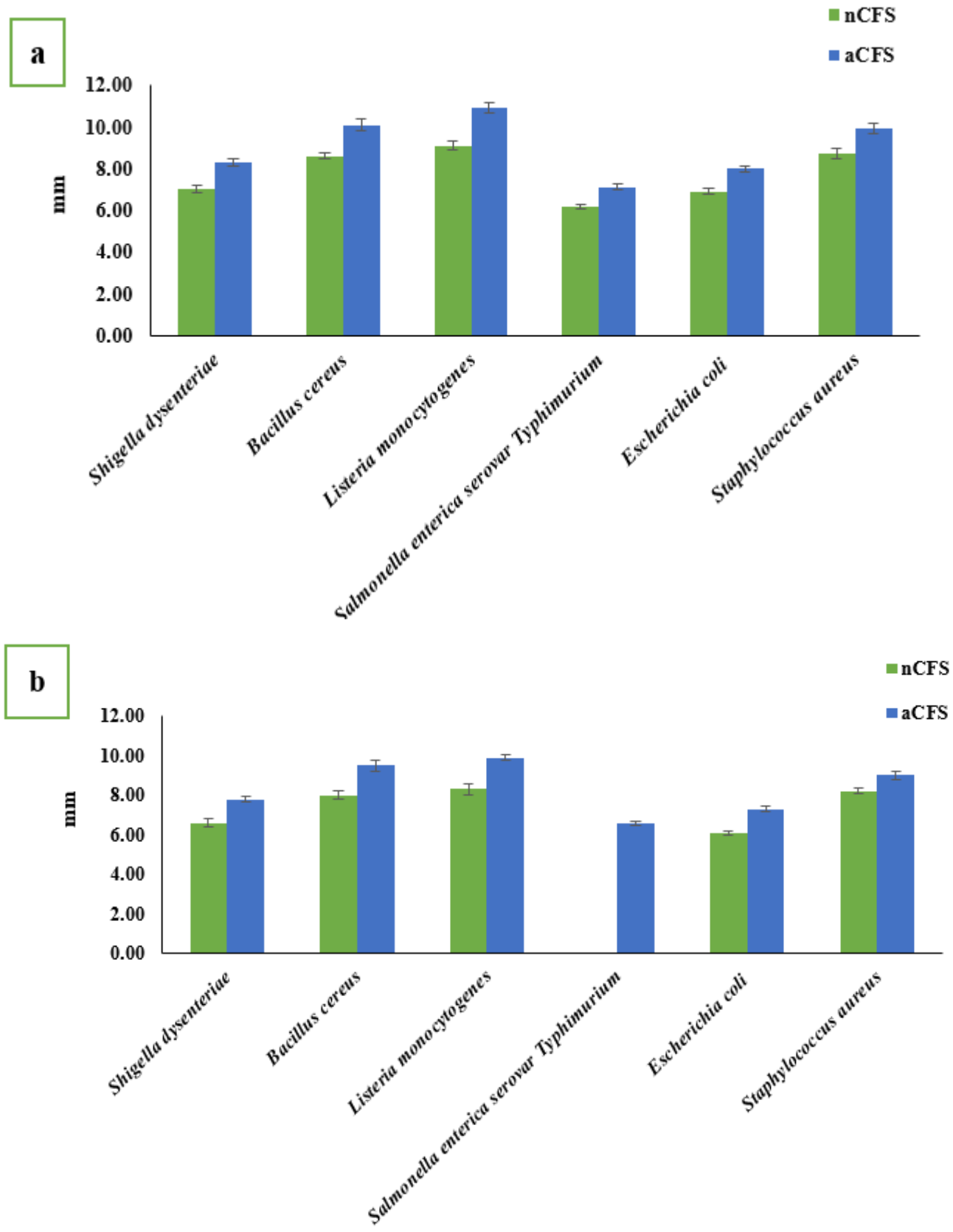

Figure 2. Antimicrobial activity of *Lactobacillus acidophilus* strain ABC240 based on (a) well diffusion agar and (b) disk diffusion agar methods. aCFS, acid cell-free supernatant; nCFS, neutralized cell-free supernatant

Antioxidant activity

Antioxidant molecules play an important role in maintaining balance of the intestinal microbiome by modulating oxidative stresses. In this study, antioxidant potential of the isolated bacterial strain was assessed using DPPH and ABTS free radical scavenging methods (Figure 3). The antioxidant activities of *Lb. acidophilus* strain ABC240 were detected as 44.69% ±0.89 and 49.80% ±0.79, respectively, indicating a high potential of the isolated strain in inhibiting free radicals.

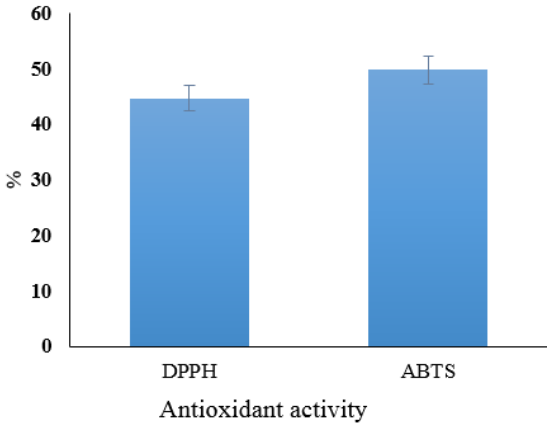

Figure 3. Antimicrobial activity of *Lactobacillus* and ABTS *acidophilus* strain ABC240 based on DPPH methods

Antibiotic sensitivity

Table 3 presents results of antibiotic resistance of *Lb. acidophilus* strain ABC240 against various antibiotics. Diameter of the non-growth halo of the strain ranged from 13.20 mm ±0.55 (vancomycin) to 24.50 mm ±0.66 (chloramphenicol). Resistance of *Lb. acidophilus* strain ABC240 against other antibiotics was as follows: gentamicin (22.60 mm ±0.39), nitrofurazone (20.20 mm ±0.41), nalidixic acid (17.10 mm ±0.52), penicillin (19.80 mm ±0.28), imipenem (14.90 mm ±0.57) and ciprofloxacin (17.70 mm ±0.25).

Table 3. Effects of common antibiotics on growth of *Lactobacillus acidophilus* strain ABC240

Antibiotic	Diameter of growth inhibition (mm)
Vancomycin	0.55±13.20
Gentamicin	0.39±22.60
Chloramphenicol	0.66±24.50
Nitrofurazone	0.41±20.00
Nalidixic acid	0.52±17.10
Penicillin	0.28±19.80
Imipenem	0.57±14.90
Ciprofloxacin	0.25±17.70

DNase activity

Results of this study indicated that *Lb. acidophilus* strain ABC240, isolated from local yogurts, showed no DNase activity, verifying its safety for use as a probiotic strain.

Hemolytic activity

Findings of this study demonstrated absence of hemolytic activity in *Lb. acidophilus* strain ABC240.

Biogenic amines

Findings of the present study revealed that *Lb. acidophilus* strain ABC240 was unable to produce biogenic amines; thereby, verifying its status as a safe probiotic strain for food and medicinal uses.

Discussion

Stability against acid and bile salts is one of the most important factors for selecting a probiotic strain, as these are key determinants in the survival of LAB in digestive system, ensuring their viability and efficacy as food supplements. Generally, LAB are microorganisms with high stability against acidic pH levels, allowing them to tolerate adverse conditions such as food fermentation or human digestive environments (14). The exceptional stability of *Lb. acidophilus* strain ABC240 under acidic conditions might be attributed to the constant gradient between intracellular and extracellular pH created by the F0F1-ATPase multicomponent system in Gram-positive bacteria. Additionally, LAB have developed various regulatory mechanisms to cope with acid stress, including preserving pH homeostasis through metabolic pathways, modifying cell membrane compositions (e.g., fatty acids) and preserving repairing macromolecules (e.g., DNA and proteins) with the help of chaperones, which enhance recovery of damaged proteins and facilitate appropriate folding of newly synthesized proteins (5, 14).

Ability of microorganisms to detoxify bile salts through enzymes that hydrolyze these salts (bile salt hydrolase, BSH) constitutes a critical characteristic for selecting probiotic strains. This detoxification process enables probiotics to survive and thrive in the digestive environment. Bile salts are secreted from the gallbladder into the small intestine, where they are hydrolyzed by microorganisms. The hydrolyzed bile acids can easily be excreted from the body, which may lead to the synthesis of new bile salts from serum cholesterol, decreasing the cholesterol concentration (14, 15).

Previous studies have revealed BSH activity in a portion of *Lb. acidophilus* strains isolated from various sources, with demonstrating robust bile salt hydrolyzing activity even after a prolonged culture time. The LAB strains have extensively been studied for their cholesterol-lowering activity under *in vitro* and *in vivo* conditions. One suggested mechanism for this decrease includes deconjugation of the bile by BSH, which increases bile acid excretion and decreases reabsorption. Cholesterol, a precursor of bile acids, is converted into bile acids to offset the lost molecules; thereby, lowering overall cholesterol levels. This mechanism contributes to the regulation of serum cholesterol levels through the actions

of colonic microorganisms (10, 13, 15). Similar findings were reported for other *Lb. acidophilus* strains isolated from traditional yogurts, which successfully decreased cholesterol levels in media containing bile salts (13).

Cell surface hydrophobicity (CSH) refers to the non-specific interactions between the bacterial cells and host cells. This characteristic is assessed using solvents such as xylene, chloroform and n-hexane. The CSH is critical for assessing a LAB strain ability to adhere to intestinal cells and proliferate within the host (16). Comparatively, previous studies have shown that strains such as *Levilactobacillus brevis* HL6, isolated from yogurts, have demonstrated significant CSH values (10). Variations in CSH within various LAB species could be attributed to differences in the chemical and structural characteristics of their surfaces, which could include cell wall proteins, hydrophobic amino acids and polysaccharides. Furthermore, environmental factors and growth conditions can affect CSH levels (9, 16, 17).

Presence of antimicrobial activity in *Lb. acidophilus* strain ABC240 plays a critical role in its colonization within the digestive tract through the elimination of competing microorganisms. The LAB can synthesize a range of metabolites with antimicrobial characteristics, including organic acids, hydrogen peroxide and bacteriocins, that inhibit growth of pathogenic bacteria. Studies have verified that *Lactobacillus* strains isolated from dairy products demonstrate antimicrobial activity against various pathogenic bacteria, indicating a potential for these isolates to be used as probiotics (5, 14, 18). Antioxidant activity is another critical characteristic in *Lactobacillus* strains. Recent studies detected that the culture supernatants of several *Lactobacillus* strains showed significant antioxidant activity that was attributed to phenolic and flavonoid compounds. Probiotics can modulate the host oxidation state by chelating metal ions and enhancing antioxidant systems, providing additional health benefits (13, 19).

Safety assessment is essential for probiotics. It generally depends on two mechanisms: natural resistance and acquired resistance through genetic mutation. Antibiotic sensitivity profiles of LAB strains can vary significantly, with several strains showing resistance to certain antibiotics while are sensitive to others (20). This study has shown that *Lb. acidophilus* strain ABC240 shows a desirable safety profile, as evidenced by the absence of hemolytic activity, DNase activity and production of biogenic amines. Biogenic amines, typically formed during the fermentation process, pose safety concerns in food products. Absence of these compounds in *Lb. acidophilus* strain ABC240 further verifies its potential as a safe probiotic strain for use in foods and medicine (21). Recent studies have demonstrated that probiotic bacteria possess both probiotic and antimicrobial properties, effectively combating foodborne pathogens. These beneficial microorganisms not only promote gut health but also can be employed in various food applications to enhance safety and quality. Their ability to inhibit harmful bacteria makes them a valuable resource in the food industry, potentially reducing the risk of foodborne illnesses and improving overall food preservation (22-36).

Conclusion

In conclusion, this study highlights significance of isolating and identifying probiotic strains from traditional fermented dairy products to enhance their nutritional values and health benefits. Successful molecular identification of *Lb. acidophilus* strain ABC240 from traditional yogurts demonstrates its significant probiotic potential, characterized by high stability in acidic and bile salt environments, favorable cell surface hydrophobicity and cholesterol-lowering capabilities. Moreover, its strong antimicrobial characteristics against pathogenic bacteria and antioxidant activity against free radicals underscore its functional benefits. Absence of hemolytic activity, DNase production and biogenic amines further supports safety profile of this strain. Overall, *Lb. acidophilus* strain ABC240 is a promising candidate for incorporation into food products and health uses, contributing to the promotion of traditional dairy consumption and improved consumer health. Furthermore, findings advocate for further traditional fermented dairy products to enhance public health through the integration of probiotics. As urbanization and industrialization continue to affect dietary habits, promoting consumption of traditional yogurts can help restore their nutritional benefits. Demonstrated characteristics of *Lb. acidophilus* strain ABC240 suggested that it could play a critical role in addressing health issues associated to gut microbiome, cholesterol management and prevention of gastrointestinal infections. Future studies should investigate potentials for commercial uses of this probiotic strain in various food matrices, as well as carrying out clinical trials to further assess its health benefits in diverse populations. Incorporation of such strains in dairy products can provide consumers with functional foods that support their overall well-being. *Lb. acidophilus* strain ABC240 represents a valuable contribution to the field of probiotics, illustrating the importance of safeguarding traditional food production methods while including their potentials for modern health solutions. Continued investigation and commercialization of such strains can promote healthier dietary practices, reinforcing the integral role of fermented products in nutrition and wellness. Additionally, it is essential to broader incorporate probiotic strains such as *Lb. acidophilus* strain

ABC240 into diets. This can lead to enhanced consumer awareness about the benefits of probiotics, encouraging healthier eating habits that prioritize fermented foods. Educational initiatives and marketing strategies can further amplify these benefits, making traditional dairy products further appealing to modern consumers, who may favor convenience over nutrition. In conclusion, identification and characterization of *Lb. acidophilus* strain ABC240 help greater understanding of the health benefits associated with traditional fermented dairy products. As research advances, it is critical to investigate innovative formulations and delivery systems that maximize the functional characteristics of such probiotics. By embracing the heritage of traditional yogurts while advancing scientific knowledge, researchers can create a healthier future where the benefits of probiotics are accessible and appreciated by all.

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References

1. FAO. 2019. Climate change and the global dairy cattle sector. United Nations Food and Agriculture Organisation. https://www.fao. org/3/CA2929EN/ca2929en.pdf. Accessed 20 May 2021.
2. Rashwan, A.K., Osman, A.I. and Chen, W., 2023. Natural nutraceuticals for enhancing yogurt properties: A review. *Environmental Chemistry Letters*, *21*(3), pp.1907-1931.
3. García-Burgos, M., Moreno-Fernández, J., Alférez, M.J., Díaz-Castro, J. and López-Aliaga, I., 2020. New perspectives in fermented dairy products and their health relevance. *Journal of Functional Foods*, *72*, p.104059.
4. Savaiano, D.A. and Hutkins, R.W., 2021. Yogurt, cultured fermented milk and health: A systematic review. *Nutrition Reviews*, *79*(5), pp.599-614.
5. Saboktakin-Rizi, M., Alizadeh Behbahani, B., Hojjati, M. and Noshad, M., 2021. Identification of Lactobacillus plantarum TW29-1 isolated from Iranian fermented cereal-dairy product (Yellow Zabol Kashk): probiotic characteristics, antimicrobial activity and safety evaluation. *Journal of Food Measurement and Characterization*, *15*(3), pp.2615-2624.
6. Gao, H., Li, X., Chen, X., Hai, D., Wei, C., Zhang, L. and Li, P., 2022. The functional roles of *Lactobacillus acidophilus* in different physiological and pathological processes. *Journal of Microbiology and Biotechnology*, *32*(10), p.1226.
7. Sornsenee, P., Chatatikun, M., Mitsuwan, W., Kongpol, K., Kooltheat, N., Sohbenalee, S., Pruksaphanrat, S., Mudpan, A. and Romyasamit, C., 2021. Lyophilized cell-free supernatants of Lactobacillus isolates exhibited antibiofilm, antioxidant and reduces nitric oxide activity in lipopolysaccharide-stimulated RAW 264.7 cells. *PeerJ*, *9*.
8. Abd Elmacksood, A., Basha, O., Talat, D., Ahmed, H., & Ibrahim, M. (2021). In Vitro evaluation of antimicrobial activity of *Lactobacillus acidophilus* against some pathogens. *Damanhour Journal of Veterinary Sciences*, *6*(1), 21-28.
9. Barzegar, H., Alizadeh Behbahani, B. and Falah, F., 2021. Safety, probiotic properties, antimicrobial activity and technological performance of Lactobacillus strains isolated from Iranian raw milk cheeses. *Food Science & Nutrition*, *9*(8), pp.4094-4107.
10. Alizadeh Behbahani, B., Jooyandeh, H., Hojjati, M. and Ghodsi Sheikhjan, M., 2024. Evaluation of probiotic, safety and anti-pathogenic properties of *Levilactobacillus brevis* HL6 and its potential application as bio-preservatives in peach juice. *LWT*, 191, p.115601.
11. Hojjati, M., Alizadeh Behbahani, B. and Falah, F., 2020. Aggregation, adherence, anti-adhesion and antagonistic activity properties relating to surface charge of probiotic *Lactobacillus brevis* gp104 against Staphylococcus aureus. *Microbial Pathogenesis*, *147*, p.104420.
12. Alizadeh Behbahani, B., Barzegar, H., Mehrnia, M.A. and Ghodsi Sheikhjan, M., 2023. Probiotic characterization of *Limosilactobacillus fermentum* isolated from local yogurt: Interaction with pathogenic bacteria and Caco-2 enteric cell line. *Nutrition and Food Sciences Research*, *10*(1), pp.37-45.
13. Farid, W., Masud, T., Sohail, A., Ahmad, N., Naqvi, S.S., Khan, S., Ali, A., Khalifa, S.A., Hussain, A., Ali, S. and Saghir, M., 2021. Gastrointestinal transit tolerance, cell surface hydrophobicity and functional attributes of *Lactobacillus acidophilus* strains isolated from Indigenous Dahi. *Food Science & Nutrition*, *9*(9), pp.5092-5102.
14. Bachtarzi, N., Gomri, M.A., Meradji, M., Gil-Cardoso, K., Ortega, N., Chomiciute, G., Del Bas, J.M., López, Q., Martínez, V. and Kharroub, K., 2024. In vitro assessment of biofunctional properties of *Lactiplantibacillus plantarum* strain Jb21-11 and the characterization of its exopolysaccharide. *International Microbiology*, *27*(1), pp.239-256.
15. Horackova, S., Vesela, K., Klojdova, I., Bercikova, M. and Plockova, M., 2020. Bile salt hydrolase activity, growth characteristics and surface properties in *Lactobacillus acidophilus*. *European Food Research and Technology*, *246*, pp.1627-1636.
16. Saboori, B., Shahidi, F., Hedayati, S. and Javadmanesh, A., 2022. Investigating the probiotic properties and antimicrobial activity of lactic acid bacteria isolated from an Iranian fermented dairy product, kashk. *Foods*, *11*(23), p.3904.

17. Sathiyaseelan, A., Saravanakumar, K., Han, K., Naveen, K.V. and Wang, M.H., 2022. Antioxidant and antibacterial effects of potential probiotics isolated from Korean fermented foods. *International Journal of Molecular Sciences*, *23*(17), p.10062.

18. Ghobadi Dana, M., 2019. A survey on the antimicrobial activity of Lactobacillus strains isolated from traditional dairy products in the historical district of Ilam, Iran. *Microbiology, Metabolites and Biotechnology*, *2*(1), pp.15-26.

19. Torshabi, M., Bardouni, M.M. and Hashemi, A., 2023. Evaluation of antioxidant and antibacterial effects of lyophilized cell-free probiotic supernatants of three *Lactobacillus* spp. and their cytocompatibility against periodontal ligament stem cells. *Iranian Journal of Pharmaceutical Research: IJPR*, *22*(1).

20. Lashani, E., Davoodabadi, A. and Soltan Dallal, M.M., 2020. Some probiotic properties of *Lactobacillus* species isolated from honey and their antimicrobial activity against foodborne pathogens. *Veterinary Research Forum : An International Quarterly Journal*, *11*(2), pp.121-126.

21. Turna, N.S., Chung, R. and McIntyre, L., 2024. A review of biogenic amines in fermented foods: Occurrence and health effects. *Heliyon*.

22. Falah, F., Vasiee, A., Behbahani, B.A., Yazdi, F.T., Moradi, S., Mortazavi, S.A. and Roshanak, S., 2019. Evaluation of adherence and anti-infective properties of probiotic *Lactobacillus fermentum* strain 4-17 against Escherichia coli causing urinary tract infection in humans. *Microbial pathogenesis*, 131, pp.246-253.

23. Vasiee, A., Falah, F., Behbahani, B.A. and Tabatabaee-Yazdi, F., 2020. Probiotic characterization of Pediococcus strains isolated from Iranian cereal-dairy fermented product: Interaction with pathogenic bacteria and the enteric cell line Caco-2. *Journal of Bioscience and Bioengineering*, *130*(5), pp.471-479.

24. Vasiee, A., Alizadeh Behbahani, B., Tabatabaei Yazdi, F., Mortazavi, S.A. and Noorbakhsh, H., 2018. Diversity and probiotic potential of lactic acid bacteria isolated from horreh, a traditional Iranian fermented food. *Probiotics and antimicrobial proteins*, *10*, pp.258-268.

25. Vasiee, A.R., Tabatabaei Yazdi, F., Mortazavi, A. and Edalatian, M.R., 2014. Isolation, identification and characterization of probiotic Lactobacilli spp. from Tarkhineh. *International Food Research Journal*, *21*(6).

26. Alebooye, P., Falah, F., Vasiee, A., Yazdi, F.T. and Mortazavi, S.A., 2023. Spent coffee grounds as a potential culture medium for γ-aminobutyric acid (GABA) production by *Levilactobacillus brevis* PML1. *Lwt*, *189*, p.115553.

27. Behbahani, B.A., Jooyandeh, H., Vasiee, A. and Zeraatpisheh, F., 2023. Evaluation of anti-yeast metabolites produced by Lactobacillus strains and their potential application as bio-preservatives in traditional yogurt drink. *LWT*, *188*, p.115428.

28. Vasiee, A.R., Mortazavi, A., Tabatabaei-yazdi, F. and Dovom, M.R., 2018. Detection, identification and phylogenetic analysis of lactic acid bacteria isolated from Tarkhineh, Iranian fermented cereal product, by amplifying the 16s rRNA gene with universal primers and differentiation using rep-PCR. *International Food Research Journal*, *25*(1).

29. Vasiee, A., Falah, F., Sankian, M., Tabatabaei-Yazdi, F. and Mortazavi, S.A., 2020. Oral immunotherapy using probiotic ice cream containing recombinant food-grade Lactococcus lactis which inhibited allergic responses in a BALB/c mouse model. *Journal of Immunology Research*, *2020*(1), p.2635230.

30. Falah, F., Zareie, Z., Vasiee, A., Tabatabaee Yazdi, F., Mortazavi, S.A. and Alizadeh Behbahani, B., 2021. Production of synbiotic ice-creams with *Lactobacillus brevis* PML1 and inulin: functional characteristics, probiotic viability, and sensory properties. *Journal of Food Measurement and Characterization*, *15*(6), pp.5537-5546.

31. Falah, F., Vasiee, A., Tabatabaei-Yazdi, F., Moradi, S. and Sabahi, S., 2024. Optimization of γ-aminobutyric acid (GABA) production by Lactobacillus spp. from agro-food waste. *Biomass Conversion and Biorefinery*, *14*(3), pp.3425-3437.

32. Falah, F., Vasiee, A., Alizadeh Behbahani, B., Tabatabaee Yazdi, F. and Mortazavi, S.A., 2021. Optimization of gamma-aminobutyric acid production by Lactobacillus brevis PML1 in dairy sludge-based culture medium through response surface methodology. *Food science & nutrition*, *9*(6), pp.3317-3326.

33. Vasiee, A., Falah, F. and Mortazavi, S.A., 2022. Evaluation of probiotic potential of autochthonous lactobacilli strains isolated from Zabuli yellow kashk, an Iranian dairy product. *Journal of Applied Microbiology*, *133*(5), pp.3201-3214.

34. Vasiee, A., Mortazavi, S.A., Sankian, M., Yazdi, F.T., Mahmoudi, M. and Shahidi, F., 2019. Antagonistic activity of recombinant Lactococcus lactis NZ1330 on the adhesion properties of Escherichia coli causing urinary tract infection. *Microbial Pathogenesis*, *133*, p.103547.

35. Falah, F., Vasiee, A., Yazdi, F.T. and Behbahani, B.A., 2021. Preparation and functional properties of synbiotic yogurt fermented with Lactobacillus brevis pml1 derived from a fermented cereal-dairy product. *BioMed research international*, *2021*(1), p.1057531.

36. Ghazanfari, N., Fallah, S., Vasiee, A. and Yazdi, F.T., 2023. Optimization of fermentation culture medium containing food waste for l-glutamate production using native lactic acid bacteria and comparison with industrial strain. *LWT*, *184*, p.114871.