Nutrition and Food Sciences Research Vol 10, No 1, Jan-Mar 2023, pages: 37-45

Original Article

Probiotic Characterization of *Limosilactobacillus fermentum* Isolated from Local Yogurt: Interaction with Pathogenic Bacteria and Caco-2 Enteric Cell Line

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Received: August 2023 **Accepted:** September 2023

ABSTRACT

Background and Objectives: The term "probiotic," which is relatively novel and means "for life," is typically used to refer to bacteria that include positive effects on humans and animals. In this study, probiotic characteristics of *Limosilactobacillus fermentum* IMAU70160, isolated from local yogurt were investigated.

Materials and Methods: In this study, molecular identification of a strain isolated from local yogurt was carried out by 16S rRNA gene analysis using 27FYM and 1492R universal primers. Probiotic potential of *L. fermentum* IMAU70160 was carried out.

Results: Strain was resistant to low pH, bile salts and simulated gastrointestinal conditions. The strain cell surface hydrophobicity, auto-aggregation, co-aggregation, adhesion capacity, anti-adhesion effect, antioxidant activity and cholesterol removal ability were 44.49, 33.29, 20.11, 10.30, 19.16–42.40, 48.40 and 43.20%, respectively. *Staphylococcus aureus* and *Escherichia coli* were the most sensitive and the most resistant bacterial species to cell-free supernatant (CFS) of *L. fermentum* IMAU70160, respectively. The two aCFS and nCFS were capable of inhibiting growth of pathogens. In general, aCFS included a greater antimicrobial activity than that nCFS did. Similar results were observed in the modified double-layer method. It showed no hemolytic or DNase activity and was unable to generate biogenic amines. The strain was greatly sensitive to imipenem (22.10 mm), chloramphenicol (22.80 mm), nitrofurantoin (24.50 mm) and ciprofloxacin (28.20 mm) with inhibition zones.

Conclusions: Further studies are needed to verify biological and functional characteristics of *L. fermentum* IMAU70160-loaded food products.

Keywords: *Limosilactobacillus fermentum*, Antioxidant activity, Caco-2 cells, Hydrophobicity

1. Introduction

Over the past two decades, probiotic development has indicated significant improvements in the food industry. As a result of factors such as existing scientific and clinical discoveries made with well-researched probiotic microorganisms, number of scientific publications on probiotics has significantly increased (1–8). The term "probiotic," which is relatively novel and means "for life," is typically used to refer to bacteria that include positive effects on humans and animals. Probiotics are live microorganisms that when consumed in certain quantities include health benefits beyond those of basic nutrition (9–14). This needs that the microorganisms must be active and present in large quantities-generally more than 10^9 cells per daily dose-to be effective. Each product should specify daily doses needed to provide desired health benefits (15, 16). Most of the strains of probiotic bacteria belong to the Genera *Lactobacillus* and *Bifidobacterium*. Probiotic strains with the following ideal characteristics can be used in the probiotic industry, benefiting human health. They are resistant to acid and bile, adhere to human epithelial cells, colonize the human intestine and produce bacteriocins, which are antimicrobial substances (2,3,17)

Lactic acid bacteria (LAB) can improve food nutrition, help in lactose digestion, prevent cancers, inhibit intestinal infections and control serum cholesterol levels. One of the most common subgroups of the LAB family is *Lactobacillus* genus, which shares traits with other members of the LAB family such as facultative anaerobicity, non-spore

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formation, rod shape, Gram positivity, acid resistance and catalase negativity. The ideal growth temperature for these microorganisms is 30–40 °C (18–20). Of the *Lactobacillus* species, *L. fermentum* strains have been shown to include probiotic effects because of their capacity to improve gut microbiota composition, modulate the intestinal immune system, decrease blood cholesterol, trigger release of immunoglobulin A, boost activity of antioxidant enzymes and lessen intestinal inflammation (21).

The objective of this study was to assess potential of *L. fermentum* IMAU70160, isolated from Iranian Tashan yogurt, as a probiotic. Assessment included analyzing various factors such as pH and bile resistance, as well as strain physicochemical characteristics such as hydrophobicity, auto-aggregation and co-aggregation. Additionally, the current study investigated the strain cholesterol removal ability, hydroxyl radical scavenging activity, adhesion capacity to Caco-2 cell monolayers and adhesion competition with *S. aureus* through competition, inhibition and replacement assays. Furthermore, the study explored DNase activity, haemolytic activity, biogenic amine production and antibiotic susceptibility of the strain. The aim of these comprehensive investigations was to provide insights into the probiotic potential and safety profile of *L. fermentum* IMAU70160.

2. Materials and Methods

2.1. Chemicals

The PCR and Genomic DNA Isolation VI kits were purchased from Parstous Biotech, Mashhad, Iran, and Asian Dena-Zist, Mashhad, Iran, respectively. Peptone water, Muller-Hinton agar and de Man-Rogosa-Sharpe (MRS) agar and broth were purchased from Merck, Darmstadt, Germany. Standard antibiotics (nitrofurantoin, chloramphenicol, imipenem, erythromycin, ampicillin, ciprofloxacin and nalidixic acid), trypsin-EDTA, pepsin, bile salt, trypsin, Triton X-100, Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS) and penicillin-streptomycin (10,000 U/mL) were purchased from Sigma-Aldrich, USA. Moreover, DNase media were purchased from HiMedia, Mumbai, India.

2.2. Isolation and identification of the strain

Method of Saboktakin-Rizi et al. (2021) was used to isolate and identify the probiotic strain (18). Samples of yogurt were randomly collected from a local market in Tarshan, Iran, and stored refrigerated until analysis. The serial dilutions (10^{-1} to 10^{-6}) were prepared and cultured on MRS agar after homogenizing samples in peptone water. The strain was isolated from the culture media and its catalase and Gram-staining abilities were assessed. Genomic DNA of the strain was extracted using Genomic DNA Isolation VI kit, followed by overnight culturing in MRS broth. For the amplification of 16S rRNA genes, universal primers of 27FYM (5′-AGAGTTTGATYMTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) were used. The PCR reaction mixture consisted of 25.15 µl, which included 2 µl of dNTP, 16.5 µl of water, 1.25 µl of each primer, 2.5 µl of 10× buffer, 1.5 µl of the DNA template, 1.2 µl of $MgCl_2$ and 0.2 µl of Taq polymerase. The PCR conditions were as follows (i) initial denaturation cycle at 95 °C for 5 min, (ii) 35 cycles of 94 °C for 30 s, 54 °C for 30 s and 72 °C for 2 min and (iii) a final elongation cycle at 72 °C for 10 min. To verify amplification of the targets, PCR products were electrophoresed on agarose gels at 95 V for 45 min. (18). The resulting nucleotide were sequenced to investigate their homology. An isolate with catalase-negative and Gram-positive characteristics belonged to *L. fermentum* IMAU70160 with 99% similarity.

2.3. Tolerance assay

Acid, bile salt and simulated gastrointestinal tract (GIT) juice tolerance assessments were carried out as described by Mousanejadi et al. (2023) with some modification (22). Strain (10^8 CFU/mL) was suspended in phosphate buffer (pH 2, 3 and 4) and incubated at 37 °C for 0, 1, 2 and 3 h. Number of the viable cells was counted in logarithmic colony forming units (log CFU/mL). For bile salt stability study, microbial suspensions were cultured using MRS agar with various bile salt concentrations (0.1, 0.4, 0.7 and 1% w/v). Results were visually observed after 24 h of incubation at 37 °C.

In the simulated GIT fluid assessment, 9 mL of the simulated gastric fluid (3 g/l pepsin, 7 mM KCl, 125 mM NaCl and 45 mM NaHCO3; pH 2.5) were added to 1 mL of the strain (9 log CFU/mL). Suspension was incubated at 37 °C for 3 h and centrifuged at 3800 rpm for 10 min. Then, supernatant was removed and the pellet washed with phosphate buffer. Pellet was redissolved in 1 mL of the simulated intestinal fluid (pH 8), containing 0.15% of bile salts and 0.1% of pancreatin, and the suspension was incubated at 37 °C for 3 h. Viable bacteria were counted after incubation and expressed as log CFU/mL (22).

2.4. Cell surface hydrophobicity

The isolate cell surface hydrophobicity was assessed using procedure by Kumari et al. (2022) (23). One milliliter of xylene was added to a total of 3 mL of 10^8 CFU/mL cell suspensions and then vortexed for 2 min. To easily separate the two phases, it was set at 37 °C for 2 h. The aqueous phase absorbance (600 nm) was then calculated (A).

$$\text{Surface hydrophobicity (\%)} = \left(\frac{\text{Initial absorbance - Final absorbance}}{\text{Initial absorbance}}\right) \times 100$$

2.5. Aggregation characteristics

Auto-aggregation and co-aggregation characteristics of *L. fermentum* were assessed based on the methods by Zareie et al. (2023) with minor modifications (24). To prepare bacterial suspension, vortex was used to thoroughly mix a

suspension of 10^8 CFU/mL. Absorbance of the suspension was measured at 600 nm and this initial absorbance value was recorded as Ai. After incubating the suspension at 37 °C for 2 h, supernatant was collected and its absorbance was measured at a similar wavelength (600 nm). This absorbance was recorded as A2h after 2 h of incubation. The following formula was used to calculate the auto-aggregation coefficient:

$$\text{Auto-aggregation (\%)} = \left(1 - \frac{A_{2h}}{A_i}\right) \times 100$$

After incubation at 0 and 5 h, absorbance values of the probiotic strain (A_x), pathogenic strain (A_y) and mixture of probiotic and pathogenic strains (A_{x+y}) were measured at 600 nm to assess the co-aggregation of *L. fermentum* and *S. aureus*:

$$\text{Co-aggregation (\%)} = [(A_x + A_y) / 2 - A_{x+y}] / [(A_x + A_y)/2] \times 100$$

2.6. Adhesion capacity

Procedure described by Vasiee et al. (2020) was used to assess adhesion ability of *L. fermentum* (25). Cells were cultured at 37 °C in a humidified atmosphere of 5 °C carbon dioxide (CO_2) using DMEM (1% penicillin-streptomycin and 10% heat-inactivated FBS). Caco-2 cells were trypsinized with 1% trypsin-EDTA when they were 80% confluent and then transferred to 6-well tissue plates at a density of 30,000 cells/cm^2. Up the formation of a differentiated cell monolayer, cells were incubated and media were frequently refreshed. The old media, particularly penicillin-streptomycin, was then removed from the wells using sterile phosphate buffer. The overnight bacterial culture was centrifuged (6,000 rpm, 10 min) and the pellet washed twice with cold phosphate buffer. Suspension of the bacteria was added to confluent Caco-2 monolayers as a suspension in DMEM at a concentration of 10^8 bacteria/mL. After incubation (1 h, 37 °C, 95% air/5% CO_2), unbounded bacteria were removed from the plates by sequentially emptying and washing the wells with phosphate buffer. The Caco-2 cells were lysed using Triton X-100 (0.1% v/v) after incubating for 10 min and counts of bacteria on MRS agar were reported. Adhesion capacity of the strain was assessed using the following formula:

$$\text{Adhesion capacity (\%)} = \left(\frac{A}{B}\right) \times 100$$

Where, A was the number of bacteria that adhered and B was all the bacteria that incorporated into the wells.

2.7. Anti-adhesion activity

Competition, inhibition and replacement studies were carried out to assess *L. fermentum* anti-adhesion characteristics against *S. aureus* adhesion to intestinal cells based on the authors' previous study (22).

2.8. Antibacterial activity

Antibacterial efficacy of *L. fermentum* against a variety of food-borne pathogenic bacteria such as *Escherichia coli*, *S. aureus*, *S. epidermidis*, *Salmonella enterica* serovar Typhi, *Bacillus subtilis*, *Enterobacter aerogenes* and *Listeria monocytogenes* was assessed.

2.9. Disc diffusion agar and well diffusion agar methods

Strain was cultured using MRS broth media (28 h, 37 °C), followed by centrifugation at 5,000 rpm for 20 min at 4 °C. To neutralize acidic effects of the organic acids, pH of half of the cell-free supernatant (CFS) was set intact while pH of the other half was shifted to 5.5. Mixtures were filtered (0.22 µm) and then freeze-dried to achieve neutralized and acid CFSs (nCFSs and aCFSs). The CFS fractions were hydrated in 2 mL of sterile distilled water (DW) before using in disc diffusion agar and well diffusion agar methods. Inhibition zones surrounding the discs/wells were measured after 48 h of incubation at 37 °C (22).

2.10. Modified double-layer method

Antimicrobial activity of the isolate was assessed using modified agar overlay. After culturing the isolate in MRS broth, it was used as an inoculum onto the MRS agar plates. These plates were then incubated for 24 h under microaerophilic conditions at 37 °C. Following the incubation, a broth culture of the pathogens was prepared and 10 mL of the nutrient soft agar (0.8% agar) were inoculated with the pathogens. This agar mixture was layered onto the pre-incubated MRS agar plates. Plates were incubated at 37 °C for 24 h. After the incubation, zones of inhibition were measured to assess inhibitory effects of the isolate on the pathogens (24).

2.11. Assimilation of cholesterol

Ability of *L. fermentum* to assimilate cholesterol was investigated using method of Pereira and Gibson (2002) (26). Briefly, polyoxyethanyl-cholesteryl sebacate, a water-soluble form of cholesterol, and oxgall at a concentration of 0.3% were added to the MRS broth. Thus, 100 mg/l of cholesterol were available in the media. In triplicate, the culture was inoculated at 1% (v/v) and incubated in anaerobic jars at 30 °C for 24 h. Sterile broth that was been uninoculated served as the control.

2.12. Antioxidant activity

Scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was assessed as described by Yang et al. (2020) with some modifications (27). Two milliliters of methanolic DPPH solution (0.14 mM) and 2 mL of the bacterial sample were mixed to assess the strain antioxidant capacity. This was incubated at 37 °C for 30 min in dark. By measuring absorbance of the supernatant at 517 nm and using the following equation, DPPH radical scavenging activity of the isolate was calculated:

$$\text{Antioxidant activity (\%)} = \left(1 - \frac{\text{Abs sample}}{\text{Abs control}}\right) \times 100$$

2.13. Antibiotic susceptibility

To assess antibiotic resistance of the isolate, agar overlay diffusion method was used. First, a bacterial suspension containing 10^6–10^7 cells/mL was prepared. A volume of 200 µl of this suspension was mixed with 4 mL of soft agar (0.8% w/v) and spread on a 15-mL MRS agar plate. Then, antibiotic discs were placed on the culture, covering a range of routine antibiotics of nitrofurantoin, chloramphenicol, imipenem, erythromycin, ampicillin, ciprofloxacin and nalidixic acid. Plates were incubated at 37 °C for 24 h. After the incubation, diameters of the zones of inhibition around the antibiotic disc were measured. Based on the measurements, isolate susceptibility to the antibiotics was classified into three categories of resistant (< 10.5 mm), intermediate (10.5–20.5 mm) and sensitive (> 20.5 mm) (24).

2.14. DNase activity

The DNase activity of *L. fermentum* was assessed using DNase media at 37 °C for 48 h. The presence of a clear pink zone around the colony indicated DNase activity of the strain (25).

2.15. Hemolytic activity

To assess hemolytic activity, method described by Pino et al. (2019) (28) was used. Cell cultures were plated overnight on blood agar plates that contained 5% of sheep blood. After incubating plates at 37 °C for 48 h, hemolytic activity was assessed visually. Colonies were investigated for the presence of clearing zones, green halos or colorless appearance around them. Based on the observations, the bacterial hemolytic activity was categorized as follows: 1) β-hemolysis, clearing zones around the colonies; 2) α-hemolysis, green halos around the colonies; and 3) γ-hemolysis, no changes in color around the colonies (colorlessness).

2.16. Biogenic amine production

By adding 0.5% (w/v) of the amino acid precursors of L-ornithine, L-tyrosine and L-histidine to MRS agar plates containing 0.06% of bromocresol purple, productions of putrescine, tyramine and histamine were assessed. Purple color suggested that biogenic amines were produced (29).

2.17. Statistical analysis

Using Minitab software v.19, analysis of variance (ANOVA) was carried out. To ensure differences between the means, Duncan test was used with a 95% level of confidence ($p < 0.05$). All the experiments were repeated three times.

3. Results

3.1. Tolerance to acid, bile salt and simulated GIT juices

As shown in Fig. 1, cell viability of the isolate depended on the pH value and exposure time. Cell viability of the isolate significantly decreased as pH value decreased from 4 to 2 and exposure time increased from 0 to 3 h. However, cell viability was not affected significantly by pH 4. Viability of the isolate cells was affected by the GIT media and decreased from 9.24 to 6.53 log CFU/mL under such conditions. Viability of isolate cells decreased as a function of bile salt concentration (Table 1); however, isolate included high survivability at 0.1–0.4% bile salt levels.

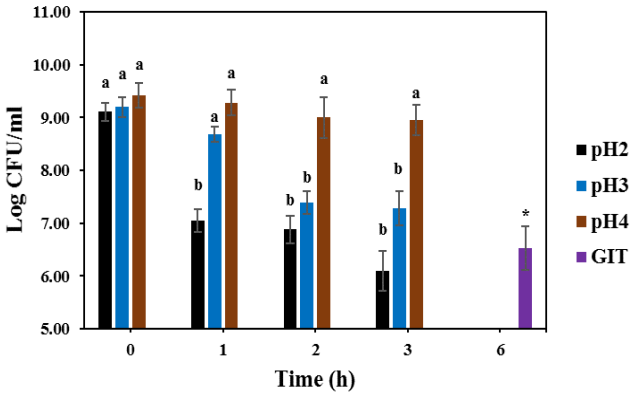

Figure 1. Survivability of *L. fermentum* IMAU70160 under acidic pH and simulated gastric and intestinal fluids.
Means with different superscript letters differ significantly ($p < 0.05$).
*This sample was not included in the statistical analysis.

Table 1. Survivability of *L. fermentum* IMAU70160 under various bile salt concentrations

	0.1%	0.4%	0.7%	1%
Survivability	++++	+++	++	+

+ Low survivability
++ Moderate survivability
+++ High survivability
++++ Very high survivability

3. 2. Cell surface hydrophobicity, auto-aggregation, co-aggregation and adhesion capacity

The probiotic cell capacity to adhere to epithelial cell surfaces naturally depends on their surface hydrophobicity. The cell surface hydrophobicity of the strain was 44.49% ±0.29 (Fig. 2). Auto-aggregation capacity of the strain was 33.29% ±0.25 (Fig. 2). Co-aggregation of the pathogens and strains might express moderate co-aggregation capacity and the isolate showed a co-aggregation of 20.11% ±0.47 (Fig. 2). Ability of the probiotics to adhere, at least momentarily, to the cells of the epithelia could be addressed when choosing them. It was found that the acidophilus microorganism included an 10.30% adhesion capacity to Caco-2 cells (Fig. 2).

3.3. Anti-adhesion characteristics

Figure 3 shows anti-adhesion ability of the isolate against *S. aureus* adhesion to Caco-2 cells. As seen in the figure, anti-adhesion effects of *L. fermentum* IMAU70160 included

42.40, 36.19 and 19.16% for competition, inhibition and displacement anti-adhesion methods, respectively.

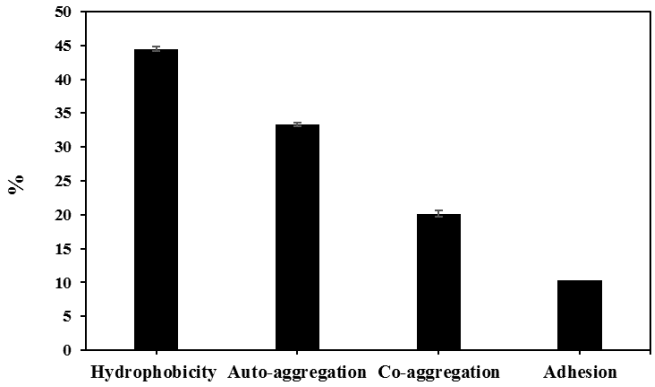

Figure 2. Hydrophobicity, co-aggregation, auto-aggregation and adhesion ability of *L. fermentum* IMAU70160.

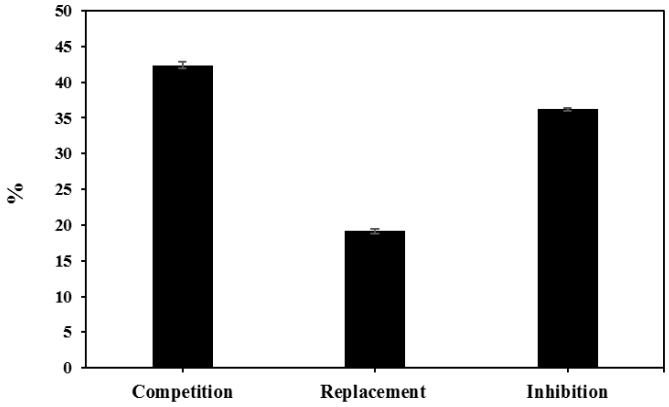

Figure 3. Anti-adhesion capacity of *L. fermentum* IMAU70160 based on competition, replacement and inhibition assays.

3.4. Antibacterial effects

Figure 4 illustrates antimicrobial activity of the aCFS and nCFS fractions of *L. fermentum* against pathogenic bacteria using disc and well diffusion agar methods. The aCFS and nCFS were capable of inhibiting growth of the pathogens. In general, aCFS included a greater antimicrobial activity than that nCFS did and *S. aureus* and *E. coli* were the most sensitive and the most resistant bacteria to CFSs. Similar results were observed in the modified double-layer method (Fig. 5).

3.5. Safety assessment

The *L. fermentum* IMAU70160 showed no hemolytic or DNase activity and was unable to generate biogenic amines. Therefore, it might be addressed as a safe strain for health promotion uses. Moreover, strain was resistant to nalidixic acid (10.10 mm), semi-sensitive to ampicillin (11.90 mm) and erythromycin (19.40 mm) and sensitive to imipenem (22.10 mm), ciprofloxacin (28.20 mm), chloramphenicol (22.80 mm) and nitrofurantoin (24.50 mm) (Fig. 6).

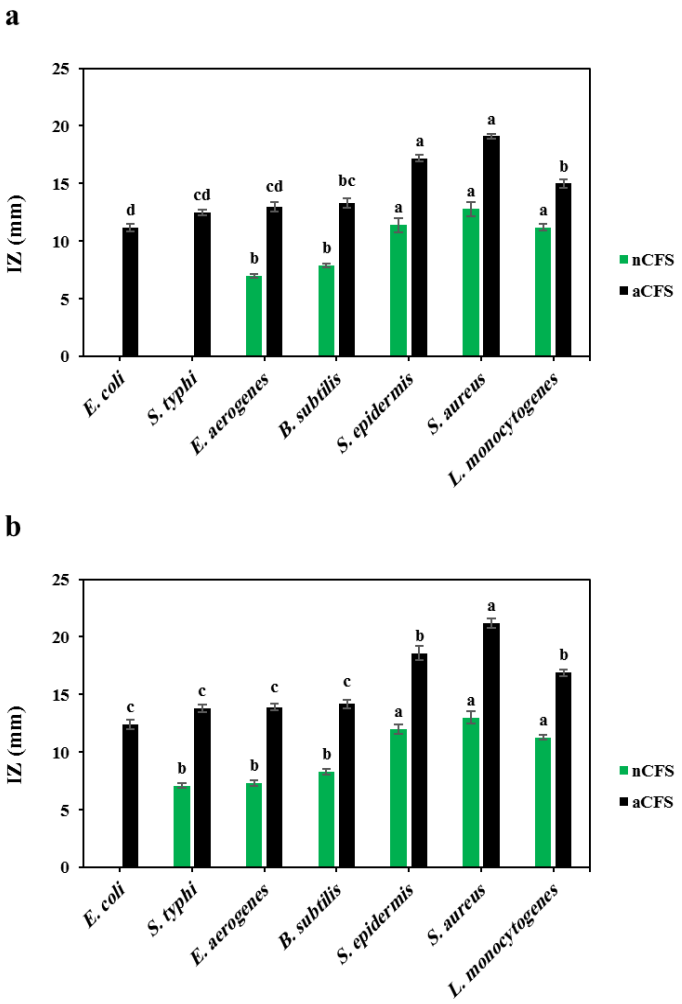

Figure 4. Antimicrobial activity of *L. fermentum* IMAU70160 based on disk diffusion agar (a) and well diffusion agar (b) methods. aCFS, acid cell-free supernatant; and nCFS, neutralized cell-free supernatant. Means with different superscript letters differ significantly ($p < 0.05$).

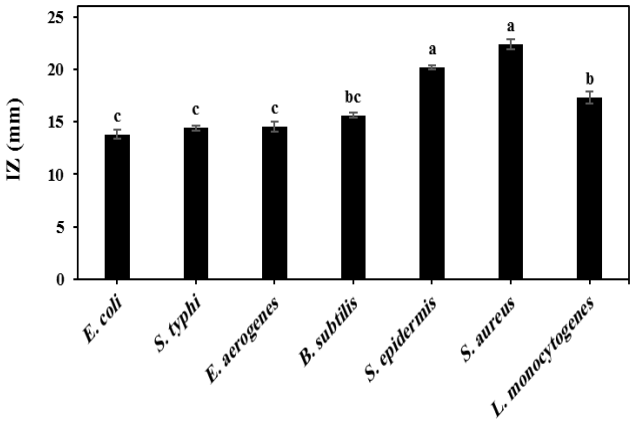

Figure 5. Antimicrobial activity of *L. fermentum* IMAU70160 based on the modified double-layer method. Means with different superscript letters differ significantly ($p < 0.05$).

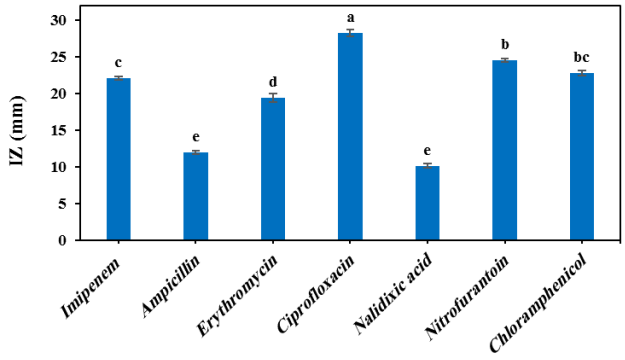

Figure 6. Effects of common antibiotics on growth of *L. fermentum* IMAU70160. Means with different superscript letters differ significantly ($p < 0.05$).

3. 6. Cholesterol assimilation and antioxidant activity

The isolate cholesterol assimilation activity was reported as 43.20% ±0.47. The antioxidant activity of *L. fermentum* was recorded as 48.40% ±0.53.

4. Discussion

By measuring resistance of the bacteria to bile salts and acidic pH, it was possible to predict the bacterial survival in the GIT and selecting isolates with probiotic qualities needing this quality (30, 31). Based on the reports, F_1F_0-ATPase can protect Gram-positive bacteria (e.g., LAB) from the harmful effects of acidic environments by increasing the intracellular pH. This decreases the lethal effects caused by acidic conditions (32, 33). Because of their ability to convert bile salts into cholesterol and amino acids, probiotic strains include the potential of resistance to bile salts (34). The acid/bile tolerance of *L. fermentum* KGC1601 has been reported by Kim et al. (2022) (35).

The *L. fermentum* strains derived from piglet feces have been detected to include hydrophobicity levels ranging 53.90–90.16% (36). Probiotics and intestinal epithelial cells may first come into contact with one another more easily if cell surfaces are hydrophobic (37). Additionally, hydrophobicity affects bacterial auto-aggregation (38). Auto-aggregation and co-aggregation abilities of *L. fermentum* strains have been reported in the literature (23, 36, 39). Probiotics help prevent pathogen colonization due to their hydrophobicity, co-aggregation and auto-aggregation characteristics. Hydrophobicity and auto-aggregation of the colonies of similar bacterial groups allow microorganisms to adhere to the intestinal layers (24). Co-aggregation is cell-to-cell adhesion ability of various strains; hence, probiotic microorganisms can adhere to pathogens via cell-to-cell manners (23).

Adhesiveness of *L. fermentum* was strain dependent, with < 2 and 30% reported values (40, 41). The probiotic capacity for *in-vitro* adhesion and their stability/colonization in the GIT have directly been correlated. Glyceraldehyde-3-phosphate dehydrogenase, S-layer, lipoteichoic acid and lectin-like proteins can all help probiotics adhere to intestinal receptor cells. The immune system might be strengthened as a result of probiotic cells subsequently colonizing the intestine (18).

Prior to colonization of the host's gut cells and releasing toxins to cause infections, pathogenic bacteria must first adhere to those cells. By blocking receptors and secreting antimicrobial substances, probiotics include the potential to lessen attachment of the pathogenic bacteria (42). Potential anti-adhesive effects of *L. fermentum* and other LAB on the pathogen adhesion to the Caco-2 cells have been reported in the literature (43, 44). The probiotics ability to produce antimicrobial compounds or the competition for nutrients and receptors between the probiotics and pathogens might be responsible for the probiotics anti-adhesive effects (44).

Test cultures of the antibiotic-resistant Gram-positive and Gram-negative pathogens have been used to assess antibacterial characteristics of *L. fermentum* strain 3872. Technically, *S. aureus* strain 8325-4 and *S. aureus* strain IIE CI-SA 1246 were two Gram-positive pathogens that were extremely sensitive to the strain bacteriolytic action. In comparison to Gram-positive pathogens, Gram-negative pathogens (e.g., *E. coli*, *Salmonella* spp. and *Campylobacter jejuni*) were further resistant to the bacteriolytic action of *L. fermentum* 3872 (45). The LAB-produced antimicrobial compounds are highly efficient as a barrier against pathogens and food spoilage by the bacteria. Nutrient competition and production of organic acids (lactic and acetic acids), proteinaceous compounds, diacetyl, fatty acids, hydrogen peroxide, bacteriocins and bactericidal proteins are a few factors that contribute to the antimicrobial activity of LAB (24). While hydroxyl fatty acids, organic acids and hydrogen peroxide include greater antimicrobial activities against the Gram-negative pathogens, bacteriocins are further effective against the growth of Gram-positive pathogens (46).

The *L. fermentum* IMAU70160 showed no hemolytic or DNase activity and was unable to generate biogenic amines; similar to a study by Kumari et al. (2022) (23). Similarly, *L. fermentum* isolates showed sensitivity to a variety of antibiotics, including erythromycin, streptomycin, gentamicin, ampicillin, clindamycin, tetracycline and azithromycin (23). Although LAB include a long reliable history of use in the production of fermented foods and beverages, recent research have indicated that when used in starter cultures or co-cultures, these bacteria may carry genes of antibiotic resistance. Presence of intrinsic resistance genes on the chromosome, which are not transferable and are present in LAB species, can be addressed (24, 47, 48).

Potential of *L. fermentum* strains in decreasing cholesterol has been reported by Palaniyandi et al. (2020)

(49) and Pan et al. (2011) (50). Mechanisms of bile salt hydrolase, cholesterol-to-coprostanol conversion, cholesterol coprecipitation with deconjugated biles, cholesterol binding to probiotic cellular surfaces and incorporation of cholesterol into their cell membranes are the mechanisms; by which, probiotics can contribute to decreasing cholesterol levels. These mechanisms act together to decrease cholesterol absorption, increase cholesterol excretion and prevent cholesterol from entering the bloodstream (26, 51).

Similarly, ABTS radical scavenging and ferric reducing ability of *L. fermentum* FTL2311 and *L. fermentum* FTL10BR have been reported by Klayraung and Okonogi (2009) (52). Numerous studies have demonstrated presence of antioxidative characteristics in specific strains of lactobacilli and bifidobacteria (53–55). These strains can be used to produce probiotic dairy products and fermented foods that enhance antioxidant status and decrease oxidative stress agents in healthy people. Antioxidant effects of the probiotics may result from ascorbate autoxidation reduction, metal ion chelation, reactive oxygen species (RO_X) scavenging and enzyme inhibition (56, 57).

Conclusion

In this study, probiotic status of *L. fermentum* IMAU70160 was verified. Strain was resistant to low pH, bile salts and simulated gastrointestinal conditions. The strain cell surface hydrophobicity, auto-aggregation, co-aggregation, adhesion capacity, anti-adhesion effect, antioxidant activity and cholesterol removal ability were reported as 44.49, 33.29, 20.11, 10.30, 19.16–42.40, 48.40 and 43.20%, respectively. Furthermore, *S. aureus* and *E. coli* were the most sensitive and the most resistant bacterial species to cell-free supernatants of *L. fermentum* IMAU70160, respectively. The bacterial strain showed no hemolytic or DNase activity and was unable to generate biogenic amines. To verify biological and functional characteristics of *L. fermentum* IMAU70160-loaded food products, further studies are necessary.

Acknowledgement

The authors would like to express their sincere gratitude to Vice-chancellor for Research and Technology, Agricultural Sciences and Natural Resources University of Khuzestan, for supporting this study (project no. 1400.35).

Financial disclosure

The authors declared no financial interest.

Ethical approval

This article does not contain any studies with human or animal subjects.

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