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*Original Article*

# *Limosilactobacillus fermentum* RSB9: Isolation and Identification, Viability in Acidic and Bile Conditions, Antimicrobial Activity, Antioxidant Power, and Safety Properties

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## ABSTRACT

**Background and Objectives:** Probiotics are an important group of living microorganisms that improve the host's metabolism, strengthen the immune system, modulate the intestinal microbiota, and demonstrate its beneficial effects in the host. Therefore, the present study was conducted with the aim of molecular identification of *Limosilactobacillus fermentum* RSB9 isolated from traditional yogurt and evaluation of its viability in acidic and bile conditions, antimicrobial activity, antioxidant power, and safety properties.

**Materials and Methods:** The strain's identification involved the utilization of the polymerase chain reaction (PCR) method. The viability of the strain was assessed in both acidic conditions (with pH values of 3, 4, and 5) and bile conditions (at concentrations of 0.3%, 0.5%, and 0.7%). Further evaluations included antimicrobial activity utilizing the well diffusion agar and disc diffusion agar methods, determination of antioxidant power through ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) and DPPH (2,2-Diphenyl-1-picrylhydrazyl), analysis of cell surface hydrophobicity, cholesterol absorption capabilities, and safety aspects such as antibiotic sensitivity, hemolytic activity, DNase activity, and biogenic amine (BAs) production.

**Results:** The findings of this research show the high stability of this strain against different concentrations of acid (pH values of 3, 4, and 5) and bile salts (0.3%, 0.5%, and 0.7%). The surface hydrophobicity of *L. fermentum* RSB9 was measured to be 54.09 ± 0.40%, with its ability to absorb cholesterol measured as 43.60 ± 0.45%. Additionally, the antioxidant activity of this isolate using DPPH and ABTS methods was determined to be 48.90% ± 0.57 and 52.50% ± 0.43, respectively, demonstrating a high potential for inhibiting free radicals. The antimicrobial potential of *L. fermentum* RSB9 was evaluated using agar diffusion methods with wells and disc diffusion in agar, revealing strong antimicrobial activity against the investigated pathogenic species. This bacterium was also found to be sensitive to most of the tested antibiotics. *L. fermentum* RSB9 did not exhibit any hemolytic or DNase activity and was unable to produce biogenic amines (BAs).

**Conclusions:** Therefore, the study confirmed the probiotic potential of Limousin *L. fermentum* RSB9, highlighting its stability against different concentrations of acid and bile salts, acceptable cell surface hydrophobicity, cholesterol reduction ability, free radical inhibition, antimicrobial activity, lack of hemolytic or DNase activity, and absence of biogenic amine production. These findings support the introduction of *L. fermentum* RSB9 as a new probiotic strain with suitable biological and functional characteristics.

**Keywords:** Probiotic properties, Antioxidant activity, Safety evaluation, *Limosilactobacillus fermentum* RSB9



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## Highlights

- The antimicrobial potential of *Limosilactobacillus fermentum* RSB9 was evaluated using agar diffusion methods with wells and disc diffusion in agar, revealing strong antimicrobial activity against the investigated pathogenic species.
- *L. fermentum* RSB9 did not exhibit any hemolytic or DNase activity and was unable to produce biogenic amines.
- The antioxidant activity of *L. fermentum* RSB9 using DPPH and ABTS methods was determined to be 48.90% ± 0.57 and 52.50% ± 0.43, respectively, demonstrating a high potential for inhibiting free radicals.
- The surface hydrophobicity of *L. fermentum* RSB9 was measured to be 54.09 ± 0.40%, with its ability to absorb cholesterol measured as 43.60 ± 0.45%.

## Introduction

Beneficial nutrients fermented dairy products containing probiotics are important sources of nutritional compounds, the consumption of which promotes health by creating an environment that protects against various diseases. Plants and the treatment of foodborne diseases require interdisciplinary methods that use beneficial living microorganisms (probiotics) in order to reduce the number of pathogenic microorganisms and their associated health risks. Recently, due to consumers' preference for food products with fewer chemical preservatives, the use of beneficial microorganisms to preserve nutrients has increased. On the other hand, animals have been given preventive doses of antibiotics for several decades, resulting in the creation of drug resistance in both human and animal hosts, multiple antibiotic resistance in pathogenic microorganisms, and potentially harmful treatment with drug residues. Probiotics offer an alternative solution to reduce such problems and have a positive impact on health (1, 2).

In general, probiotics are an essential group of living microorganisms that improve the host's metabolism, strengthen the immune system, modulate the intestinal microbiota, and provide various benefits to the host. These benefits include reducing symptoms of intestinal diseases, irritable bowel syndrome, constipation, acute antibiotic-associated diarrhea (AAD), high blood pressure, restoring and maintaining balance in intestinal flora homeostasis, enhancing defense functions of the body, increasing levels of folate, niacin, and riboflavin in the diet, improving lactose intolerance, reducing allergic reactions, controlling blood cholesterol levels, and more (2 and 3).

Lactic acid bacteria (LAB), as protective cultures, are common probiotic microorganisms known for their safety and specific characteristics. Within the Bacillota branch, there are about 20 distinct genera, including Lactobacillus, Lactococcus, Leuconostoc, Streptococcus, Enterococcus, Pediococcus, Tetragenococcus, and Vagococcus. Lactobacillus, the most well-known genus in the LAB group, is commonly used in dairy products, vegetables, and fruits. These bacteria also constitute the natural mucosal microbiota of humans and animals. Lactobacillus species are Gram-positive, non-sporing, catalase-negative facultative anaerobes or anaerobes, not pathogenic, and thrive in acidic gastrointestinal (GI) conditions (3, 4). The most important probiotic lactobacillus species used in the food industry include Lactobacillus acidophilus, *Lactiplantibacillus plantarum*, *Lacticaseibacillus casei*, *Lacticaseibacillus paracasei*, *Limosilactobacillus reuteri*, *Lacticaseibacillus rhamnosus*, *Limosilactobacillus fermentum*, and more. *L. fermentum*, a probiotic strain with various strains, can be found naturally in dairy products, fermented vegetables, bread, fermented sausages, human milk, and saliva (3, 5, 6).

Therefore, the present study was conducted with the aim of molecular identification of *L. fermentum* RSB9 isolated from traditional yogurt and evaluation of its viability in acidic and bile conditions, antimicrobial activity, antioxidant power, and safety properties.

## Materials and Methods

### Chemicals and culture media

Genomic DNA isolation kit and PCR kit were purchased from Dena-Zist Asian (Iran) and Parstous Biotech (Iran) companies, respectively. The culture media used, including de Man-Rogosa-Sharpe (MRS) agar, MRS broth, Müller Hinton agar (MHA), and sheep blood agar, were obtained from Merck (Germany). DNase culture medium was purchased from HiMedia (India). Bile salt from Sigma-Aldrich (United States) and standard antibiotics (vancomycin, gentamicin, penicillin, ciprofloxacin, imipenem, chloramphenicol, nitrofurazone, nalidixic acid) were obtained from Padtan Teb (Iran).

### Isolation of *L. fermentum* RSB9 strain from local yogurt and its molecular identification using polymerase chain reaction (PCR) method

To isolate and identify the *L. fermentum*, traditional yogurt samples were homogenized using peptone water and successive dilutions were prepared. After cultivation on the surface of MRS agar culture medium and incubation at 37°C for 48 hours, Gram staining and catalase tests were

performed on the strain. Genomic DNA was then extracted from the strain using a DNA isolation kit and cultured in MRS broth for 24 hours. For 16S rRNA gene amplification, universal primers, including 27FYM (5'-AGA GTT TGATYMTGG CTC AG-3') and 1492R (5'-GGT TAC CTT GTT ACG ACT T-3'), were used. The DNA was amplified using a PCR kit in a reaction solution with a volume of 25.15 µL (16.5 µL water, 2 µL deoxyribonucleotide triphosphate (dNTP), 2.5 µL 10x buffer, 1.25 µL primers, 1.2 µL magnesium chloride (MgCl2), 1.5 µL template DNA, 0.2 µL Taq polymerase enzyme). The PCR conditions were carried out according to the protocol, and the PCR products were electrophoresed on a 1.5% agarose gel at a voltage of 95 V for 45 minutes. The results indicated that the isolate with catalase-negative and Gram-positive properties, with a similarity rate of 97%, belonged to the *L. fermentum* RSB9 strain (7).

**The stability of the *L. fermentum* RSB9 strain in different acid ratios**

Initially, the *L. fermentum* RSB9 strain was cultured in MRS broth medium, and after being kept in the incubated for 24 hours at 37°C, microbial cells were separated using a refrigerated centrifuge (Hermle, Germany). The centrifugation was carried out at 9000 × g at a temperature of 4°C for 5 minutes to separate the cells from the liquid culture medium. To completely remove the culture medium, the local sediment was washed with sterile phosphate buffer saline (PBS). The sample was centrifuged again, and after discarding the supernatant, the formed precipitate was mixed with phosphate buffer until the absorbance spectrophotometer read 0.6 at 600 nm. Subsequently, 50 µL of the microbial suspension was added to 450 µL of acidic phosphate buffer solution with pH values of 3, 4, and 5, and the mixture was incubated at 37°C for 3 hours. After preparing successive dilutions of the sample using sterile PBS, cultures were performed on the surface of MRS agar. The plates were then incubated at 37°C for 24 hours, and after this period, the colonies were counted using a colony counter machine. Finally, the survival percentage of the *L. fermentum* RSB9 strain compared to the initial sample count was calculated (8).

**The stability of the *L. fermentum* RSB9 strain in different concentrations of bile salts**

To conduct this test, the *L. fermentum* RSB9 strain was cultured on the surface of the culture medium containing varying concentrations of bile salts (0.03%, 0.5%, and 0.7%). The plates were then incubated at 37°C for 24 hours, and visual observations were made to assess bacterial growth or non-growth (7).

**Cell surface hydrophobicity**

To determine the cell surface hydrophobicity, cell cultures of *L. fermentum* RSB9 were centrifuged at 5000 × g at 4°C for 15 minutes to separate the cells from the suspension. The cells were then washed with phosphate buffer (pH 7.2) and 6 mL of phosphate buffer was added to the sample. The absorbance was measured at 600 nm ($OD_{initial}$). In the next step, 3 mL of the microbial suspension was mixed with 0.6 mL of n-hexane and incubated at 25°C for 1 hour. The absorbance of the aqueous phase was then measured at 60 nm ($OD_{final}$). The level of cell surface hydrophobicity was calculated using the following equation (3):

(%) cell surface hydrophobicity = (($OD_{initial}$ - $OD_{final}$)/$OD_{initial}$) × 100

**Cholesterol uptake assay**

To investigate the cholesterol absorption capabilities of *L. fermentum* RSB9, polyoxyethyl-cholesteryl sebacate and Oxgall (3%) were added to the MRS broth. The initial cholesterol concentration in the culture medium was 100 µg/mL. Subsequently, microbial culture (1%) was added to the prepared medium, and the sample was incubated at 37°C for 24 hours. A control sample without microbial culture was also prepared (9).

**Assessment of antioxidant activity**

**ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) assay:** A stock solution of ABTS (7 mM) was prepared using potassium persulfate solution (5.5 mM) and allowed to equilibrate at room temperature in the dark for at least 6 hours before use. The solution was diluted with distilled water to achieve an absorbance of approximately 1 at 734 nm. Then, 0.3 mL of cell-free supernatant (CFS) was added to 2.7 mL of the ABTS solution, and the mixture was incubated for 45 seconds. After 1 minute, the absorbance was measured at 734 nm using a spectrophotometer. The inhibitory activity of *L. fermentum* RSB9 was calculated using the following formula (10):

(%) Free radical inhibition = ((Abs (s)/Abs (c)) - 1) × 100

where Abs (s) is the absorption rate of the sample and Abs (c) is the absorption rate of the control sample.

**DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay:** To assess the ability to inhibit DPPH radicals, 2 mL of methanolic DPPH solution (0.14 mM) was mixed with 2 mL of microbial suspension. The mixture was then incubated at 37°C for 30 minutes in a warm, dark environment. The DPPH radical scavenging activity of *L. fermentum* RSB9 strain was determined by measuring the absorbance of the supernatant at 517 nm and calculating the percentage of free radical inhibition using the formula (11):

(%) Free radical inhibition = ((Abs (s)/Abs (c)) - 1) × 100

where Abs (s) and Abs (c) represent the absorption rates of the sample and the control sample, respectively.

**Antimicrobial activity using well diffusion agar and disc diffusion agar methods**

To evaluate the antimicrobial activity of *L. fermentum* RSB9 against important food-borne pathogenic bacteria such as *Staphylococcus aureus*, *Enterobacter aerogenes*, *Bacillus subtilis*, *Salmonella typhi*, *Shigella dysenteriae*, and *Listeria monocytogenes*, well diffusion agar and disc diffusion agar were employed. Initially, the strain was inoculated into MRS broth culture medium and incubated at 37°C for 48 hours. The sample was then centrifuged (5000 × g at 4°C for 15 min), and part of the CFS was utilized as is, while the pH of another part was adjusted to 5.5. Next, the CFS samples were filtered through a syringe filter and freeze-dried to powder form. Sterile distilled water was added to the samples, and 100 µL of each neutral and acidic CFS (nCFSs and aCFSs) were placed in well (diameter of 6 mm) on MHA. Subsequently, pathogenic bacteria (at a concentration of 0.5 McFarland) were added. For the disc diffusion agar, discs (diameter of 6 mm) were coated with CFS and placed on MHA. Following incubation at 37°C for 48 hours, the diameter of the inhibition zone around the well/disk was measured (7).

**Safety assessment of *L. fermentum* strain RSB9**

**Antibiotic sensitivity:** Antibiotic sensitivity of *L. fermentum* RSB9 against 8 common antibiotics (vancomycin, gentamicin, chloramphenicol, nitrofurazone, nalidixic acid, penicillin, imipenem, and ciprofloxacin) was assessed using the disc diffusion test in agar. The strain was cultured on MRS agar culture medium, and antibiotic discs were placed on the surface. After incubation at 37°C for 48 hours, the diameter of the inhibition zone around the discs was measured, and results were categorized as resistant (R), semi-sensitive (SS), or sensitive (S) (12).

**Hemolytic activity:** Hemolytic activity of *L. fermentum* RSB9 was evaluated by culturing the strain on Sheep blood agar containing 7% sheep blood. The plates were then incubated at 37°C for 48 hours, and examined for the presence of hemolysis areas (13).

**DNase activity:** The DNase activity of the strain was determined by inoculating it on DNase culture medium and incubating at 37°C for 48 hours. The presence of a transparent pink halo around the bacterial colony indicated DNase activity (8).

**Production of biogenic amines (BAs):** The production of biogenic amines (BAs) including putrescine, tyramine, and histamine by *L. fermentum* RSB9 was tested by adding their amino acid precursors (L-ornithine, L-tyrosine, and L-histidine) to MRS agar plates containing 0.06% bromocresol violet. The appearance of a purple color indicated the production of biogenic amines (8).

**Statistical analysis**

All experiments were conducted thrice and analyzed using SPSS software (version 22) with one-way analysis of variance (ANOVA). Differences between means were determined using Duncan's test at a 95% confidence level ($p < 0.05$).

## Results

**Stability of microbial strain against different acid concentrations**

In Figure 1, the survival percentage of *L. fermentum* RSB9 against different acidic pH levels (3, 4, and 5) during a 3-hour period is shown. According to the results obtained from this study, with the passage of time and the increase in pH, the percentage of bacterial survival also decreased.

**Stability of *L. fermentum* RSB9 against different concentrations of bile salts**

In this research, the growth of *L. fermentum* RSB9 against different concentrations of bile salts was evaluated (Table 1). The findings of this study showed that this bacterium is stable against different concentrations of bile salts; however, with the increase in bile salt concentration, the viability of the strain decreased.

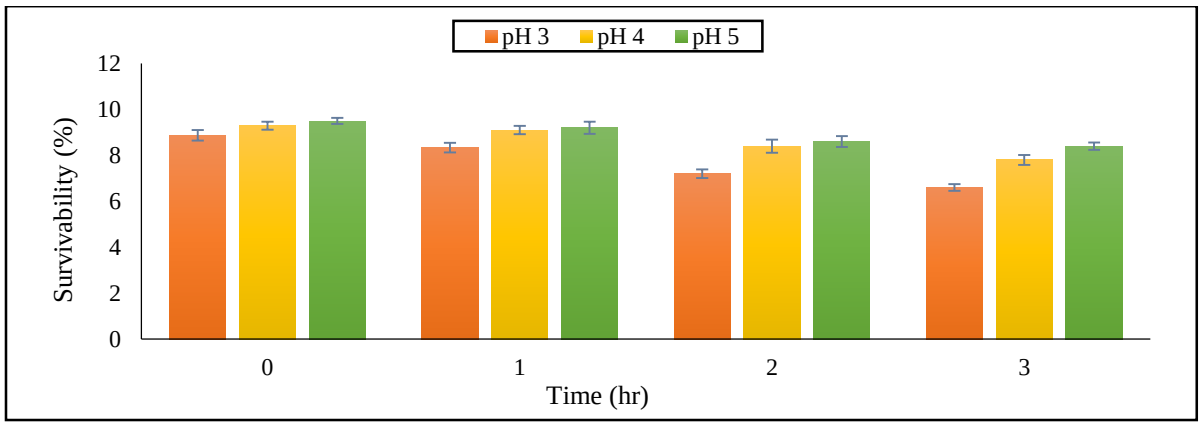



**Figure 1.** Survival (%) of *L. fermentum* RSB9 at different pH (3, 4, and 5) after 3 hours.

**Table 1.** Survivability of *L. fermentum* RSB9 at different bile salt concentrations

| | Bile salt concentrations | | | |
|---|---|---|---|---|
| | 0% | 0.3% | 0.5% | 0.7% |
| *Survivability | ++++ | +++ | ++ | + |

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

### Surface hydrophobicity

The research findings indicated that the surface hydrophobicity of *L. fermentum* RSB9 was measured at 54.90% ± 0.40 (Table 2).

**Table 2.** Hydrophobicity ability and cholesterol assimilation of *L. fermentum* RSB9

| Hydrophobicity (%) | Cholesterol assimilation (%) |
|---|---|
| 54.90 ± 0.40 | 43.60 ± 0.45 |

### Cholesterol absorption

The experiments reported that *L. fermentum* RSB9 exhibited a cholesterol absorption rate of 43.60% ± 0.45, indicating its potential in cholesterol reduction (Table 2).

### Antioxidant activity

In the current study, the antioxidant activities of isolated from yogurt were measured using DPPH and ABTS free radical methods (Figure 2). The antioxidant activities of *L. fermentum* RSB9 strain measured by DPPH and ABTS methods were calculated as 48.90% ± 0.57 and 52.50% ± 0.43, respectively, indicating the strain's potential in combating free radicals.

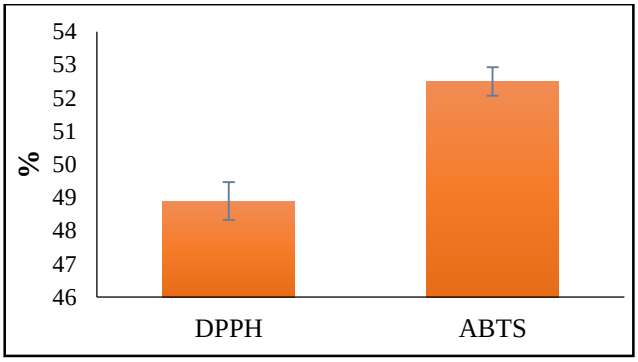
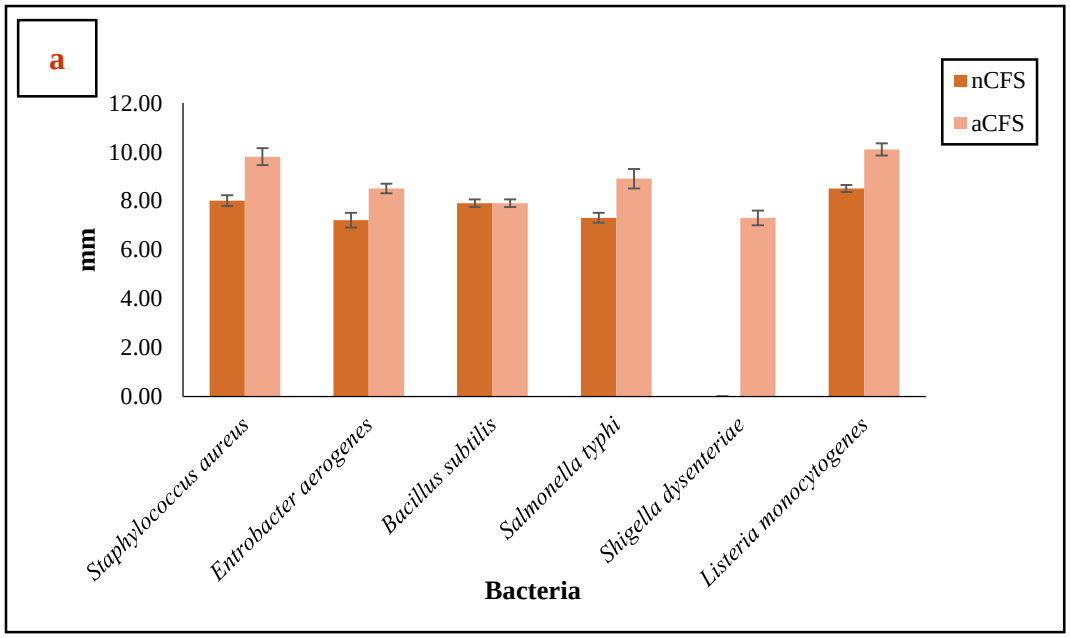



**Figure 2.** Antioxidant activity of *L. fermentum* RSB9 based on DPPH and ABTS assay

### Antimicrobial activity

Figure 3 displays the antimicrobial activity of *L. fermentum* RSB9 against important pathogenic bacteria. The strain effectively inhibited the growth of most examined pathogens. In the agar diffusion test with wells, nCFS values varied from zero to 8.50 ± 0.14 mm (*L. monocytogenes*). Additionally, aCFS values ranged from 7.30 ± 0.30 mm (*S. dysenteriae*) to 10.10 ± 0.25 mm (*L. monocytogenes*). In the disk diffusion test, nCFS values ranged from zero mm to 7.90 ± 0.23 mm (*L. monocytogenes*), with aCFS values from 6.20 ± 0.18 mm (*S. dysenteriae*) to 9.00 ± 0.27 mm (*S. aureus* and *L. monocytogenes*).



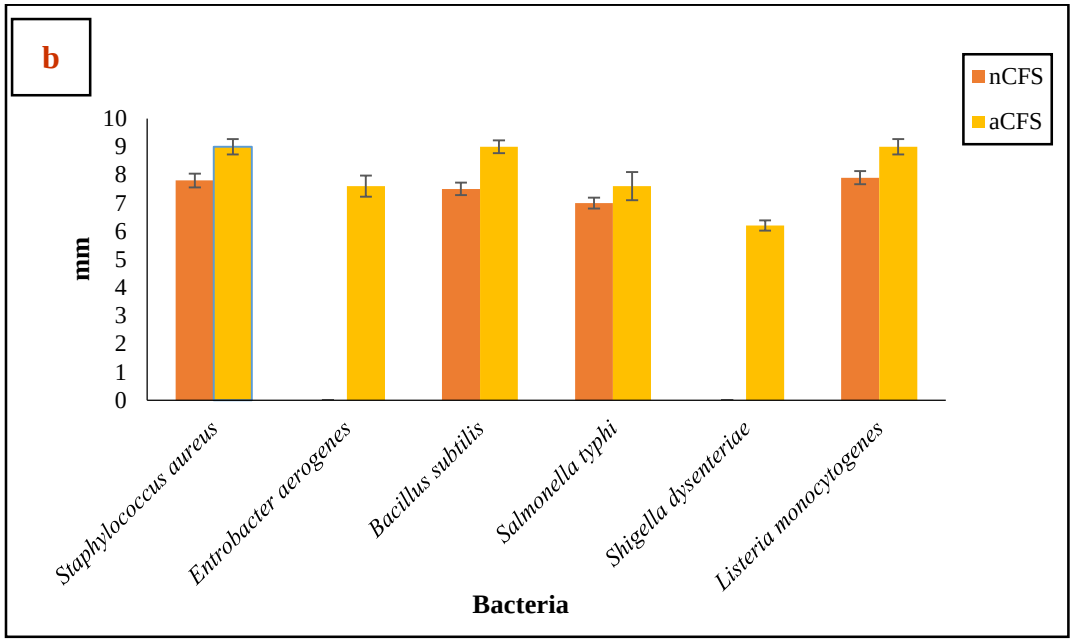



**Figure 3.** Antimicrobial activity of *L. fermentum* RSB9 based on (a) well diffusion agar and (b) disk diffusion agar methods. nCFS: neutralized cell-free supernatant, aCFS: acid cell-free supernatant.

### Antibiotic sensitivity

The results obtained from the antibiotic sensitivity testing of *L. fermentum* RSB9 against 8 common antibiotics are presented in Table 3. The strain exhibited sensitivity to all antibiotics except for penicillin and imipenem, with moderate sensitivity observed for ciprofloxacin.

**Table 3.** Antibiotic sensitivity results of *L. fermentum* RSB9

| Antibiotics | Result |
|---|---|
| Vancomycin | Sensitive |
| Gentamicin | Sensitive |
| Penicillin | Resistance |
| Ciprofloxacin | Semi-sensitive |
| Imipenem | Resistance |
| Chloramphenicol | Sensitive |
| Nitrofurazone | Sensitive |
| Nalidixic acid | Sensitive |

### Hemolytic activity

Hemolytic activity, or red blood cell lysis, is crucial to assess for safety in probiotic strains. The current study tested the hemolytic activity of *L. fermentum* strain RSB9 and found no hemolytic activity.

### DNase activity

DNase activity testing revealed that *L. fermentum* RSB9 lacks DNase activity, making it suitable for use in various food products.

### Biogenic amines (BAs)

The research findings revealed that *L. fermentum* RSB9 does not produce BAs, making it a safe probiotic strain for food and medicinal use.

## Discussion

In addition to surviving in culture or food products, probiotic bacteria must be able to survive while passing through the digestive system. Therefore, stability against acidic pH and high concentrations of bile salts are among the most important factors for choosing a probiotic strain because they are the main factors affecting the survival of probiotics in the digestive system, enabling the use of these microorganisms as food supplements (14). When microbial cells are placed in an acidic environment, the leakage of protons out of the cell exceeds the cells' ability to maintain their natural homeostasis. Proton flow at low pH disrupts pH homeostasis mechanisms, lowers intracellular pH, affects physiological processes, and causes protein denaturation and damage to DNA and cell membranes. Therefore, for a microorganism, intracellular pH changes are more critical than extracellular changes (15). Similarly, the survival ability of *Lactiplantibacillus plantarum* AR113 and AR501 strains as well as *Pediococcus pentosaceus* AR 243 strain in acidic pH has been studied. The results of this investigation revealed that the strains were not able to survive at pH equal to 2 for 48 hours. The comparison of the strains showed that the AR 113 strain had the shortest delay time, indicating the highest level of stability at low pH (16).

Bile acids cause rupture of bacterial cell membranes, denaturation of cell proteins, chelation of iron and calcium,

and oxidative damage to DNA. Unconjugated bile acids formed by hydrolysis of bile salts have stronger inhibitory effects on bacterial cells compared to conjugated bile acids. Therefore, for the survival of probiotics in the digestive system, their potential ability to resist bile and bile acids is crucial (15). In another study, among 30 strains isolated from fermented foods, only *Enterococcus faecium* CK21 and *L. fermentum* PP17 were able to tolerate bile salts up to 2.5% and pancreatin up to 2% after being kept at a temperature of 37°C for 24 hours (17). Similarly, it was found that 9 strains of probiotic Enterococcus isolated from traditional Turkish cheese exhibited a high degree of survival (77-94%) when cultured with 0.25% bile salt (18).

The non-specific interaction between microbial cells and the host is termed the hydrophobicity of the cell surface. Bacterial cells with high hydrophobicity typically exhibit strong interactions with the host's mucous cells, influencing bacterial adhesion. The level of surface hydrophobicity varies depending on cell surface expression and environmental factors (19). Features with increased water repellency facilitate better connections with intestinal epithelial cells, impacting microbial composition. In contrast, *Lactiplantibacillus plantarum* Jb21-11 strain demonstrated a surface hydrophobicity of 16.59 ± 0.012% (5), while *L. fermentum* strain IMAU70160 exhibited a hydrophobicity level of 44.49 ± 0.29% (8). These diverse results highlight the unique hydrophobic nature of each strain.

Cholesterol, an essential component of cell membranes, plays a vital role in various biological processes. Elevated cholesterol levels pose risks for conditions such as atherosclerosis, cardiovascular diseases, and hypertension. Studies have shown that consumption of fermented dairy products or those containing probiotics can lower serum cholesterol levels. Probiotics contribute to hypocholesterolemia through mechanisms like cholesterol absorption by cells, binding cholesterol to the cell surface, deconjugation of bile salts by bile salt hydrolases (BSH), simultaneous cholesterol deposition with deconjugated bile, and production of cell surface structures like exopolysaccharides (EPSs) (20). Similarly, *L. fermentum* strain IMAU70160 derived from traditional yogurt demonstrated a cholesterol absorption activity of 43.20 ± 0.47% (8). Additionally, studies showed that *Lactobacillus acidophilus* strains WFA1, WFA2, and WFA3 isolated from Dahi yogurt could effectively reduce cholesterol levels in media containing 0.2% bile salts, with the highest reduction observed after 24 hours of inoculation, reaching 26% (10). A similar study demonstrated that the culture supernatant of *Lacticaseibacillus casei*, *Limosilactobacillus reuteri*, *Lactobacillus acidophilus*, and *Lactococcus lactis* exhibited antioxidant activities attributed to phenolic and flavonoid compounds (21). Probiotics exhibit antioxidant activities through various mechanisms, including the synthesis of specific metabolites with antioxidant properties (e.g., glutathione, butyrate, and folate), ion chelation, possession of antioxidant enzymes, upregulation of host antioxidant activities, and modulation of microbiota to regulate oxidative stress (22).

Similarly, the antimicrobial activity of *Lactiplantibacillus plantarum* Jb21-11 exhibited significant inhibition around *S. aureus* ATCC 6538 colonies, mirroring results from the antibiotic resistance test against gentamicin. Other studies have shown the inhibitory effects of Lactobacillus strains against various pathogens, underscoring their potential in food systems.

Antibiotic sensitivity is a critical consideration for assessing the safety and approval of using bacteria in food systems. Concerns regarding antibiotic resistance gene transfer have heightened safety considerations for probiotics containing resistant strains. However, studies indicate that antibiotic resistance in Lactobacillus strains is chromosomally coded and non-transferable, reflecting intrinsic or natural resistance. Lactobacillus species are naturally resistant to certain aminoglycoside antibiotics due to cytochrome-related electron transfer mechanisms, influencing drug absorption (19).

Probiotic stability against different antibiotics can generally be explained by two mechanisms: natural or intrinsic resistance that is non-transmissible and acquired resistance typically resulting from bacterial mutations and transferable antibiotic resistance genes (23).

In another study, all six LAB species isolated from domestic fermented foods in Lithuania showed resistance to vancomycin, streptomycin, and kanamycin, with most strains sensitive to chloramphenicol. Only *Lactiplantibacillus plantarum* isolate 48C exhibited resistance to three antibiotics, while the rest were sensitive to gentamicin. These findings highlight the varied antibiotic sensitivities among LAB strains (4).

Similarly, among 26 strains isolated from raw milk, 14 isolates showed no hemolytic activity, emphasizing the non-harmful nature of these strains (12).

In another study, *Lactiplantibacillus plantarum* strain TW29-1 isolated from Zabul yellow curd also did not show DNase activity (7).

Biogenic amines (BAs) are low molecular weight compounds formed during microbial fermentation, with some fermented foods containing high levels of BAs like histamine, tyramine, or phenylethylamine. High levels of BAs in food can lead to conditions such as migraine, high blood pressure, and rapid heartbeat (24). Another study showed that some of the 16 strains isolated from Mutal cheese had the ability to decarboxylate L-tyrosine, L-ornithine, L-lysine, and L-histidine (25). Numerous studies have suggested the use of lactic acid bacteria as probiotic strains, highlighting their application in various food products, including ice cream and yogurt (26-44).

**Conclusion**

In conclusion, the study confirmed the probiotic potential of *L. fermentum* RSB9, highlighting its stability against different concentrations of acid and bile salts, acceptable cell surface hydrophobicity, cholesterol reduction ability, free radical inhibition, antimicrobial activity, lack of hemolytic or DNase activity, and absence of biogenic amine production. These findings support the introduction of *L. fermentum* RSB9 as a new probiotic strain with suitable biological and functional characteristics.

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## Financial disclosure

The authors declare no conflict of financial interest.

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