

Research paper

# *Lactobacillus rhamnosus* -derived from raw sheep milk as natural prebiotic carriers: Probiotic properties, cholesterol absorption, folic acid and cobalamin synthesis

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## ABSTRACT

Milk and dairy products provide a favorable environment for the growth of *Lactobacillus* species, which can produce compounds with probiotic properties vital to the food and pharmaceutical industries.

Twelve *Lactobacillus rhamnosus* strains obtained from raw sheep milk were evaluated for cholesterol assimilation, folic acid production, cobalamin synthesis, antibiotic resistance, hemolytic activity, bile salt and acid tolerance, and cell surface hydrophobicity. The applicability of the isolated strains in functional dairy products was also investigated.

In addition to exhibiting antagonistic activity against *Streptococcus pyogenes*, the strains exhibited varying capacities for cholesterol assimilation and caused changes in folate production. Specifically, *L. rhamnosus* T5 and T11 strains exhibited the highest cholesterol assimilation and folic acid production; however, cobalamin production was not detected in any strain. The results suggest that T5 and T11 strains may be suitable candidates for probiotic and starter cultures in functional food production. The data obtained will enable the development of new probiotics that have beneficial health effects and are of industrial importance.

## 1. Introduction

Sheep milk is an important source of income in countries with arid climates, and Türkiye is one of the countries with the most developed dairy sheep farming. Production has increased significantly in the last decade, and countries such as Mali, Iran, France and Spain have also increased sheep milk production considerably (Turynskiy et al., 2020).

Raw sheep milk in particular is nutritious, and is rich in lipids, proteins, minerals, vitamins and carbohydrates, making it an excellent source of nutrition and energy (Dinkçi et al., 2023; Maduko & Park, 2011).

Rich in nutrients and vitamins, minerals and trace elements, sheep milk is very effective in bone health, immune system, muscle development and digestive health. In addition to having conjugated alpha-linolenic and linoleic acids that prevent cancer, coronary artery disease, atherosclerosis and osteoporosis, its low lactose content relieves digestive irritation (O'Shea et al., 2004).

Milk and dairy products are also high in probiotics because animals have different bacterial flora. Consuming different dairy products with

probiotic qualities continues to have an impact on health by reducing inflammation, improving the digestive system, and preventing neurodegenerative diseases like Alzheimer's and type 2 diabetes (Flis & Molik, 2021). Lactic acid bacteria (LAB), the primary probiotic actors, are well-known for their beneficial effects on the immune and digestive systems. They also continue to play a significant role in the health field by producing B group vitamins like cobalamin and folate. Recently, LAB species such as *Lb. Pentosus/plantarum/paraplantarum*, *Lb. Casei/paracasei/rhamnosus*, *Lb. Brevis*, *Lb. Curvatus*, *E. hirae*, and *W. confusa* have been frequently isolated from sheep milk that the general public prefers (Quintana et al., 2020).

Especially strains of *L. rhamnosus* belonging to the *Lactobacillus* genus are widely used as probiotics due to their effects on digestive health. Additionally, these strains adhere to the digestive system, regulating intestinal health and preventing infections (Nath et al., 2020).

Cardiovascular disease, a leading cause of mortality globally, is linked to excessive blood pressure (WHO, 2002). The World Health Organisation predicts cardiovascular disease will affect 23.6 million people globally by 2030, ranking as a leading cause of death. Probiotics,

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particularly *Lactobacillus*, can help reduce cholesterol levels, but the relationship between high-saturated-fat diet and heart disease risk is controversial (Zong et al., 2016).

Sheep milk, which can help people to lose weight, reduce cholesterol and reduce heart disease risks, is rich in omega-3 fatty acids. In addition, the features of the probiotic properties still attract the attention of researchers, as the folate deficiency, osteoporosis, Alzheimer's, coronary heart disease and colorectal and breast cancer, which we include in our study, still attract the attention of researchers. (Patel et al., 2013). In studies, the health benefits of probiotic strains and their benefits in food applications, as well as their cholesterol-lowering, antioxidant and enzymatic activities, have been evaluated (Chauhan et al., 2025; Modasiya et al., 2024).

Although most vitamins are found in various foods, malnutrition, unbalanced nutrition or inadequate food intake continues to be the cause of vitamin deficiency in humans in some countries. Vitamin B 12 (Cobalamin) is a complex compound naturally produced only by bacteria and archaea. In addition, Cobalamin synthesis (vitamin B12) is an essential vitamin in human health and some probiotic bacteria can synthesize this vitamin (Martens et al., 2002). Cobalamin synthesis by probiotic bacteria provides a potential benefit, especially for vegan and vegetarian individuals. It has been determined in the literature reviews that raw sheep milk is a source that has not yet been fully discovered in terms of probiotic potential. Probiotic properties, cholesterol assimilation, folic acid and cobalamin synthesis of *Lactobacillus* isolated from sheep milk have not been investigated to a great extent.

The aim of this article is to evaluate cholesterol assimilation and folic acid and cobalamin production by determining the probiotic properties of *Lactobacillus* spp. isolated from raw sheeps milk offered for public consumption in İzmir province in western Türkiye.

## 2. Materials and methods

### 2.1. Isolation and identification

This study analyzed raw milk samples from healthy sheep under sterile conditions using De-Man Rogosa Sharpe (MRS) agar. Bacteria were grown on MRS agar and their carbohydrate fermentation profile was determined using commercial API 50 CHL strips and API 50 CHL Medium (Jeyagowri et al., 2023).

Pure cultures were also identified using MALDI-TOF MS (Matrix Supported Laser Desorption/Ionization Flight Time Mass Spectrometry, Bruker, Germany) method. The samples were automatically analyzed using a MALDI-TOF mass spectrometry (Bruker, Germany) running Flexcontrol 3.4 software. The mass spectrometry was calibrated using the Bruker's bacterial test standard (Bruker Daltonics) in accordance with the method described by Alatoom et al., (2011).

In this work, twelve *Lactocaseibacillus rhamnosus* (*L. rhamnosus*) were discovered. At  $-20^{\circ}\text{C}$ , the identified *Lactobacillus* isolates were kept in cryogenic tubes (Cryobank, Mast Diagnostics, France). Indicators for this investigation included *Streptococcus pyogenes* ATCC 19615 bacterium and *S. pyogenes* strains S1-S12. The microbiology culture collection of Dr. Behçet Uz Children's Hospital provided the bacteria. Probiotic characteristics of isolated microorganisms are tested.

### 2.2. Tolerance for bile salts and acids

The study involved reducing MRS broth's pH to 2.0, adding 0.3 % bile salt, and autoclaving it. The cultures were inoculated with active cultures, incubated at  $37^{\circ}\text{C}$  for 3 h, and seeded on MRS agar. After 24 h, the colonies were measured for log CFU/ml in forming colonies (Tulumoğlu et al., 2023).

### 2.3. Assays for auto-aggregation and co-aggregation

*L. rhamnosus* and *S. pyogenes* ATCC 19615 isolates were activated by

a 24-h incubation period at  $37^{\circ}\text{C}$ , vortexed for 15 s, and ODs reduced to 0.5 using PBS.

Auto-aggregation; *Lactobacillus* strains were incubated at  $25^{\circ}\text{C}$ , then observed at various intervals and quantified at 600 nm using a spectrophotometer before recording (Collado et al., 2007). The following formula was used to determine the auto-aggregation:

$$(\%) \text{ Auto-aggregation} = 1 - (A_1 / A_0) \times 100$$

Where  $A_1$  is the absorbance at 7, 8, 10, and 12 h (OD) and  $A_0$  is the absorbance at 0 time.

Co-aggregation: The study combined *S. pyogenes* ATCC 19615 and *L. rhamnosus* and incubated for 6 h. The suspension was measured at 600 nm for seven, eight, ten, and 12 h (Shokrak et al., 2024). The co-aggregation percentage was calculated using a formula. The following formula was used to get the co-aggregation percentage:

$$\text{Co-aggregation } (\%) = 1 - \left( \frac{A_{\text{mix}}}{\frac{A_{\text{isolate}} + A_{\text{pathogen}}}{2}} \right) \times 100$$

Where  $A_{\text{mix}}$  = Absorbance after incubation.  $A_{\text{isolate}}$  = Absorbance of the tested isolate before mixing,  $A_{\text{pathogen}}$  = Absorbance of pathogen before mixing.

### 2.4. Activity of hemolysis

A colony was grown on 5 % sheep blood agar for 24 h at  $37^{\circ}\text{C}$  in an incubator with 5 %  $\text{CO}_2$ . Hemolysis was classified as alpha, beta, or gamma, with *S. pyogenes* ATCC 19615 as the control strain (Maragkoudakis et al., 2006).

### 2.5. Susceptibility to antibiotics

The antibiotic susceptibility of *L. rhamnosus* bacteria was assessed using the disc diffusion technique. Overnight active cultures were distributed on MRS agar medium, with antibiotic discs on them. LNZ: linezolid (10 µg), LEV: levofloxacin (5 µg), SXT: sulphamethoxazole/trimethoprim (25 µg), AM: ampicillin (2 µg), FOX: cefoxitin (30 µg), TE: tetracycline (30 mcg), and TEC: teicoplanin (30 µg) On it were antibiotic discs (BD USA). The incubation period was 24 h at  $37^{\circ}\text{C}$ , and resistance and sensitivity were assessed using the CLSI, 2023 criteria.

### 2.6. Activity that is antagonistic

The study used the agar well diffusion method to study antagonistic activity in *L. rhamnosus* cultures (Hussein et al., 2018). Cultures were grown in MRS broth and incubated in a %  $\text{CO}_2$  incubator for 24 h. The supernatants were filtered and seeded with *S. pyogenes* strains and ATCC 19615 5 % Blood Agar. 100 µl of free cell supernatant was added to 10 mm diameter wells and incubated for 2 h at room temperature and 24 h at  $37^{\circ}\text{C}$ . According to Prabhurajeshwar and Chandrakanth (2017), zones of growth inhibition were classified as low or resistant (>10 mm), moderate (10–20 mm), and sensitive (<20 mm) based on their diameter.

### 2.7. The absorption of cholesterol

After being inoculated in an MRS broth containing 0.3 % oxgall, the active *Lactobacillus* was let to grow for 24 h in an atmosphere with 5 %  $\text{CO}_2$ . The cholesterol content of the obtained supernatants was centrifuged at 5000 g for 10 min, and for this test, Unicell DXC800 model autoanalyzer (Beckman Coulter, USA) and enzymatic kit [Synchron® systems (Beckman Coulter, USA)] were used (Tulumoğlu et al., 2014).

### 2.8. Production of folic acid and cobalamin

100 µL of inoculation was applied to 5 ml of MRS medium from overnight active cultures and allowed to develop at  $37^{\circ}\text{C}$  for 5 h in a 5 %

CO<sub>2</sub> incubator. Centrifugation at 5000 rpm was done, 2 ml of the supernatant was taken from the top, and folate and Cobalamin was determined as ng/dl by making 600 nm measurements with Abbott brand Architect i1000sr Immuno Thetics Analyzer (Tulumoğlu et al., 2023). All studies were independently repeated three times.

Vitamins Folate (B9) and Cobalamin (B12) have a very important role in the general functioning of the body. They support the body in the formation of red blood cells. They also contribute to DNA and RNA synthesis. They are effective vitamins in the formation of genetic codes (Carmel et al., 2003).

### 2.8.1. Statistical analysis

Statistical analyses were performed using MINITAB (version 16) (Minitab Inc., Coventry, UK). Pearson correlation coefficients were calculated to determine the relationships between acid and bile salt tolerance, auto-aggregation, and co-aggregation among strains. Statistical significance was defined as  $p < 0.05$ .

## 3. Results and discussion

Sheep milk has gained interest as a substitute milk source due to its nutritional profiles and potential probiotic qualities. *L. rhamnosus*, a probiotic known for its beneficial effects on intestinal health and immune function, is a significant source of interest (Saleem et al., 2024). Probiotic properties of *L. rhamnosus* strains isolated from sheep milk, such as adhesion to intestinal cells and resistance to gastrointestinal diseases, have been shown in literature studies (Balthazar et al., 2017). Consumption of *L. rhamnosus* rich sheep milk and products provides a number of health benefits such as immunity, better digestion and fewer allergic reactions (Salva et al., 2011).

### 3.1. Acid and bile salt tolerance

Twelve distinct strains of *L. rhamnosus* were found to have data ranging from 10.1 % to 98 % in a low acid environment at pH 2.0 and from 20.1 % to 90 % in bile salt (0.3 % ox bile) in tests of acid and bile salt tolerance (Fig. 1).

Our findings also support this study; especially the high acid and bile tolerance of some strains emphasizes the potential of these microorganisms to be used as functional probiotics. In this context, it is thought that the observed tolerance differences may be related to the genetic

structure, cell wall properties and adaptation mechanisms of the strains. As a result, strains showing high acid and bile tolerance can be considered as strong candidates for further in vivo studies and functional food applications. It has been reported that lactobacilli in milk and dairy products can maintain their viability against low pH. In this context, even strains that cannot withstand low pH in vitro can show significant viability when consumed with milk.

Furthermore, bile salt tolerance levels are comparable to those found in milk-derived strains reported by Modasiya et al. (2024) and Maniya et al. (2024).

Similar results were reported by (Agaliya & Jeevaratnam, 2012), who also observed to survive and colonize the gastrointestinal tract, probiotic bacteria must be tolerant of low pH (pH 2.5–3.5) and gastric pepsin, bile salts, and pancreatin in the upper intestine, and must have the ability to adhere to intestinal mucus.

According to Elli et al. (2006), probiotic *Lactobacillus* found in milk and dairy products can withstand low pH values, especially in the stomach. The resistance of *L. rhamnosus* strains to bile salts and acids has been reported as an important criterion in the selection of probiotic bacteria (Lima et al., 2020). Bile and acid resistance are important determinants of many probiotic *Lactobacillus* strains (Agaliya & Jeevaratnam, 2012).

### 3.2. Coaggregation as well as autoaggregation

This study examined *L. rhamnosus* strains' auto-aggregation and co-aggregation percentages, finding variation influenced by strain-specific adhesion properties and growth conditions. Auto-aggregation was found to be highest in *L. rhamnosus* T2 (92.5 %) bacteria and lowest in *L. rhamnosus* T4 (40.0 %) bacteria. In our lab study, we took a close look at how different strains of *L. rhamnosus* clump together, and we discovered that *L. rhamnosus* T2 had the highest autoaggregation rate at an impressive 92.5 %. In contrast, strain T4 lagged behind at just 40.0 %, suggesting that the aggregation behavior varies among these bacteria. In the study, it was determined that *L. rhamnosus* T2 (56.7 %) and T10 (85.7 %) strains were effective against pathogenic *Streptococcus pyogenes* ATCC 19615. These results suggest that *L. rhamnosus* strains (T2 and T10) may help maintain microbial balance and prevent harmful bacteria from adhering to surfaces. The results showed that T10 outperformed T2 in co-aggregation. *L. rhamnosus* T10 strain is considered a potential probiotic due to its coaggregation capacity (Fig. 2).

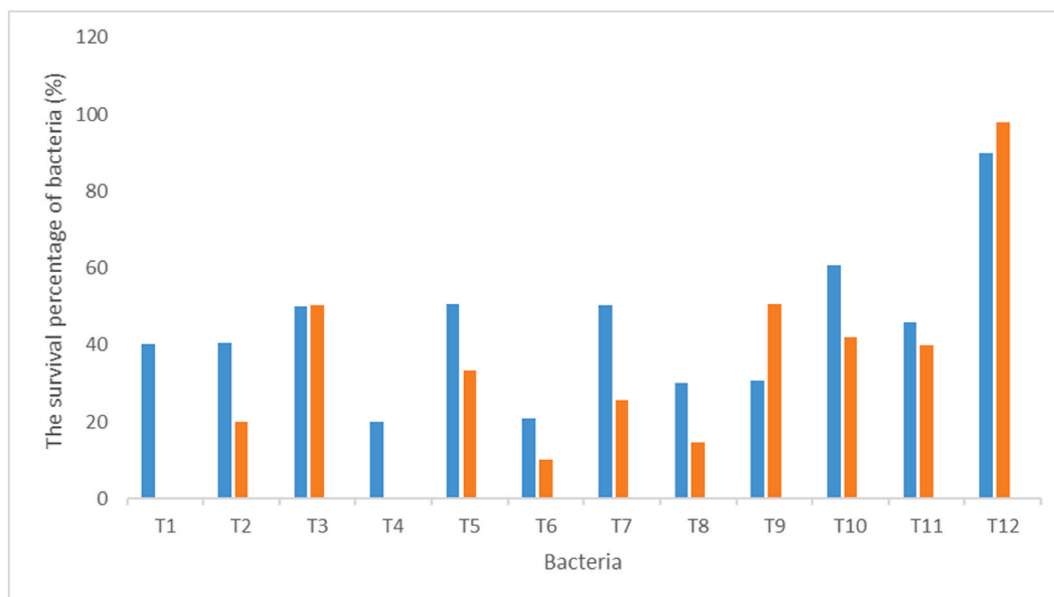


Fig. 1. *L. rhamnosus* survival rates after 4 h in bile salt (0.3 % Oxcall) and low acidic (pH 2).

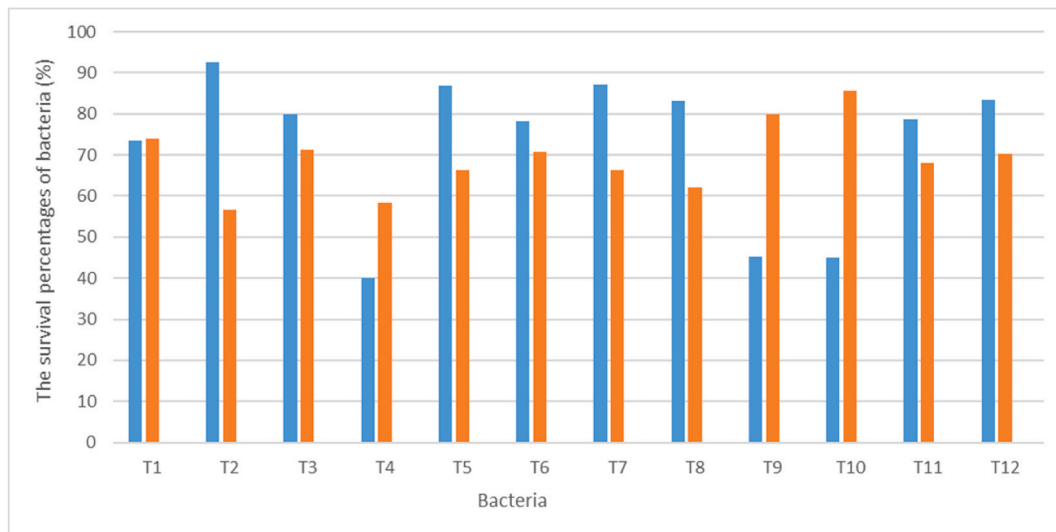


Fig. 2. *L. rhamnosus* autoaggregation and coaggregation percentages (%).

This suggests that there is no direct correlation between autoaggregation and co-aggregation in the research. By stopping harmful bacteria from sticking to the body’s mucosal surfaces, some *Lactobacillus* strains with strong co-aggregation, including T10, can lower the risk of infection. It is reported in the literature that the ability of probiotic strains to coaggregate with pathogens plays an important role in maintaining host health. For example, in one study, it was shown that *L. rhamnosus* and *L. reuteri* strains inhibited the hemolytic activity of *S. pyogenes* and that this effect occurred at the transcriptional level. It was also reported that these strains prevented *S. pyogenes* from adhering to host epithelial cells (Saroj et al., 2016).

### 3.3. Resistance to antibiotics

The study revealed that *L. rhamnosus* strains are completely resistant to teicoplanin and sulfamethoxazole/trimethoprim, suggesting that using these antibiotics with probiotics is not recommended (Table 1).

Table 1

Antibiotic susceptibility profile of *L. rhamnosus* strains isolated from sheep milk based on inhibition zone interpretation (S: Sensitive, R: Resistant, I: Intermediate).

<i>Lactobacillus</i> strains	SAM	AM	FOX	TE	LNZ	LEV	SXT	TEC
<i>L. rhamnosus</i> T1	S	S	R	S	S	S	R	R
<i>L. rhamnosus</i> T2	S	S	S	S	S	S	R	R
<i>L. rhamnosus</i> T3	S	S	S	S	S	S	R	R
<i>L. rhamnosus</i> T4	S	S	I	S	S	S	R	R
<i>L. rhamnosus</i> T5	S	S	I	S	S	I	R	R
<i>L. rhamnosus</i> T6	S	S	I	S	S	S	R	R
<i>L. rhamnosus</i> T7	S	S	S	S	S	S	R	R
<i>L. rhamnosus</i> T8	S	S	R	S	S	S	R	R
<i>L. rhamnosus</i> T9	S	S	R	S	S	S	R	R
<i>L. rhamnosus</i> T10	S	S	R	S	S	I	R	R
<i>L. rhamnosus</i> T11	S	S	I	S	S	S	R	R
<i>L. rhamnosus</i> T12	S	S	R	S	S	R	R	R
Resistance percentages (%)	0	0	41.6	0	0	8.3	100	100

Note: Antibiotic susceptibility was determined based on inhibition zone diameters measured in millimeters and interpreted according to CLSI (Clinical and Laboratory Standards Institute) guidelines. All tests were performed in triplicate. The results are presented as categorical outcomes: S (Sensitive), I (Intermediate), and R (Resistant).

SAM ampicillin/sulbactam, AM ampicillin, FOX cefoxitin, TE tetracycline, LNZ linezolid, LEV levofloxacin, SXT sulphamethoxazole/trimethoprim, TEC teicoplanin.

Recent research indicates *Lactobacillus* species may exhibit antibiotic resistance, particularly to certain antibiotics like sulfamethoxazole/trimethoprim, but are also susceptible to beta-lactam antibiotics like ampicillin (Yarahmadi et al., 2022). Furthermore, they are naturally resistant to glycopeptide antibiotics like teicoplanin, according to reports (Anisimova & Yarullina, 2019). Commercial *Lactobacillus* strains found in *L. rhamnosus* strains were found to be resistant to linezolid, tetracycline, ampicillin and ampicillin/sulbactam, indicating that there are different antibiotic resistance mechanisms depending on the drug and species. As a result, it is necessary to evaluate the antibiotic resistance profile of each probiotic strain independently (Sharma et al., 2016).

The consider uncovered that *L. rhamnosus* strains were totally safe to teicoplanin and sulfamethoxazole/trimethoprim, demonstrating that the utilize of these anti-microbials at the side probiotics isn’t prescribed. *L. rhamnosus* strains are exceedingly touchy to anti-microbials such as linezolid, tetracycline, ampicillin and ampicillin/sulbactam, making them secure to utilize as probiotics amid anti-microbial treatment (Nayyar et al., 2022). This resistance profile is reliable with past considers appearing that the helpful utilize of probiotics depends on the anti-microbial resistance of the strain. Future investigate ought to examine the atomic associations between resistance qualities and drugs. *L. rhamnosus* strains appear add up to resistance to sulfamethoxazole/trimethoprim and teicoplanin, making it hazardous to utilize them as probiotics whereas taking anti-microbials. Be that as it may, they are touchy to tetracycline, ampicillin, ampicillin/sulbactam, and linezolid (Nayyar et al., 2022). This adjusts with past inquire about appearing probiotic viability is affected by anti-microbial resistance. Future ponders ought to investigate resistance quality connections.

### 3.4. Hemolytic action

All of the *L. rhamnosus* strains exhibited alpha hemolysis in the hemolysis activity assay, but none of them displayed beta hemolytic activity in blood agar. A crucial need for possible probiotic usage is this (Maragkoudakis et al., 2006). A feature of harmful bacteria is beta hemolysis, which lyses erythrocytes. The safety of the strains under study is demonstrated by the lack of beta hemolysis. Oxidative stress in *Lactobacillus* species can naturally result in non-pathogenic alpha hemolysis. As a result, strains of *L. rhamnosus* are bacteria that have safe probiotic qualities.

### 3.5. Activity that is antagonistic

Zones of inhibition for the *S. pyogenes* strains employed in the study ranged from 16 to 26 mm, indicating significant resistance to *L. rhamnosus* strains (Table 2). *Lactobacillus* species are effective antibacterials against harmful microorganisms.

Recent research has focused on the health effects of certain probiotic strains, including their ability to ease lactose intolerance symptoms and reduce rotavirus diarrhea duration. These strains have potential in atopy prevention and treatment, but more research is needed (De Keersmaecker et al., 2006; Ouwehand et al., 2002). They contribute to food safety through the synthesis of chemicals like hydrogen peroxide, bacteriocins, and organic acids (Kongo et al., 2006).

### 3.6. Cholesterol assimilation

The study found that T1 and T7 strains had the lowest cholesterol assimilation levels, while T4 and T5 strains had the highest levels. These discoveries recommend that diverse strains of *L. rhamnosus* may have diverse capacities to acclimatize cholesterol (Table 3).

The T4 strain in our think about was compared to the T4 strain by Tulumoğlu et al. (2018) had an esteem of 70 mg/dl higher than the values of 25–59 mg/dl distributed by the creators, showing that hereditary and metabolic varieties among strains are viable in cholesterol digestion system.

A study found that 61 *Lactobacillus* strains can remove cholesterol from MRS-THIO medium after 18 h, with the first 20 strains showing a 27 % decrease. *L. plantarum* MA2 and *L. johnsonii* 3121 showed the highest cholesterol-lowering activities (over 50 %) (Lee et al., 2022). Probiotics reduce cholesterol absorption in the intestine by binding to cell membranes and assimilating during growth (Guo et al., 2011). The cholesterol assimilation ability of *L. rhamnosus* T5 and T11 determined in our study is consistent with the findings of Chauhan et al. (2025), who reported similar properties with multi-strain probiotics.

### 3.7. Production of folic acid and cobalamin

The T11 strain of *L. rhamnosus* produces the most folate (331.8 ng/dl), according to the research, although levels of folate production vary amongst strains (Table 3). Given that the T11 strain generated the most folate, it may find application in functional foods and supplements. Such low levels are produced by T2 and T8 strains, which have poor folate synthesis. The capacity of *L. rhamnosus* strains to produce folate varies and must be taken into account in both industrial and health-related applications. Strains with very high levels of folate production may be considered as functional food components or supplements. None of the strains used in the study were found to produce cobalamin, or vitamin B12.

This suggests that the ability of different strains of *L. rhamnosus* to produce folate is greatly impacted by genetic and metabolic differences

**Table 2**

The *S. pyogenes* of *L. rhamnosus* antagonistic activity.

<i>Lactobacillus</i> strains	<sup>a</sup> S1	<sup>a</sup> S2	<sup>a</sup> S3	<sup>a</sup> S4	<sup>a</sup> S5	<sup>a</sup> S6	<sup>a</sup> S7	<sup>a</sup> S8	<sup>a</sup> S9	<sup>a</sup> S10	<i>S. pyogenes</i> ATCC 19615
<i>L. rhamnosus</i> T1	25	24	22	22	22	20	20	22	20	22	24
<i>L. rhamnosus</i> T2	25	20	22	24	26	20	26	20	20	22	23
<i>L. rhamnosus</i> T3	24	20	20	20	16	19	15	15	18	20	22
<i>L. rhamnosus</i> T4	20	20	20	18	20	16	20	18	18	19	20
<i>L. rhamnosus</i> T5	20	20	22	20	26	20	20	20	22	20	20
<i>L. rhamnosus</i> T6	20	18	18	18	22	16	19	17	20	20	18
<i>L. rhamnosus</i> T7	20	18	18	20	24	18	18	17	20	22	22
<i>L. rhamnosus</i> T8	25	20	18	18	22	16	20	14	18	18	20
<i>L. rhamnosus</i> T9	18	16	16	16	22	10	20	14	18	16	17
<i>L. rhamnosus</i> T10	20	20	20	18	22	15	19	16	18	17	20
<i>L. rhamnosus</i> T11	20	20	20	20	26	18	24	20	19	20	22
<i>L. rhamnosus</i> T12	20	20	20	18	20	18	20	20	24	24	20

<sup>a</sup> *S. pyogenes* (S1, S2, S3, S4, S5, S6, S7, S8, S9, S10) test strains.

**Table 3**

Assimilation of cholesterol and production of folate and cobalamin in *L. rhamnosus*.

<i>Lactobacillus</i> strains	Cholesterol assimilation (mg/dl)	Folate (B9) production (ng/dl)	<sup>a</sup> Cobalamin (B12) production (pg/mL)
<i>L. rhamnosus</i> T1	20	198,2	ND
<i>L. rhamnosus</i> T2	42	56,1	ND
<i>L. rhamnosus</i> T3	50	203,4	ND
<i>L. rhamnosus</i> T4	70	115,2	ND
<i>L. rhamnosus</i> T5	60	290,4	ND
<i>L. rhamnosus</i> T6	30	185,7	ND
<i>L. rhamnosus</i> T7	8	95,0	ND
<i>L. rhamnosus</i> T8	35	73,3	ND
<i>L. rhamnosus</i> T9	55	153,6	ND
<i>L. rhamnosus</i> T10	54	194,8	ND
<i>L. rhamnosus</i> T11	65	331,8	ND
<i>L. rhamnosus</i> T12	58	112,4	ND

<sup>a</sup> ND: No production.

(Rossi et al., 2011). Since not all *Lactobacillus* strains are able to produce significant amounts of folate, strain selection has a big impact on the functional properties of probiotic products. But strains with low production capacity might have unique probiotic properties, like the ability to adhere to intestinal epithelium or have an anti-inflammatory effect (Sanders et al., 2019). Many species of the *Lactobacillus* genus are incapable of synthesising cobalamin, and only a few species, including *Propionibacterium*, *Pseudomonas*, and some strains of *L. reuteri*, are known to be able to generate cobalamin.

In conclusion, the lack of cobalamin production by the *L. rhamnosus* strains used in the study suggests that this species' natural biosynthetic capability is restricted. Therefore, choosing the right microbial species is crucial for solutions that aim to supplement with vitamin B12.

This study provides information on the probiotic properties of *L. rhamnosus* strains isolated from raw sheep milk. However, there are some limitations that must be acknowledged. First, the study included only 12 strains, which may not fully represent the broader diversity of *L. rhamnosus* isolates. Second, cobalamin (vitamin B12) production was not detected under the applied conditions, indicating the need for optimization of culture parameters or genomic analysis to identify relevant biosynthetic pathways. Third, probiotic properties were only evaluated in vitro; therefore, further in vivo studies are required to confirm their health benefits.

## 4. Conclusions

Probiotic qualities were demonstrated by strains of *L. rhamnosus* that were isolated from raw sheep milk. *L. rhamnosus* T5 and T11 have the

potential to become functional foods since they can synthesize folic acid and cobalamin and decrease the absorption of cholesterol. The high dry matter content and nutritional profile of sheep milk make it a valuable raw material for fermented beverages, which form the basis of the functional food market and are a promising matrix for probiotic carriers and functional dairy products. Sheep milk, rich in vitamins, minerals and bioactive peptides, can provide therapeutic benefits, maintain intestinal homeostasis and act as a probiotic matrix. Fatty acid composition and bioactive substances are important for human health since they are found in Sheep milk. It is suitable to add sheep milk to diets because it can help digestion, strengthen the immune system and provide you with plenty of vitamins and minerals. Future studies should aim to investigate the genomic profiles of promising strains (such as T5 and T11), assess their stability and functionality in real food matrices, and investigate their efficacy in animal models or human trials. These studies are expected to contribute to a better understanding of the probiotic potential and industrial applicability of the isolated strains.

### CRedit authorship contribution statement

**Şener Tulumoğlu:** Writing – original draft, Validation, Software, Methodology, Data curation, Conceptualization. **Belgin Erdem:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Investigation, Formal analysis, Conceptualization.

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### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data availability

The authors do not have permission to share data.

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