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Investigation of *Lactobacillus* spp. bacteria in infants consuming breast milk and formula and determination of some probiotic characters

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ABSTRACT

Objectives: It was aimed to investigate some probiotic properties of Lactic Acid Bacteria (LAB) isolated from stool samples taken from 45 healthy 0-4 month old babies, who did not take antibiotics or probiotic supplements in the last 3 months, and who did not have any health problems.

Methods: Six different species were obtained from 21 isolates selected by the method of Mass Spectrometry (MALDI-TOF MS).

Results: The most common strain was *Lactobacillus rhamnosus* with a rate of 59%, followed by *Lactobacillus paracasei* with a rate of 13.6%. Vancomycin, tetracycline, gentamicin, netilmicin, tobramycin, penicillin, ampicillin, teicoplanin and amikacin antibiotics were used to evaluate the antimicrobial activities of the strains. In our study, while all strains were resistant to the antibiotic amikacin, they were sensitive to tetracycline, penicillin, gentamicin, netilmicin, teicoplanin, vancomycin, ampicillin and tobramycin antibiotics. In the evaluation of the antagonistic activities of LAB, 6 different pathogens (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *S. aureus* ATCC 25923, *Candida albicans* ATCC 10231, *Listeria monocytogenes* ATCC 19111) were used and it was determined that the strains showed antimicrobial effects on all pathogenic microorganisms. Cholesterol assimilation abilities, T21 and T22 strains achieved the highest cholesterol assimilation rate of 39.1%.

Conclusions: It is thought that most of the isolated strains have probiotic potential, and especially *Lactobacillus gasseri* T21 and *Lactobacillus paracasei* T22 strains may be probiotic strains that can be used in the production of preparations alone or together with other *Lactobacillus strains*.

Keywords: Lactic acid bacteria, breast milk, probiotic

One of the most basic events that shape the intestinal microbiota is breastfeeding [1]. Breast milk; Milk containing 10-105 CFU of microorganisms per ml from which *Streptococcus* and *Staphylococcus* are the most, *Lactobacillus*, *Bifidobacterium*, *Enterococcus* and short-chain fatty acid producing bacteria such as *Veilonello*, *Propionibacterium*, *Faecalibacterium* are easily isolated. Human milk plays an important

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role in the development of the neonatal gut microbiota, as it provides a continuous supply of microorganisms to the infant gut a few weeks after birth [2].

An effective probiotic agent is known to be microorganisms that can survive the host's digestive process, colonize the gut, and produce a beneficial response in the host without pathogenic or toxic side effects [3]. Each probiotic strain is known to have its own characteristics and should be investigated in detail in this context. It is necessary to determine the types of probiotic characters to be used especially in infant formula.

For this purpose, in our study, it was aimed to determine the various probiotic characterizations of lactic acid bacteria (LAB) isolated from the stools of 0-4 month-old infants who received only breast milk and supplemented with formula in addition to breast milk.

METHODS

Isolation of Bacteria

In our study, isolated from the stools of 51 healthy babies who were admitted to Sivas Cumhuriyet University Health Services Application and Research Hospital, aged 0-4 months, born vaginally, who did not receive antibiotic and/or probiotic treatment in the last 3 months, took only breast milk or were fed with formula in addition to breast milk. The obtained LAB was used. During the collection of stool samples, the baby's gender, week of birth, birth weight, current weight, feeding style (whether he took only breast milk or infant formula in addition to breast milk) and the number of daily defecations were obtained. The ethics committee of our study was received from Kırşehir Ahi Evran University on 24.09.2019 (Decision No 2019-16/165). The samples were brought to Sivas Cumhuriyet University Faculty of Medicine, Department of Medical Microbiology, Microbiology Laboratory, under sterile conditions, and evaluated within the same day. MRS (De Man Ragosa Sharpe, Merck) broth and solid media were used in the development and activation of LAB. Each sample was incubated in MRS solid medium for 48 hours at 37°C in anaerobic conditions. Large, small, white, off-white and opaque colonies formed on the media as a result of incubation were selected and stored in TSB (Tryptic Soy Broth) medium with glycerol at -18°C until studied. *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *S. aureus* ATCC 25923, *Listeria moncytogene*s strains used to determine the antagonistic effects of LAB were obtained from Amasya University Microbiology Laboratory Culture Collection. Species of isolated strains were determined by Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) (Bruker Biotyper 3.0 Mikroflex LT Bruker Daltonics GmbH Bremen Germany). MALDI-TOF MS detects the protein profiles of existing microorganisms and compares the obtained profiles with the microorganisms in the current library [4].

Determination of Probiotic Properties of LAB

Acid and Bile Tolerance

In order to determine the accessibility of LAB isolated from the stool of infants to the intestines by surviving in the acidic environment of the stomach, an environment similar to the gastric fluid environment was prepared. Cultures activated in broth for 18 hours were precipitated by centrifugation at 3000 × g for 15 minutes at 4^{0}C. After the precipitate was washed twice with sterile phosphate buffered saline (PBS), PBS buffer (1N NaOH and 1N HCl) was prepared in 3 different ways with pH levels of 2.0, 2.5,3.0 and the strains were incubated in low pH PBS buffers for 3 hours at 37^{0}C. has been done. PBS with a pH of 7.2 was used for the control. Serial dilutions were made by taking 1 ml from the samples that were incubated at low pH at the 0th and 3rd hours of the incubation, and they were incubated by making triple parallel cultivations on MRS agar media. At the end of the period, the colonies in the control and test groups were counted and the % viability rates were calculated. % Viability = X/X0 (X: Number of viable microorganisms in the test group, XO: Number of viable microorganisms in the control group) [5].

Bile Salt Tolerance: 0.3% by weight to determine the viability of the isolated LAB in a medium containing bile salt; MRS broths containing 0.5% and 1% bile salts (oxgall, sigma) were prepared and the other stages of the study were applied as stated above.

% Vitality = X/X0 ×100 [6].

pH Change

Each sample was inoculated into MRS broth, and after 18 hours of incubation at 37^{0}C, it was measured

with a pH meter (AZ Instrument) to determine the acidic pH values of the cultures. As a control, the pH of sterile MRS broth without inoculation was checked. Measurements were carried out in 3 repetitions [7].

Determination of Antibiotic Sensitivities

Activated cultures in MRS medium were adjusted to 0.5 McF (625 nm absorbance = 0.08-0.1) with physiological saline and spread homogeneously on sterile MRS agar with a sterile drigalski spatula. Antibiogram discs were placed in Petri dishes at appropriate intervals and incubated at 37°C for 24 hours. The diameters of the zones formed around the antibiotic discs as a result of incubation were measured in millimeters with a caliper. Measurements were evaluated as Resistant (R), Semi-Fine (I), and Sensitive (S) according to NCCLS (National Committee for Clinical Laboratory Standards) criteria. Ampicillin (AM) (10 mcg) in the study; Penicillin (P) (10 U); Teicoplanin (TEC) (30 mcg); Gentamicin (CN) (120 mcg); Tetracycline (T) (30 mcg); Netilmicin (NET) (30 mcg); Vancomycin (VA) (30 mcg); Tobramycin (TOB) (10 mcg); Amikacin (AK) (30 mcg) discs were used.

Determination of Lactic Acid Amounts

5 mL of distilled water and 250 microliters of phenolphthalein were added to each of the 5 mL samples activated for 18 hours at 37 C in MRS broth. It was titrated by adding 0.1 N NaOH dropwise. The number of drops is multiplied by 4. The lactic acid content of each sample was determined so that each 1 ml of 0.1 N NaOH consumed was equivalent to 0.009 g of lactic acid [8, 9].

Determination of Cholesterol Assimilation Capacities

In order to determine the cholesterol assimilation capacities of LAB isolated from the stool microflora of healthy infants, the total cholesterol level of serum collected from patients with serum cholesterol level of 250-300 mg/dL who applied to Amasya University Sabuncuoğlu Şerefeddin Training and Research Hospital was measured. 1 mL of cultures activated for 18 hours in MRS broth was taken and added to [0.3% (Oxgall, Sigma)] MRS broth (3 mL) containing bile salt. Each sample, 1 mL of sterile serum, was collected into 5 ml tubes by passing it through a 0.45 micro m disposable (Milipore, USA) filter with a final concentration of 100 mg/mL. After incubation at 37ºC for 24 hours, the final cholesterol values of the supernatants were determined by centrifugation at 5000 × g at 4ºC for 10 minutes. Measurements were carried out in the Biochemistry Laboratory of Amasya University Sabuncuoğlu Şerefeddin Training and Research Hospital on a Roche HITACHI cobas 8000 device. Cholesterol reduction rates were determined by comparing the cholesterol amount of the samples before incubation with the amount of cholesterol after incubation.

RESULTS

Collection of Stool Samples and Identification of LAB

Only commercial infant formula, who applied to Sivas Cumhuriyet University Hospital Pediatrics outpatient clinic for routine controls, was born vaginally, did not use antibiotics and probiotics in the last 3 months, did

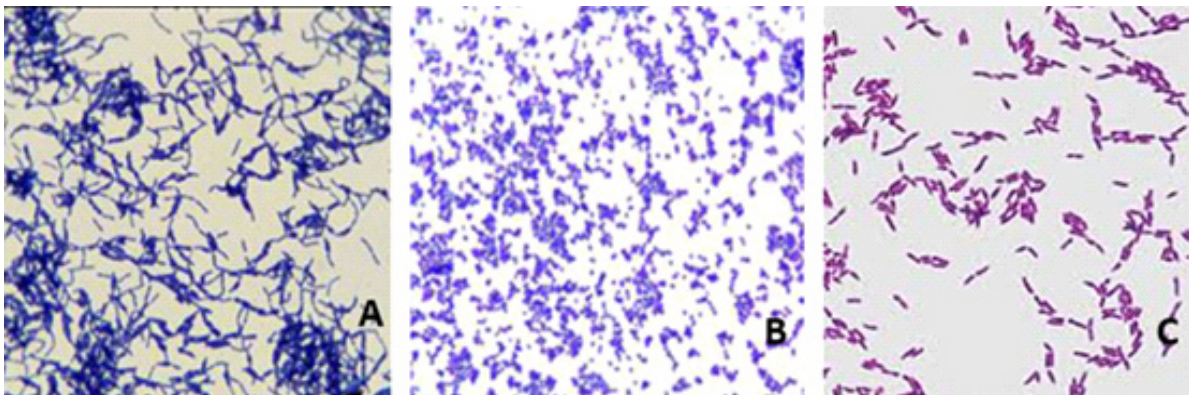

Fig 1. Gram stain images of 3 different species isolated from stool microflora, respectively (A) *L. rhamnosus*, (B) *P. acidilactici*, and (C) *L. reuteri*.

not have a history of hospitalization, took only breast milk, received and/or receives commercial infant formula in addition to breast milk. For LAB isolation, 22 gram positive, white, opaque-looking pure isolates were selected. It was observed that the babies from whom 22 selected isolates were obtained were 36 days old on average.

Gram stain images of some isolates are given in Fig. 1. Identification of isolates was performed using MALDI-TOF MS.

According to the MALDI-TOF MS method, which is based on looking at the protein profiles of microorganisms (protein, peptide, sugar) and large organic molecules after they are ionized and passed through electric and/or magnetic fields, Lactobacillus rhamnosus is the most common strain with a rate of 59%. Lactobacillus paracasei follows with 13.6%. Then there were 9% Lactobacillus reuteri, Lactobacillus gasseri and 4.5% Enterococcus faecalis, Pediococcus acidilactici strains. Gram stain images of some species are given in Fig. 1.

Determination of Probiotic Properties of LAB

Acid and Bile Tolerance of Stool LAB

Determination of resistance to acid and bile salts of LAB isolated from stool swabs was performed in vitro by exposing the strains to pH (pH 2.0, 2.5 and 3.0) adjusted PBS. The survival rates of LAB in pH

Table 1. Survival rates of LAB in a low pH environment

	pH = 2.0			pH = 2.5			pH = 3.0		
	Beginning	**End**	**%Life**	**Beginning**	**End**	**%Life**	**Beginning**	**End**	**%Life**
***L. rhamnosus* T1**	8.60	-	-	8.68	5.57	64.1	8.75	8.33	95.2
***L. rhamnosus* T2**	8.58	-	-	8.63	5.42	62.8	8.52	7.81	91.6
***L. rhamnosus* T3**	8.63	2.87	33.2	8.72	6.46	74	8.75	8.38	95.7
***L. rhamnosus* T4**	8.55	2.35	27.4	8.58	5.73	66.7	8.63	7.94	92
***L. rhamnosus* T5**	8.87	-	-	8.90	6.65	74.7	8.75	8.23	94
***L. rhamnosus* T6**	8.60	-	-	8.62	5.81	67.4	8.69	8.17	94
***L. reuteri* T7**	8.50	-	-	8.52	5.45	63.9	8.71	8.43	96.7
***L. paracasei* T8**	8.60	2.56	29.7	8.63	6.81	78.9	8.74	8.52	97.4
***P. acidilactici* T9**	8.90	-	-	8.92	6.72	75.3	8.83	8.25	93.4
***L. rhamnosus* T10**	8.89	-	-	8.75	6.38	72.9	8.72	8.28	94.9
***L. rhamnosus T*11**	8.77	-	-	8.70	6.41	73,6	8.81	8.31	94.3
***L. reuteri* T12**	8.55	-	-	8.45	5.71	67.5	8.61	8.15	94.6
***E. faecalis* T13**	8.65	2.80	32.3	8.73	6.29	72	8.77	8.01	91.3
***L. rhamnosus* T14**	8.72	-	-	8.95	6.76	75.5	8.91	8.12	91.1
***L. rhamnosus* T15**	8.90	-	-	8.98	6.72	74.8	8.87	8.18	92.2
***L. gasseri* T16**	8.65	-	-	8.83	6.61	74.8	8.75	8.02	91.6
***L. paracasei* T17**	8.75	2.51	28.6	8.80	6.56	74.5	8.86	8.09	91.3
***L. rhamnosus* T18**	8.80	-	-	8.92	6.87	77	8.72	8.35	95.7
***L. rhamnosus* T19**	8.75	-	-	8.63	5.97	69.1	8.66	8.21	94.8
***L. rhamnosus* T20**	8.80	2.29	26	8.90	6.91	77.6	8.82	8.35	94.6
***L. gasseri* T21**	8.60	-	-	8.55	5.79	67.7	8.58	7.83	91.2
***L. paracasei* T22**	8.52	-	-	8.68	5.89	67.8	8.73	8.03	91.9

Survival number (log cfu/mL) and Rate (%) of Isolates at the end of the 3rd hour in media with different pH value.

medium are given in Table 1 and the survival rates in MRS medium containing low bile salt are given in Table 2.

pH Change

After 18 hours of incubation at 37ºC in MRS broth, the mean pH values of L. rhamnosus strains were 4.36; The average pH value of L. reuteri strains was 4.04; The mean pH value of L. paracasei strains was 4.03; The average pH value of L. gasseri strains was measured as 3.9.

Determination of Antibiotic Sensitivities

In our study, while T3 and T18 strains were susceptible and semi-susceptible to ampicillin antibiotic, all other strains were resistant; T2, T12, T13, T17, T18 strains were resistant to Penicillin antibiotic, while all other strains were found to be susceptible. Although resistance to antibiotics varies according to strains, resistance to ampicillin was 91% for all strains; resistance to teicoplanin 59%; gentamicin resistance 36%; resistance to netilmicin 54%; vancomycin resistance 64%; tobramycine resistance 95%; resistance to amikacin was observed at a rate of 100%

Determination of LAB's Lactic Acid Amounts and Cholesterol Removal

The amount of rubbery acid was calculated with

Table 2. Survival rates of LAB in a low bile salt MRS medium

	%0.3			%0.5			%1		
Lactic acid bacteria	**Beginning**	**End**	**%Life**	**Beginning**	**End**	**%Life**	**Beginning**	**End**	**%Life**
***L. rhamnosus* T1**	8.92	7.74	86.7	8.73	5.46	62.5	8.56	5.21	60.8
***L. rhamnosus* T2**	8.65	-	-	8.96	-	-	9.12	6.36	69.7
***L. rhamnosus* T3**	8.81	7.85	89.1	9.25	6.21	67.1	8.87	-	-
***L. rhamnosus* T4**	8.46	7.43	87.8	8.99	6.32	70.3	8.60	5.76	66.9
***L. rhamnosus* T5**	9.23	8.56	92.7	9.17	5.52	60.1	8.79	4.24	48.2
***L. rhamnosus* T6**	8.96	5.23	58.3	8.65	5.47	63.2	9.14	-	-
***L. reuteri* T7**	9.12	6.79	74.4	9.33	6.36	68.1	8.54	4.79	56
***L. paracasei* T8**	9.17	6.84	74.5	8.87	5.94	66.9	8.98	4.93	54.8
***P. acidilactici* T9**	8.97	5.92	65.9	9.26	6.83	73.7	8.59	5.23	60.8
***L. rhamnosus* T10**	9.24	7.26	78.5	8.75	5.77	65.9	8.96	6.02	67.1
***L. rhamnosus T*11**	9.11	8.35	91.6	8.83	4.89	55.3	8.64	6.11	70.7
***L. reuteri* T12**	8.82	6.54	74.1	8.24	5.26	63.8	8.47	6.39	75.4
***E. faecalis* T13**	8.98	2.80	31.1	8.75	5.96	68.1	8.63	5.42	62.8
***L. rhamnosus* T14**	9.02	7.32	81.1	9.26	6.85	73.9	9.25	6.82	73.7
***L. rhamnosus* T15**	9.36	8.21	87.7	8.98	6.47	72	9.36	5.97	63.7
***L. gasseri* T16**	8.35	6.67	79.8	8.74	5.71	65.3	8.96	-	-
***L. paracasei* T17**	8.52	2.51	29.4	9.02	-	-	8.81	6.28	71.2
***L. rhamnosus* T18**	9.18	8.43	91.8	8.96	6.32	70.5	8.79	5.86	66.6
***L. rhamnosus* T19**	8.69	-	-	9.11	7.12	78.1	8.93	4.54	50.8
***L. rhamnosus* T20**	8.73	2.29	26.2	8.99	-	-	8.86	-	-
***L. gasseri* T21**	8.67	7.58	87.4	8.88	6.47	72.8	9.11	6.12	67.1
***L. paracasei* T22**	9.28	-	-	9.17	-	-	9.02	-	-

Survival number (log cfu/mL) and rate (%) of isolates at the end of the 3rd hour in MRS media containing different bile salts

the help of phenolphthalein and NaOH for the cultures activated in MRS broth at 37°C for 18 hours. The average amount of lactic acid produced by LAB and probiotic bacteria after being activated in MRS broth at 37°C for 18 hours is 361.2. It was observed that *L. rhamnosus* T11 strain had the highest lactic acid production capacity with 496.8. *L. paracasei* T17 strain had the lowest lactic acid production with 86.4.

Among the strains used in our study, the strains with the highest cholesterol assimilation capacity were T21 and T22 strains with a rate of 39.1%. These strains are followed by T18, T16, T17, T17, T4, T9, T13 strains. The strain with the least cholesterol assimilation capacity was found to be the T20 strain. The lactic acid amounts and cholesterol removals of LAB are shown in Table 3.

DISCUSSION

There are many factors that shape the baby's microflora immediately after birth. Some of those; The birth type of the baby, health and immunological status, whether the baby receives breast milk, the mother's diet, the GIS (Gastro-intestinal System) transition time and GIS pH are factors such as stress [10].

In another study evaluating the microbiota of infants fed with formula (only or with breast milk), the bacterial groups that colonize formula-fed infants more frequently were *Enterococcus, C. coccoides, Atopobium cluster, B. vulgatus*, *B. longum* subsp. *longum* is indicated [11].

E.coli, C. difficile, B. fragilis species are less in breastfed infants, but *Bifidobacteria*, especially *B.*

Table 3. LAB's lactic acid production and cholesterol removal rates

Lactic acid bacteria	Lactic acid amount (mg/dL)	Cholesterol ratio (mg/dL)	Cholesterol removal (%)
	74		
L. rhamnosus T1	417.6	59	20.27
L. rhamnosus T2	255.6	53	28.3
L. rhamnosus T3	147.6	54	27.02
L. rhamnosus T4	446.4	49	33.7
L. rhamnosus T5	273.6	57	22.9
L. rhamnosus T6	360	52	29.7
L. reuteri T7	453.6	50	32.4
L. paracasei T8	360	54	27.02
P. acidilactici T9	432	50	32.4
L. rhamnosus T10	432	58	21.6
L. rhamnosus T11	496.8	56	24.3
L. reuteri T12	113.4	52	29.7
E. faecalis T13	453.6	51	31.08
L. rhamnosus T14	482,6	55	25.67
L. rhamnosus T15	252	48	35.13
L. gasseri T16	468	48	35.13
L. paracasei T17	86.4	48	35.13
L. rhamnosus T18	345.6	46	37.83
L. rhamnosus T19	396	52	29.7
L. rhamnosus T20	374.4	69	6.75
L. gasseri T21	417.6	45	39.1
L. paracasei T22	482.4	46	39.1

breve and *B. longum*, are early colonizers in infants, but *B. animalis* subsp. lactis occurs only with the type of diet and is not the common infant gut microorganism [12, 13].

Similar to our study, fecal *Bifidobacterium* and *Lactobacillus/Enterococcus* counts were found to be higher in breastfed infants compared to formula-fed infants at 6 months [14]. *L. acidophilus* (20%), *L. acidophilus-3* (10%), *L. brevis* (30%), *L. casei* (15%) bacteria were isolated from colostrum. *L. brevis* (41.2%), *L. fermentum* (11.8%), *L. reuteri* (5.9%), *L. rhamnosus* (11.8%), *L. plantarum* (29.4%) were detected in stool [15]. The results of the study are similar to the results of our study. In our study, stool samples were collected from 45 healthy babies and 22 bacterial isolates were evaluated. 54.5% of the babies to whom these bacterial isolates belong are exclusively breastfed; While 31.8% are fed with commercial infant formula, 13.6% are fed with infant formula as well as breast milk. Of the strains isolated from the stools of exclusively breastfed babies, 41.6% were identified as *L. rhamnosus,* 16.6% *L. reuteri,* 25% *L. paracasei,* 12.5% *L. gasseri,* 12.5% *P. acidilactici*. Of the strains isolated from the stools of infants fed only infant formula, 62.5% *L. rhamnosus*, 12.5% *L. paracasei,* 12.5% *L. gasseri,* 12.5% *E. faecalis;* All of the strains isolated from the stools of infants who received infant formula in addition to breast milk were identified as *L. rhamnosus.* Similar to the studies, lactic acid bacteria were also found in our study. However, the stools of exclusively breastfed babies have greater microbiological diversity.

In a study of *L. rhamnosus, L.casei, L. paracase*i strains obtained from different sources (meat and dairy products, sourdough doughs, wine, beverages, vegetables and human body), survival and growth were improved after exposure to low pH values for 2 hours. 61% of strains evaluated for their restart capacity 2 hours at pH =2.5; 3.3% continued to grow after incubation at pH = 1.5. All strains survived in the presence of 1.5% bile salt after 24 hours of incubation at 37 °C [16].

The viability rates of *L. rhamnosus* 19 strain obtained from Kenya's traditional fermented food (two) at pH =2.0 decreased from 7.29 log10 cfu/ml to 4.11 log10 cfu/ml at the end of the 3rd hour. *L. rhamnosus* 19 strain was incubated at pH = 2.0 for 3 hours, and the survival rate of *L. rhamnosus* in MRS broth with added 0.3% bile salt was reported to be 3.85 log10 cfu/mL at the end of 48 hours [17].

In one study, a total of 22 *Lactobacillus strains* isolated from infant feces, low pH and resistance to bile salts, as well as 8 isolates (*L. reuteri* 3M02, 3M03; L. gasseri 4M13, 4R22, 5R01, 5R02, 5R13; *L. rhamnosus* 4B15) were evaluated.) has high tolerance to acids (99.1%) and bile salts (99.9%) [18]. It was stated that there was no significant decrease in the amount of *P. acidilactici* strain kept in acidic salt solutions (pH = 2.0) [19].

107 lactic acid bacterial isolates were isolated from 6 donor infant meconium from the Roubaix hospital in northern France. Some of the *E. faecalis* strains produced lactic acid up to 7.06g/l after 24 hours of incubation [20]. In our study, the lactic acid amount of *E. faecalis* strain obtained from a baby who received formula was 453.6 mg/dL, and lactic acid production was below this result. In another study, the average amount of lactic acid produced by *L. rhamnosus* strains obtained from vaginal swabs was 585 mg/dL; The average amount of lactic acid produced by *L. paracasei* strains was 458 mg/dL; The average lactic acid content of *P. acidilactici* strains was found to be 682 mg/dL [21]. In our study, while the lactic acid production amount of *L. rhamnosus* strains was found to be 360 mg/dL on average, it was determined that the strain with the highest lactic acid production capacity was *L. rhamnosus* T11 with 496.8.

In our study, it was determined that LAB isolated from baby feces was resistant to low pH environment and showed 100% viability especially in pH 2.5-3.0 environments. It was observed that especially *L. rhamnosus* strains survived better in pH 2.0 environment. *L. paracasei* strains isolated from Italian Castelmagno cheese after 24 hours of incubation, mean pH values of *L. paracasei* strains were found to be 3.87.30 Average pH values of *L. rhamnosus* strains isolated in our study were 4.36; The average pH value of *L. reuteri* strains was 4.04; The mean pH value of *L. paracasei* strains was 4.03; The average pH value of *L. gasseri* strains was measured as 3.9. [22].

L. rhamnosus, *L. paracasei* strains obtained from human faeces showed resistance to vancomicin, colistin sulfate, oxalinic acid, gentamicin, oxalinic acid, kanamicin under high in vitro conditions [23]. In our study, *L. rhamnosus, L. rhamnosus, L. paracasei* strains were similarly resistant to vancomicin but

highly sensitive to gentamicin. In our study, while *P. acidilactici* was resistant to ampicillin, tobramycin, amikacin, it showed sensitivity to penicillin and teicoplanin. These results are similar to other studies [24, 25]. In our study, the *E. faecalis* strain isolated from a baby who received only formula was found to be resistant to all antibiotics used in the study. Similarly, in other studies, it was determined that the *E. faecalis* strain was resistant to many antibiotics [26, 27].

L. paracasei subsp. *paracasei* (41), *L. fermentum* (24), *L. rhamnosus* (11), *L. casei* (17), *Lactobacillus* spp. (11) strains have been reported to have strong antimicrobial effects, especially on *S. aureus*. 28 *L. monocytogenes* ATCC 3512, *L. innocus* 103982, B. subtilis ATCC 6633, S. aureus ATCC 3386, *E. coli* CIPI 103982 have been reported to show antagonistic activity against pathogenic bacteria [18].

It is quite remarkable that LAB has potential cholesterol-lowering effects in recent studies [28]. High cholesterol is associated with cardiovascular diseases, a major cause of death worldwide. Current therapeutic measures, lifestyle changes, dietary interventions, pharmaceutical agents are insufficient to regulate cholesterol level. Probiotic bacteria show the potential to lower cholesterol levels through inhibition of 3-hydroxy-3-methylglutaryl coenzyme A enzymes by different mechanisms, including bile salt hydrolase.

In the study, a total of 22 *Lactobacillus* strains isolated from infant feces were evaluated, 8 isolates (*L. reuteri* 3M02, 3M03; *L. gasseri* 4M13, 4R22, 5R01, 5R02, 5R13; *L. rhamnosus* 4B15), especially *L. rhamnosus* 4B15 and *L. gasseri* 4M13 strains have been shown to have significant cholesterol-lowering capacity compared to other strains [18].

In one study, it was stated that *L. rhamnosus* strain showed less cholesterol assimilation with a cholesterol assimilation value of 13.21% compared to *L. acidophilus, L. fermentum, B. Lactis* [29]. In our study, *L. rhamnosus, L. reuteri, L. gaseri, L. paracasei*. Among the strains, the strains with the highest cholesterol removal were *L. gasseri* (39.1%) and *L. paracasei* (39.1%) isolated from exclusively breastfed infants. The mean cholesterol assimilation percentage of the *L. paracasei* strain was 28.5%; The mean cholesterol assimilation percentage of the *L. reuteri* strain was calculated as 31.05%. With these values, previous studies showed, on average, higher cholesterol removal.

Limitations

The biggest limitation of our study is that the study is limited to Sivas province and it is necessary to study with more stool samples in order to fully reveal the microbial difference between breast milk and formula babies.

CONCLUSION

In the light of our results, it is thought that the isolated strains have strong probiotic potential, especially *L. gasseri* T21 and *L. paracasei* T22 strains, which can be used in the production of preparations alone or together with other *Lactobacillus* strains. In recent years, with the addition of prebiotics and probiotics to commercial infant formulas, the difference between infants receiving breast milk and infant formula has decreased. However, breast milk, which is a cheap, reliable, natural food that meets all the needs of the baby on its own, should always be the first choice. It is important to evaluate the use of strains as oral preparations clinically well.

Authors' Contribution

Study Conception: TMC, EK, EK; Study Design: TMC, EK, EK; Supervision: TMC, EK, EK; Funding TMC, EK, EK; Materials: TMC, EK, EK; Data Collection and/or Processing: TMC, EK, EK; Statistical Analysis and/or Data Interpretation: TMC, EK, EK; Literature Review: TMC, EK, EK; Manuscript Preparation: TMC, EK, EK and Critical Review: TMC, EK, EK.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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This article describes *Lactobacillus* spp. in breastfed and non-breastfed infants. It was prepared from the master's thesis named research of bacteria and determination of some probiotic characters.

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