JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

IDENTIFICATION AND CHARACTERIZATION OF *LIMOSILACTOBACILLUS FERMENTUM* ISOLATED FROM KANGRA CURD

Disha Chauhan[1] and Ranjit Kumar[2]*

Address(es):
[1] Department of Animal Sciences, Central University of Himachal Pradesh, Dharamshala, Himachal Pradesh, India.
[2] Department of Zoology, Nagaland University, Lumami, Nagaland.

*Corresponding author: ranjitzool17@gmail.com

https://doi.org/10.55251/jmbfs.12381

ARTICLE INFO

Received 14. 2. 2025
Revised 10. 2. 2026
Accepted 23. 2. 2026
Published 1. 4. 2026

Regular article

OPEN ACCESS

ABSTRACT

Curd, commonly called “Dahi," is a fermented milk product widely consumed in India for its probiotic benefits. The composition of the probiotic strains in curd varies with geographical location. This study aimed to identify the most predominant strain of *Lactobacillus* in the curd collected from Chhattri village, Shahpur tehsil, Kangra district, Himachal Pradesh. The sample was inoculated in MRS broth and streaked on the MRS agar plate. The most prevalent isolate was characterized on morphological, biochemical, and molecular bases. Comprehensive analysis, including Gram staining, catalase test, oxidase test, motility test, and indole test, as well as evaluations of pH, bile, salt, and temperature tolerance was performed. The isolate was gram-positive, catalase, oxidase, sulfide, and indole-negative. It showed maximum growth at 0.5% salt, 0.5% bile salts, and 37ºC. Optimal growth occurred between pH 6-8, with peak growth at pH 7. Molecular identification and phylogenetic analysis confirmed the isolate as an *L. fermentum* strain. This strain exhibited the highest prevalence in Kangra and showed promising probiotic traits with potential gastrointestinal health benefits. Its dominance highlights the probiotic richness of curd obtained from the lower hills of Himachal Pradesh.

Keywords: Characterization, Curd, *Lactobacillus*, Probiotic

INTRODUCTION

The Kangra district of Himachal Pradesh, India is renowned for its rich dairy heritage. Kangra curd, which is prepared traditionally, provides an ideal environment for the proliferation of beneficial microorganisms. The consumption of such fermented dairy products has been associated with various health benefits in Indian traditional medicine **(Bajaj *et al*., 2022)**. Curd is an ideal medium for delivering beneficial microbes to the human gut (**Kariyawasam *et al*., 2021**). The presence of probiotic strains varies geographically. The presence of specific probiotic strains in curd can enhance its nutritional profile and contribute to its potential health benefits. Probiotics may aid in better nutrition, growth, and prevention of several gastrointestinal diseases by increasing the number of useful microorganisms, and strengthening the functioning of the gut mucosal barrier (**Verna and Lucak, 2010; Zeng *et al*., 2008**). This can be used to treat irritable bowel syndrome, enteritis, other gastrointestinal problems, and lactose intolerance (**Markowiak and Slizewska, 2017**). It has been shown to reduce the risk of respiratory and urinary tract infections (**Amara and Shibl, 2015**) and positively influence cognitive function through the gut-brain axis, which is a two-way communication system connecting the gut and the brain. Probiotics may be helpful against the symptoms of anxiety, depression, and stress (**Nagpal *et al*., 2012**). It stimulates the production of antimicrobial peptides, enhances the activity of immune cells, and regulates the inflammatory response (**Mazziotta *et al*., 2023**). It also enhances the absorption of minerals, including calcium, iron, and magnesium (**Shi *et al*., 2016**). Most probiotics are classified within the *Lactobacillus* genus (**Van Baarlen *et al*., 2013**). Different studies have described the well-established *Lactobacillus* species, but the isolation and characterization of relatively lesser-explored members like *Limosilactobacillus fermentum* from curd is required (**De *et al*., 2017; Goyal, 2012**).
Limosilactobacillus fermentum, formerly known as *Lactobacillus fermentum*, is a species of lactic acid bacteria (LAB) commonly found in fermented food items, including curd (**Ao *et al*., 2012; Kumara *et al*., 2019**). This study is designed to isolate and identify the predominant probiotic strain in Kangra curd based on morphological, biochemical, and molecular characteristics.

MATERIALS AND METHODS

Sample collection

Samples were aseptically collected in a sterile plastic sample container from Chhatri village of Shahpur tehsil of Kangra, Himachal Pradesh (Latitude: **32°13'27.1"N, Longitude: 76°09'02.7"E, Elevation 760 M**) and transferred immediately to the laboratory.

Isolation of probiotic strain:

A 1 g of curd sample was suspended in 9 ml of Phosphate Buffer saline (pH=7.2). The suspension was homogenized and serially diluted to 10^2. 9 ml of de Man, Rogosa, and Sharpe (MRS) broth was supplemented with 1 ml of the diluted suspensions. This was incubated at 37^0C for 24 hours. On completion of the incubation period the culture in broth was streaked on MRS agar by quadrant plate method. Plates were then incubated at 37°C for 48 hours to collect the most prevalent colony, the process was repeated to get isolation of the single most prevalent strain.

Morphological and Biochemical Characterization:

Pure colonies were transferred into MRS broth and grown for 24 hours, then it was further characterized morphologically and biochemically to understand its physicochemical characteristics. Gram staining was performed to determine the Gram reaction of the isolates. The isolates were then subjected to catalase, oxidase, sulfide, indole, and motility tests.

Salt tolerance test

Growth at 0.5%, 1%, 1.5%, and 2% salt was observed by adjusting the salt concentration of MRS broth to 0.5%, 1%, 1.5%, and 2%. Sterile test tubes were filled with 9 ml of the medium. 1 ml of broth containing *L. fermentum* was further added to the test tube and properly mixed. The tubes were then incubated at 37ºC for 24 hours and analyzed for growth after measuring optical density at 600 nm (OD600) using a UV-Vis Spectrophotometer.

Bile tolerance test

MRS broth containing 0.5, 1, 1.5, and 2% bile salts was prepared and 1 ml of broth containing *L. fermentum* was added to 9 ml of medium with varying concentrations of bile. The test tubes, after incubation for 24 hours at 37ºC were observed for growth after measuring OD600 using a UV-Vis Spectrophotometer.

Temperature tolerance test

Growth at different temperatures was observed by mixing 9 ml of medium with 1 ml of broth containing *L. fermentum*. The tubes were incubated at 25 ºC, 37ºC, and 45 ºC for 24 hours and observed for growth after measuring OD600 using a UV-Vis Spectrophotometer.

pH tolerance test

Growth at different pH will be observed by adjusting the pH of MRS broth to 2 to 8 using 1N hydrochloric acid (HCl) or 10% w/v sodium hydroxide (NaOH). 9 ml of the medium was distributed into each sterile test tube and 1 ml of broth containing *L. fermentum* was added to these tubes. The tubes, after incubation at 37ºC for 24 hours, were observed for growth after measuring OD600 using a UV-Vis Spectrophotometer.

Survival Percentages (SR) of the isolate were calculated as follows:

$$SR = \frac{\text{OD600 of the strain (in different conditions)}}{\text{OD600 of the strain (control)}} * 100$$

Molecular Identification

Genomic DNA was extracted from the culture by ZR Fungal/Bacterial DNA MiniPrep kit. This was further subjected to PCR amplification of the 16S rRNA gene by using the universal primers: 27F (AGAGTTTGATCCTGGCTCAG) and 1492R (TACGGYTACCTTGTTACGACTT), the resulting product was observed on Agarose gel electrophoresis. It was purified by gel elution; the PCR product was subjected to Sanger sequencing. Sequences were then observed and examined with the assistance of Finch TV software version 1.4. The nucleotide sequences were compiled and subjected to a similarity search using the BLAST tool.

Phylogenetic analysis

Homologous sequences were identified through BLASTn searches in the NCBI GenBank database, selecting sequences with ≥95% similarity. Sequence alignment was performed using Clustal W software. Phylogenetic analysis was conducted using with Neighbour joining method to compute evolutionary distances (Saitou and Nei, 1987). Bootstrap analysis with 1000 replicates was applied to assess the statistical support of the tree topology. Evolutionary analyses were conducted in MEGA11 (**Tamura *et al*., 2021**)

RESULTS

The colony obtained was circular, elevated, and creamy white, margins were smooth (Figure - 1). It was gram-positive bacteria while it showed negative results for catalase, oxidase, indole, and sulfide tests. It was non-motile (Table - 1). The colony obtained from three different plates of DC1 showed varied degrees of survival in different salt (NaCl) concentrations (Figure - 2). It was 94.716±0.525 % at 0.5% NaCl, 89.565±0.262 % at 1% NaCl, 85.158±0.348 % at 1.5% NaCl and 40.214±5.514 % at 2% NaCl (Table - 2). Maximum growth was found at 0.5% salt. The colony of DC1 showed a variable range of survival percentage in different concentrations of bile. It was 26.311±0.483 % at 0.5% bile, 20.874±0.320 % at 1% bile, 20.565±0.788 % at 1.5% bile, and 16.502±0.428 % at 2% bile. Maximum survival was found at 0.5% bile concentration. DC1 strain showed a variable range of survival percentages at different temperatures. It was 95.021±0.086 % at 25°C, 99.977±0.015 % at 37°C, and 70.984±0.309 % at 45°C. Maximum growth was found at 37°C. DC strain showed variable survival percentages at different pH values. It was 25.786±1.509 % at pH 2, 33.746±3.145 % at pH 3, 61.023±0.745 % at pH value 4, 90.724±2.649 % at pH 5, 95.623±2.733 % at pH 6, 96.353±2.886 % at pH value 7 and 95.369±5.152 % at pH 8. The maximum survival was obtained at pH 7.

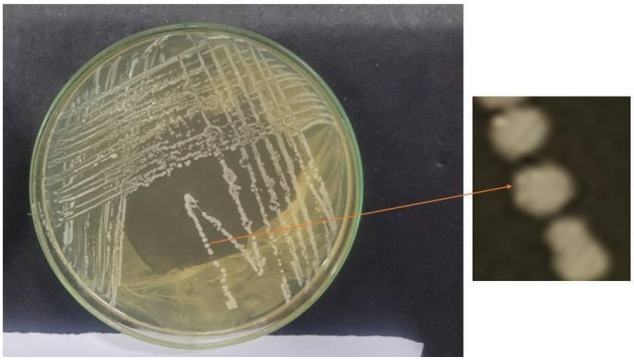

Figure 1 Colony of DC1 isolated from Kangra curd on agar plate after incubation at 37^{0}C for 48 hours. The isolate collected from the centre shows a circular shape, creamy white color, and smooth margins.

Table 1 shows the physical and chemical characteristics of the DC1 isolate obtained from Kangra curd.

Microbial Colony	**DC1**
Colony morphology	Circular
Color	Creamy white
Elevation	Convex
Margins	Smooth
Gram	Positive
Biochemical tests:	
Catalase	Negative
Oxidase	Negative
Sulphide	Negative
Indole	Negative
Motility	Negative
Phenotypic characterization	*Lactobacillus*

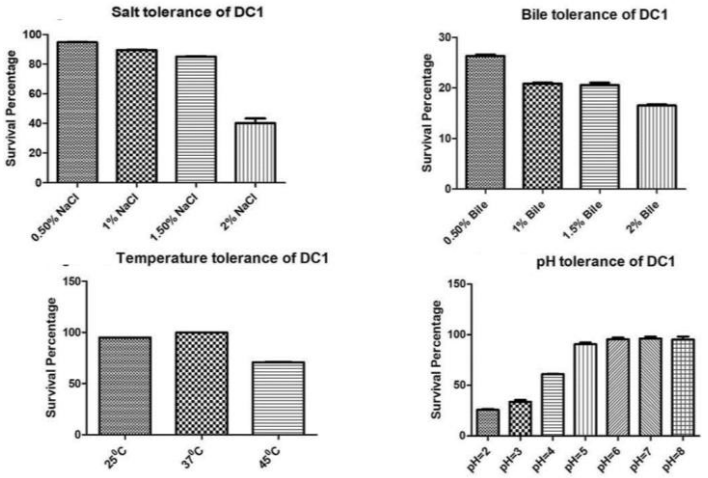

Figure 2 The survival percentages of DC1 in different salt (NaCl), bile, temperature, and pH values.

Table 2 The values of survival rates (%) of DC1 isolates in different salt (NaCl),bile, temperature and pH values.

Condition		**Survival percentage (%)**
Salt	**0.50% NaCl**	94.716±0.525
	1% NaCl	89.565±0.262
	1.50% NaCl	85.158±0.348
	2% NaCl	40.214±5.514
Bile	**0.50% Bile**	26.311±0.483
	1.0% Bile	20.874±0.320
	1.50% Bile	20.565±0.788
	2.0% Bile	16.502±0.428
Temperature	**25°C**	95.021±0.086
	37°C	99.977±0.015
	45°C	70.984±0.309
pH	**pH-2**	25.786±1.509
	pH-3	33.746±3.145
	pH-4	61.023±0.745
	pH-5	90.724±2.649
	pH-6	95.623±2.733
	pH-7	96.353±2.886
	pH-8	95.369±5.152

Molecular Analysis through DNA sequencing and analysis for homology through BLAST confirmed the presence of *Limosilactobacillus fermentum,* it showed 99.93% similarity. The hit strain was CECT 562(T) and the accession was AJ575812.

Table 3 BLAST results of the obtained sequence

Hit taxon name	*Limosilactobacillus fermentum*
Hit strain name	CECT 562(T)
Accession	AJ575812
% Similarity	99.93

The phylogenetic tree (Figure 3) illustrates the relationship of the isolate *Limosilactobacillus fermentum* C with other closely related strains based on 16S

rRNA sequences. Bootstrap values support the reliability of each branch. The short branch lengths between *L. fermentum* C and other *L. fermentum* reference strains (CIP 102980, NBRC 15885, NCDO 1750) indicate high genetic similarity, suggesting close evolutionary relationships. The relatively short branch lengths indicated genetic similarity while minor divergence suggested potential strain specific adaptations. These findings align with bacterium's ecological niche.

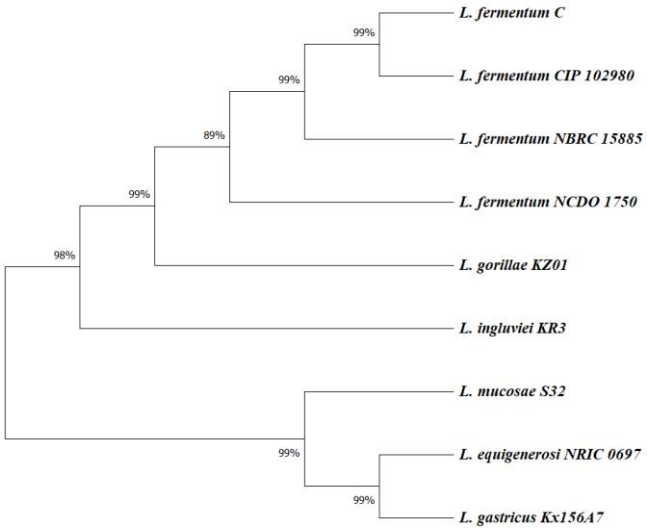

Figure 3 The results of phylogenetic tree analysis of *Limosilactobacillus fermentum* (denoted as *L. fermentum C*) based on 16s rRNA sequences.

DISCUSSION

The probiotic isolate has been identified based on morphological, biochemical, and molecular characterization. In China, 22.6% of the strains of probiotics used to produce conventionally fermented milk are *L. fermentum* (**Ao *et al*., 2012**). *L. fermentum* strains from dairy products have been also isolated in Ethiopia, China, and Karnataka state of India (**Ao *et al*., 2012; Bao *et al*., 2010; Kumara *et al*., 2019**). The physiochemical characteristics of *L. fermentum* include circular morphology, creamy white color, convex elevation, and smooth margins along with Gram-positive, catalase, oxidase, sulfide, and indole-negative tests (**Karami *et al*., 2017**). Our isolate had similar characteristics obtained from Kangra curd. On molecular characterization, it was identified as *Limosilactobacillus fermentum*. This is the first report on the dominance of this strain in the Kangra district of Himachal Pradesh.

The probiotic potential of a bacterial isolate is based on the ability to tolerate different physiological conditions like the human body's temperature, bile salts, and the acidic environment with low Ph levels that are commonly found in the GI tract. These features increase the likelihood that bacterial strains will reach the intestines and provide health benefits like assisting with digestion and maintaining a healthy gut microbiome (**Argyri *et al*., 2013; Shokryazdan *et al*., 2014**). The ability to tolerate NaCl concentrations can vary within the *L. fermentum* species. Some *L. fermentum* isolates could tolerate NaCl concentrations up to 6.5% (**Adikari *et al*., 2021**). *L. fermentum* showed only 60% survival at 1% NaCl (**Sharafi *et al*., 2015**). Our strain showed 94.71% survival at 0.5% salt and 89% survival at 1% salt concentration. The strain obtained from Kangra showed effective growth at both 0.5% and 1% salt concentration.

The capability of LAB to withstand bile is pivotal for their passage through the harsh conditions of the small intestine (**Khushboo *et al*., 2023**). The potential mechanisms include active expulsion of bile salts, hydrolysis of bile salt, and alteration in the structure and composition of the cellular membrane and cell wall (**Urdaneta and Casadesús, 2017; Ruiz *et al*., 2013**). The survival percentage of *L. fermentum in* previous studies showed a decrease with an increase in bile concentration; maximum growth was found at 0.3% bile (**Srinu *et al*., 2013**). These results are similar to our findings where higher bile concentrations are associated with decreased bacterial survival, demonstrating the inhibitory effect of bile on bacterial viability. Similar trends were also observed by Tulumoglu *et al*. 2014 on the growth of *L. fermentum* with different concentrations of bile.

L. fermentum is shown to exhibit good survival at 37°C (**Patil *et al.* 2010**). According to a study conducted by Riaz *et al*., the growth of *L. fermentum* was highest at 25°C (**Riaz *et al*., 2010**) and weak at 45°C (**Patil *et al.* 2010**). Our isolate was able to survive best at 37°C, and survival dropped to about 95% at 25°C and 70% at 45°C. These findings support the findings by **Patil *et al.* 2010**.

Table 4 Comparative analysis of tolerance traits of *L. fermentum* with other common probiotic strains.

Trait	*L. fermentum*	Other probiotic strains	References
pH tolerance	Grow optimally at 6-8. Moderate survival at pH 2.	*L. rhamnosus, L. casei, L. paracasei*: consistent values from pH 3 to 7. But at pH 2, lower survival than *L. fermentum.*	**Reale *et al*., 2014; Sharobe *et al*., 2015**
Bile tolerance	Can tolerate 0.5% bile, drastic reduction above 1% bile	*L. acidophilus*: Highly sensitive; *L. rhamnosus*: can tolerate 1.0–1.5% bile	**Mirlohi *et al*., 2009; Reale *et al*., 2014**
Temperature tolerance	Highly viable at 37^0C	*L. rhamnosus, L. fermentum, L. plantarum*: optimal survival at 37°C	**Katiku *et al*., 2022; Palaniyandi *et al*., 2017.**
Salt tolerance	Good growth at 0.5 and 1% NaCl	*L. acidophilus*: tolerates up to 0.5% NaCl; *L. plantarum*: shows moderate growth at 1% NaCl	**Reale *et al*., 2014**

The adeptness of LAB strains to persist in acidic environments is required for their viability in the gastrointestinal tract (**Khushboo *et al*., 2023**). Different strains of *Lactobacillus* differ in their ability to tolerate acid (**Sharma *et al*., 2021**). **Riaz *et al*., 2010** reported the best survival of *L. fermentum* strains at pH 5.5. *L. fermentum* has shown good survival capabilities within the acidic pH range of 1.5, 2.0, 3.0, and 3.5 (**Srinu *et al*., 2013**). Our isolate exhibits 25% survival at pH 2. Previous studies stated that the survival of probiotic strains decreased with decreasing pH (**Sahadeva *et al*., 2011**). Loss in viability was observed at lower pH values (**Tulumoglu *et al*., 2014**). These results are consistent with our findings where survival percentages increased as pH transitions from highly acidic (pH 2) to neutral (pH 7) and slightly decreased with alkaline (pH 8) conditions. The best survival of our isolate was found at pH 6 and 7. This pH-dependent trend indicates the potential adaptability of this strain to the dynamic environments within the human digestive tract. This opposes the findings of Patil *et al*., 2010 where *L. fermentum* had no growth at 4.4 pH. While in our study 61% growth was observed at pH 4. The phylogenetic tree shows evolutionary relationships among strains.

CONCLUSION

The present study reports, for the first time, the presence of *Limosilactobacillus fermentum* species in curd samples from Kangra district of Himachal Pradesh. Identification was confirmed through phenotypic characterisation and 16S rRNA sequencing. The isolated strain showed significant probiotic potential, demonstrating high tolerance to 0.5% and 1% NaCl, exceptional survival at 37°C and optimal growth within pH range of 6 to 8 conditions representative of human gastrointestinal environment. All these attributes suggest the potential application of this strain as a functional probiotic. *Limosilactobacillus fermentum* was predominant probiotic species found in Kangra curd, which enhances probiotic potential of curd obtained from lower hills of Himachal Pradesh and opens avenues for its further exploration in health promoting formulations.

DECLARATIONS

- **Ethics approval and consent to participate:** Approval- granted by the Institutional Ethics Committee of the Central University of Himachal Pradesh. (Date: 23.08.2022/ Approval Number: IAEC/CUHP/2022/I-2.3)
- **Consent for publication:** Not applicable.
- **Competing interests:** None.
- **Funding:** Author Disha Chauhan is getting senior research fellowship from CSIR.
- **Authors' contributions: Disha Chauhan (DS):** Manuscript writing, and experimental work. **Ranjit Kumar (RK):** Conceptualisation, manuscript editing, rewriting, and revision.

Acknowledgments: The authors are thankful to the Central University of Himachal Pradesh for their support during the study and the CSIR-Institute of Microbial Technology for 16s rRNA sequencing. Author Disha Chauhan also expresses gratitude to CSIR (Council of Scientific and Industrial Research) for the aid in the form of Junior Research Fellowship (JRF) and Senior Research Fellowship (SRF), which supported the study.

REFERENCES

Adikari, A. M. M. U., Priyashantha, H., Disanayaka, J. N. K., Jayatileka, D. V., Kodithuwakku, S. P., Jayatilake, J. A. M. S., and Vidanarachchi, J. K. (2021). Isolation, identification and characterization of *Lactobacillus* species diversity from Meekiri: traditional fermented buffalo milk gels in Sri Lanka. Heliyon, 7(10). https://doi.org/10.1016/j.heliyon.2021.e08136

Amara, A. A., and Shibl, A. (2015). Role of Probiotics in health improvement, infection control and disease treatment and management. Saudi pharmaceutical journal, 23(2), 107-114. https://doi.org/10.1016/j.jsps.2013.07.001

Ao, X., Zhang, X., Shi, L., Zhao, K., Yu, J., Dong, L., ... and Cai, Y. (2012). Identification of lactic acid bacteria in traditional fermented yak milk and evaluation of their application in fermented milk products. Journal of dairy science, 95(3), 1073-1084. https://doi.org/10.3168/jds.2011-4224

Argyri, A. A., Zoumpopoulou, G., Karatzas, K. A. G., Tsakalidou, E., Nychas, G. J. E., Panagou, E. Z., and Tassou, C. C. (2013). Selection of potential probiotic lactic acid bacteria from fermented olives by in vitro tests. Food microbiology, 33(2), 282-291. https://doi.org/10.1016/j.fm.2012.10.005

Bajaj, K. K., Chavhan, V., Raut, N. A., and Gurav, S. (2022). Panchgavya: A precious gift to humankind. Journal of Ayurveda and integrative medicine, 13(2), 100525.

Bao, Y., Zhang, Y., Zhang, Y., Liu, Y., Wang, S., Dong, X., ... and Zhang, H. (2010). Screening of potential probiotic properties of *Lactobacillus fermentum* isolated from traditional dairy products. Food control, 21(5), 695-701. https://doi.org/10.1016/j.foodcont.2009.10.010

De, S., Pramanik, A., Das, A. K., Paul, S., Jana, S., and Pramanik, P. (2017). Isolation and characterization of Lactobacillus spp. from curd and its pharmacological application in probiotic chocolate. The Journal of Phytopharmacology, 6(6), 335-339.

Goyal, R., Dhingra, H., Bajpai, P., and Joshi, N. (2012). Characterization of the *Lactobacillus* isolated from different curd samples. African Journal of Biotechnology, 11(79), 14448-14452. https://doi.org/10.5897/ajb11.310

Karami, S., Roayaei, M., Hamzavi, H., Bahmani, M., Hassanzad-Azar, H., Leila, M., and Rafieian-Kopaei, M. (2017). Isolation and identification of probiotic *Lactobacillus* from local dairy and evaluating their antagonistic effect on pathogens. International journal of pharmaceutical investigation, 7(3), 137. https://doi.org/10.4103/jphi.jphi_8_17

Kariyawasam, K. M. G. M. M., Lee, N. K., and Paik, H. D. (2021). Fermented dairy products as delivery vehicles of novel probiotic strains isolated from traditional fermented Asian foods. Journal of Food Science and Technology, 58, 2467-2478. https://doi.org/10.1007/s13197-020-04857-w

Katiku, M. M., Matofari, J. W., and Nduko, J. M. (2022). Preliminary evaluation of probiotic properties and safety profile of *Lactiplantibacillus plantarum* isolated from spontaneously fermented milk, *Amabere amaruranu*. *Heliyon, 8*(11), e10342. https://doi.org/10.1016/j.heliyon.2022.e10342

Khushboo, Karnwal, A., and Malik, T. (2023). Characterization and selection of probiotic lactic acid bacteria from different dietary sources for development of functional foods. Frontiers in microbiology, 14, 1170725. https://doi.org/10.3389/fmicb.2023.1170725

Kumara, S. S., Bashisht, A., Venkateswaran, G., Hariprasad, P., and Gayathri, D. (2019). Characterization of novel *Lactobacillus fermentum* from curd samples of indigenous cows from Malnad region, Karnataka, for their aflatoxin B 1 binding and probiotic properties. Probiotics and antimicrobial proteins, 11, 1100-1109. https://doi.org/10.1007/s12602-018-9479-7

Markowiak, P., and Śliżewska, K. (2017). Effects of probiotics, prebiotics, and synbiotics on human health. Nutrients, 9(9), 1021. https://doi.org/10.3390/nu9091021

Mazziotta, C., Tognon, M., Martini, F., Torreggiani, E., and Rotondo, J. C. (2023). Probiotics mechanism of action on immune cells and beneficial effects on human health. Cells, 12(1), 184. https://doi.org/10.3390/cells12010184

Mirlohi, M., Soleimanian-Zad, S., Dokhani, S., Sheikh-Zeinodin, M., and Abghary, A. (2009). Investigation of acid and bile tolerance of native lactobacilli isolated from fecal samples and commercial probiotics by growth and survival studies. *Iranian Journal of Biotechnology, 7*(4), 233–240.

Nagpal, R., Kumar, A., Kumar, M., Behare, P. V., Jain, S., and Yadav, H. (2012). Probiotics, their health benefits and applications for developing healthier foods: a review. FEMS microbiology letters, 334(1), 1-15. https://doi.org/10.1111/j.1574-6968.2012.02593.x

Palaniyandi, S. A., Damodharan, K., Suh, J. W., and Yang, S. H. (2017). In vitro characterization of *Lactobacillus plantarum* strains with inhibitory activity on enteropathogens for use as potential animal probiotics. *Indian Journal of Microbiology, 57*(2), 201–210. https://doi.org/10.1007/s12088-017-0646-4

Patil, M. M., Pal, A., Anand, T., and Ramana, K. V. (2010). Isolation and characterization of lactic acid bacteria from curd and cucumber. Indian Journal of Biotechnology. 9:166-172. http://evolution.genetics.washington.edu/phylip.ht

Reale, A., Di Renzo, T., Rossi, F., Zotta, T., Iacumin, L., Preziuso, M., Parente, E., Sorrentino, E., and Coppola, R (2015). Tolerance of *Lactobacillus casei, Lactobacillus paracasei* and *Lactobacillus* rhamnosus strains to stress factors encountered in food processing and in the gastro-intestinal tract. *LWT - Food Science and Technology,* 60(2), 721–728. doi:10.1016/j.lwt.2014.10.022

Riaz, S., Kashif Nawaz, S., and Hasnain, S. (2010). Bacteriocins produced by L. Fermentum and *L. Acidophilus* can inhibit cephalosporin resistant e. Coli. Brazilian journal of microbiology : [publication of the Brazilian Society for Microbiology], 41(3), 643–648. https://doi.org/10.1590/S1517-83822010000300015

Ruiz, L., Margolles, A., and Sánchez, B. (2013). Bile resistance mechanisms in Lactobacillus and Bifidobacterium. Frontiers in microbiology, 4, 396. https://doi.org/10.3389/fmicb.2013.00396

Sahadeva, R. P. K., Leong, S. F., Chua, K. H., Tan, C. H., Chan, H. Y., Tong, E. V., ... and Chan, H. K. (2011). Survival of commercial probiotic strains to pH and bile. International Food Research Journal, 18(4), 1515-1522.

Saitou N. and Nei M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406-425.

Sharafi, H., Derakhshan, V., Paknejad, M., Alidoust, L., Tohidi, A., Pornour, M., ... and Noghabi, K. A. (2015). *Lactobacillus crustorum* KH: Novel prospective probiotic strain isolated from Iranian traditional dairy products. Applied biochemistry and biotechnology, 175, 2178-2194. https://doi.org/10.1007/s12010-014-1404-2

Sharma, A., Lavania, M., Singh, R., and Lal, B. (2021). Identification and probiotic potential of lactic acid bacteria from camel milk. Saudi journal of biological sciences, 28(3), 1622-1632.https://doi.org/10.1016/j.sjbs.2020.11.062

Sharoba, A. M., Soliman, A. H. S., Bahlol, H. E. M., Soliman, A. S., and Radi, O. M. M. (2015). Evaluation of *Lactobacillus acidophilus*, *Lactobacillus casei* and *Lactobacillus plantarum* for probiotic characteristics. *Middle East Journal of Applied Sciences, 5*(1), 10–18.

Shi, L. H., Balakrishnan, K., Thiagarajah, K., Ismail, N. I. M., and Yin, O. S. (2016). Beneficial properties of probiotics. Tropical life sciences research, 27(2), 73. https://doi.org/10.21315/tlsr2016.27.2.6

Shokryazdan, P., Sieo, C. C., Kalavathy, R., Liang, J. B., Alitheen, N. B., Faseleh Jahromi, M., and Ho, Y. W. (2014). Probiotic potential of *Lactobacillus* strains with antimicrobial activity against some human pathogenic strains. BioMed research international, 2014(1), 927268. https://doi.org/10.1155/2014/927268

Srinu, B., Rao, T. M., Reddy, P. M., and Reddy, K. K. (2013). Evaluation of different lactic acid bacterial strains for probiotic characteristics. Veterinary World, 6(10), 785. https://doi.org/10.14202/vetworld.2013.785-788

Tamura K., Stecher G., and Kumar S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution. https://doi.org/10.1093/molbev/msab120.

Tulumoğlu, Ş., Kaya, H. İ., and Şimşek, Ö. (2014). Probiotic characteristics of *Lactobacillus fermentum* strains isolated from Tulum cheese. Anaerobe, 30, 120-125. https://doi.org/10.1016/j.anaerobe.2014.09.015

Urdaneta, V., and Casadesús, J. (2017). Interactions between Bacteria and Bile Salts in the Gastrointestinal and Hepatobiliary Tracts. Frontiers in medicine, 4, 163. https://doi.org/10.3389/fmed.2017.00163

van Baarlen, P., Wells, J. M., and Kleerebezem, M. (2013). Regulation of intestinal homeostasis and immunity with probiotic lactobacilli. Trends in immunology, 34(5), 208-215. https://doi.org/10.1016/j.it.2013.01.005

Verna, E. C., and Lucak, S. (2010). Use of probiotics in gastrointestinal disorders: what to recommend? Therapeutic advances in gastroenterology, 3(5), 307–319. https://doi.org/10.1177/1756283X10373814

Zeng, J., Li, Y. Q., Zuo, X. L., Zhen, Y. B., Yang, J., and Liu, C. H. (2008). Clinical trial: effect of active lactic acid bacteria on mucosal barrier function in patients with diarrhoea-predominant irritable bowel syndrome. Alimentary pharmacology & therapeutics, 28(8), 994–1002. https://doi.org/10.1111/j.1365-2036.2008.03818.x