Isolation and Identification of Lactic Acid Bacteria from Naturally Fermented Goat Milk as Potential Goat Milk Yogurt Starter

Asma Billateh
International Program of Biotechnology, Center of Excellence in Innovative Biotechnology for Sustainable Utilizatio of Bioresources, Faculty of Agro-Industry, Prince of Songkla University

Benjamas Cheirsilp
International Program of Biotechnology, Center of Excellence in Innovative Biotechnology for Sustainable Utilizatio of Bioresources, Faculty of Agro-Industry, Prince of Songkla University

https://doi.org/10.5109/7323402

出版情報：Proceedings of International Exchange and Innovation Conference on Engineering & Sciences (IEICES). 10, pp.1146-1151, 2024-10-17. International Exchange and Innovation Conference on Engineering & Sciences
バージョン：

Isolation and Identification of Lactic Acid Bacteria from Naturally Fermented Goat Milk as Potential Goat Milk Yogurt Starter

Asma Billateh and Benjamas Cheirsilp
International Program of Biotechnology, Center of Excellence in Innovative Biotechnology for Sustainable Utilization of Bioresources, Faculty of Agro-Industry, Prince of Songkla University, Hatyai, Songkhla, 90110, Thailand.
Corresponding author email: benjamas.che@psu.ac.th

Abstract: *Proper selection and balance of starter culture are critical for manufacturing fermented products with desirable texture and flavor. This study aimed to characterize lactic acid bacteria (LAB) from naturally fermented goat milk and assess their potential as a starter culture. Thirteen LAB species were isolated from goat milk collected at Mato farm, Satun, Thailand. These isolates were phenotypically characterized, and their technological properties, including acidification, antimicrobial activity, and tolerance to acid and bile salts, were evaluated using standard procedures. The results showed that the isolated R-03 demonstrated high acidifying capability, strong antimicrobial activity against Staphylococcus aureus, Escherichia coli, and Salmonella spp., and good tolerance to acid and bile salts. Therefore, Isolate R-03 is a promising candidate for manufacturing goat milk yogurt.*

Keywords: Goat milk yogurt; Naturally fermented milk; Yogurt starter

1. INTRODUCTION

The most ambitious global development goals ever were introduced when all 193 member states of the United Nations General Assembly unanimously adopted the 2030 Agenda for Sustainable Development in 2015. These member states committed to achieving the seventeen Sustainable Development Goals (SDGs) from 2015 to 2030, aiming to end poverty, advance global sustainability, and enhance inclusion [1].

Addressing malnutrition and food insecurity among vulnerable populations requires sustainable, accessible, and affordable food production schemes to meet nutritional needs. Food scarcity is a significant problem in certain parts of the world today, and if not addressed, it could escalate into a global threat in the coming decades [2]. Food security involves ensuring that everyone has access to nutritious and healthy food. Milk, particularly goat milk, is a naturally available and nutritionally complete food, offering essential carbohydrates, proteins, fats, vitamins, and various micro and macro elements beneficial for human health [3].

Goat milk aligns with several Sustainable Development Goals (SDGs) by promoting economic growth, food security, and health. It supports smallholder farmers and communities, providing income and reducing poverty (SDG 1). As a nutritious food source, it contributes to zero hunger (SDG 2) and good health and well-being (SDG 3), especially for those with cow milk allergies or lactose intolerance [4].

A diverse range of goat milk-based products, including yogurt, cheese, fermented milk, and goat milk powder, are available in the market. The popularity of goat milk and its products is increasing due to a growing awareness of the importance of a healthy diet. Extensive research into the nutritional and therapeutic effects of goat milk has established it as a functional food [5].

The fermentation process further contributes to the utilization of the functional value of goat milk, as fermented products serve as effective carriers for probiotics. Fermented milk is a crucial component of diets in many regions worldwide. There are numerous traditional and industrialized fermented milk products, identified by over 400 generic names. These traditional products vary based on the type of milk used, the treatment of the milk, the fermentation process, and the subsequent handling of the product [6]. Traditional fermentation methods relied solely on the natural microbiota present in the milk, without the use of commercial starter cultures. The processes of acidification and gel formation were influenced by these native microorganisms and the specific method of inoculation [7].

Starter cultures are microorganisms intentionally introduced to milk to initiate fermentation and manage the process under controlled conditions. They play a crucial role in contributing to the diversity of the final product [8]. Starter cultures in yogurt primarily function to produce lactic acid and various flavor compounds, while also influencing texture. However, different strains, even within the same species, may exhibit distinct characteristics [9], After ensuring that starter cultures intended for yogurt production are safe for health, they should exhibit optimal levels of acid production and proteolytic activity. Ideally, these cultures should also be resistant to bacteriophages and capable of producing flavor compounds, exopolysaccharides (EPS), and antimicrobial substances [10]. Genetic modifications are often employed on strains with potential as starter cultures—those possessing some or all of the desired traits—to introduce or enhance specific characteristics [11]. However, consumers often favor natural organisms and foods over genetically modified alternatives. Consequently, a common strategy is the direct isolation of lactic acid bacteria from various natural and traditional fermented foods to develop starter or probiotic cultures that can impart diverse sensory properties to new products [12]. In line with the growing trend towards a healthy, natural, and balanced diet, there has been a rising demand for natural antimicrobials. As an alternative to food-borne natural antimicrobial components, it has become increasingly common to use microorganisms,

particularly lactic acid bacteria, which exhibit antimicrobial activity through various metabolites, including bacteriocins [13,14].

Additionally, it has been reported that wild strains could be more resistant to environmental conditions and survive under harsh conditions and may exhibit unique characteristics compared to their commercial counterparts [15]. In addition, traditional milk is known as one of the natural habitats of lactic acid bacteria (LAB). Functional LAB strains can be used in the developing of new types or improving the quality of fermented milk product [16]. Previous studies showed that *Lactobacillus* and *Streptococcus* were the predominant genera at the genus level in homemade yogurts of Xinjiang [17]. Similar results were obtained by Celik *et al.* [18], who isolated LAB from traditional yogurts as potential starter cultures based on their antimicrobial activity. The closest species to all of the selected isolates was determined to be *Lactobacillus bulgaricus.*

When considering the different fermentation strategies applied in the dairy industry in general, it would be useful to isolate different LAB species, including both mesophilic and thermophilic species. As it is important to have strains for rapid development of lactic acid and concomitant reduced pH as well as strains that are able to produce gas and flavors, a variety of both homofermentative and heterofermentative species would be desirable [19].

In this study, we conducted direct isolation and identification of potential lactic acid bacteria (LAB) from naturally fermented goat milk to use as a starter culture. This was done by evaluating acid production and assessing LAB properties, including antimicrobial activity, and tolerance to acid and bile salts. The strains with strong potential as yogurt starters were selected to. prepare goat milk yogurt in further study.

2. MATERIALS AND METHODS

2.1 Isolation and primary screening LAB from goat milk

The raw goat milk was collected from Mato farm, Satun province, Thailand. The raw goat milk was enriched by naturally fermentation at 37°C and 42°C under anaerobic condition for three days (pH reach 4.0). Ten milliliters of fermented goat milk were weighed and mixed with 90 mL of sterile 0.1% (w/v) peptone water (Merck, Darmstadt, Germany). The mixture was homogenized manually, and the necessary dilution rates were prepared. Appropriate dilutions of the samples were then inoculated on de Man, Rogosa, and Sharpe agar (MRS, Merck, pH 5.7 ± 0.2) containing 0.02% bromocresol purple as an indicator, using the spread plate technique to count lactic acid bacteria. The MRS agar plates were incubated at 37°C and 42°C for 48 h under aerobic conditions. The colonies that exhibit yellow colony were picked and restreaked to purify the isolates. Then, they were subjected to catalase activity analysis using 3% (v/v) hydrogen peroxide (H_2O_2). Only isolates that showed negative for catalase test were further examined with a Gram stain. Gram-positive isolates, identified by their blue-violet or purple staining, were observed for morphology under an optical microscope. Cultures that were both Gram-positive and catalase-negative were selected for further study. The step of isolation and screening of LAB as show in Fig.1

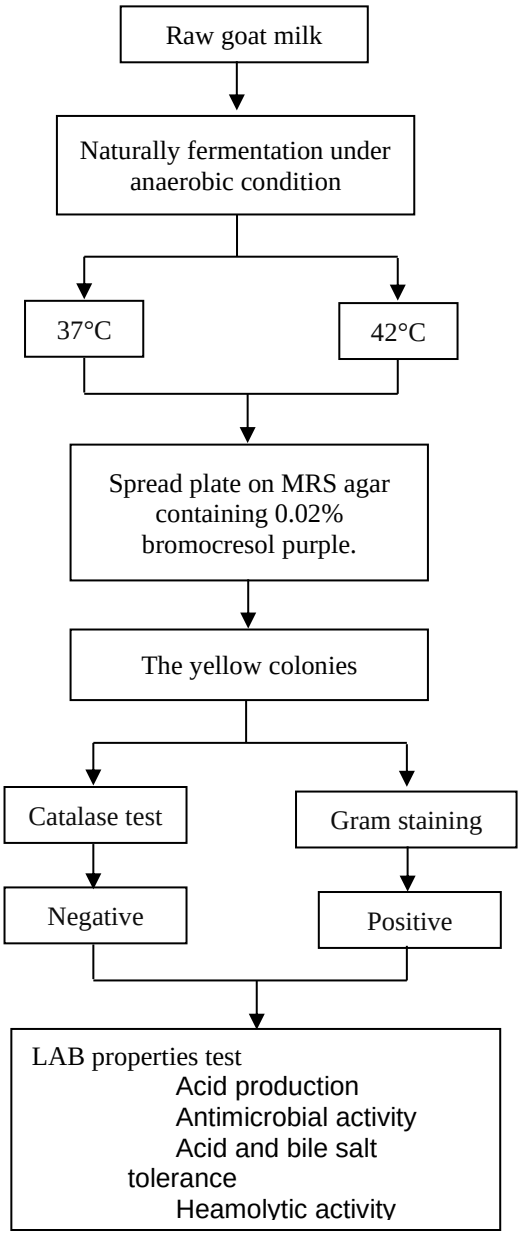

Fig.1. Isolation and screening of LAB from fermented goat milk.

2.2 Acid Production

The selected isolates from previous study were inoculated into sterile test tubes containing 10 mL of MRS broth (OD_{600nm} = 0.1), the cultures were incubated at 37°C for 48 h. The cultures were measured cell growth, pH, and acidity (%), the isolated that produce the highest acid were selected for further study [20].

2.3 Secondary screening of probiotic bacteria as potential goat milk yogurt starter

2.3.1 Antimicrobial activity

The antibacterial activity was assessed using the agar spot assay method detailed by Celik *et al.* [18]. MRS agar plates were divided into quarters, each quarter receiving 5 µL of overnight cultures of isolates grown in MRS broth, with optical densities adjusted to OD_{600nm} = 0.1 ± 0.005. Following a 24 h incubation period at 37°C, the plates were overlaid with 7 ml of fresh cultures of

indicator strains (1%) of *E. coli* BL21 and *S. aureus* NCTC 8530 prepared in Tryptic soy soft agar. Inhibition zones were subsequently measured using a digital caliper.

2.3.2 Acid tolerance

Acid tolerance was assessed following the method outlined by Argyri *et al.* [21], with minor adjustments. Bacterial overnight cultures were washed thrice with PBS (pH 7.0) to eliminate impurities, followed by centrifugation at 5,000 rpm for 10 minutes at 4°C. The resulting cell pellets were suspended in MRS broth adjusted to pH 2.5 using 3N HCl or NaOH. The cultures were then anaerobically incubated at 37°C for 24 h. Samples were collected every 2 h over a 4-h period and enumerated by spread plate counts using 10-fold serial dilutions in 0.1% peptone water. Viable microorganisms were counted in triplicate on MRS agar. The initial samples collected at 0 h served as controls. Isolates demonstrating final counts $>10^6$ CFU/mL were classified as having moderate to good resistance.

2.3.3 Bile salts tolerance

The bile salt tolerance of chosen isolates was assessed following the method outlined by Argyri *et al*. [21], with slight adjustments. Bacterial overnight cultures were washed three times with PBS (pH 7.0) to eliminate impurities, followed by centrifugation at 6,000 rpm for 10 minutes at 4°C. The resulting cell pellets were suspended in MRS broth supplemented with 0.3% oxgall bile salts. The cultures were then anaerobically incubated at 37°C for 4 h. Samples were collected at 0, 2, and 4 h and subjected to spread plate counts using 10-fold serial dilutions in 0.1% peptone water. Control samples collected at 0 h were used for comparison. Bile salt tolerance was evaluated based on viable colony counts on MRS agar in triplicate after 37°C incubation for 0, 2, and 4 h, reflecting the typical transit time of food through the small intestine. Isolates demonstrating final counts $>10^6$ CFU/ml were classified as having good resistance.

2.3.4 Heamolytic activity

The selected strains from a previous study were streaked onto blood agar and incubated at 37°C for 48 h, following the method described by Fadda *et al.* [22]. Post-incubation, the hemolytic activity of the isolates was assessed based on the lysis of red blood cells in the surrounding medium. Hemolysis was categorized as green zones around colonies (α-hemolysis), clear zones around colonies (β-hemolysis), and no zones around colonies (γ-hemolysis) on Columbia blood agar plates. Only strains exhibiting γ-hemolysis were considered safe.

3. RESULTS AND DISCUSSION

3.1 Isolation and primary screening of LAB from naturally fermented goat milk

The fermented goat milk samples were serially diluted with sterile saline (0.85%), appropriate dilutions of the samples were spread on MRS medium containing 0.02% of bromocresol purple. The incubation was performed at 37°C and 42°C for 48 h. It was found that the viability count of LAB at both temperatures has a value in the range of log 9.29±0.004 cfu/mL to log 9.33 ± 0.024 cfu/mL (Table 1). The colonies that exhibited yellow colony in each sample were pick and restreak to purify the isolated. The obtained colonies were subjected to primary screening through catalase test and gram-staining. It was found that 10 isolated LABs exhibited catalase-negative and gram-positive characteristics from the fermented goat milk sample incubated at 37°C, and 3 isolated LABs exhibited catalase-negative and gram-positive characteristics from the fermented goat milk sample incubated at 42°C (Table 2). It can be observed that most LAB strains are more abundant at 37°C than at 42°C, possibly due to their optimal growth temperature being around 37°C, where they efficiently metabolize nutrients and proliferate [23].

Table 1. Viability count of LAB from naturally fermented goat milk

Temperature (°C)	Viability count (Log cfu/mL)
37	9.33±0.024
42	9.29±0.004

Table 2. Characteristics of isolated LAB

Temp.	Catalase test		Gram-staining		Morphology			LAB (isolates)
(°C)	+	-	+	-	Cocci	rod	long rod	
37	4	10	7	0	4	3	0	10
42	4	3	3	0	3	0	0	3

3.2 Acid Production

Yogurt gel forms as an acid clot due to the denaturation of serum proteins by heat treatment and subsequent development during incubation. Thus, acid production is a crucial factor in selecting a yogurt starter culture [18]. The bacteria strains that usually used in yogurt production are *S. thermophilus* and *L. bulgaricus*, both bacteria have the ability to produce acid. The optical density (OD_{600} nm) of cell growth and pH are presented in Table 3, while the acidity production during the 48-hour incubation of the obtained isolates is shown in Table 4. The highest acidity value (higher than 2.00%) was obtained only in isolates that incubated in 37°C, which including, R-01 (2.09±0.023%), R-02 (2.21±0.045%), R-03 (2.07±0.045%), R-10 (1.96±0.068%), R-13 (2.00±0.023%) and R-15 (2.09±0.023%).

Table 3. The optical density (OD_{600} nm) of cell growth and pH by each isolate obtained from naturally goat milk after 48 h of fermentation.

Temp.(°C)	Isolates	OD_{600} nm	pH
37	R-01	5.18 ±0.02	3.66±0.015
	R-02	4.42±0.3	3.73±0.01
	R-03	5.12±0.005	3.69±0.05
	R-08	1.11±0.01	4.27±0.01
	R-09	4.99±0.36	3.65±0.01
	R-10	4.55±0.37	3.73±0.05
	R-11	1.09±0.02	4.29±0.01
	R-13	5.1±0.02	3.68±0.15
	R-14	1.07±0.15	4.32±0.03
	R-15	5.23±0.11	3.68±0.025
42	RH-01	1.08 ±0.035	4.37±0.03
	RH-02	1.15±0.07	4.35±0.03

Temp.(°C)	Isolates	OD_{600} nm	pH
	RH-07	1.25±0.005	4.31±0.01

Table 4. The acid production by each isolate obtained from naturally goat milk after 48 h of fermentation.

Temp. (°C)	Isolates	Acidity (%)
37	R-01	2.09±0.023
	R-02	2.21±0.045
	R-03	2.07±0.045
	R-08	1.31±0.045
	R-09	1.07±0.00
	R-10	1.96±0.068
	R-11	1.24±0.023
	R-13	2.00±0.023
	R-14	1.27±0.032
	R-15	2.09±0.023
42	RH-01	1.31±0.045
	RH-02	1.40±0.045
	RH-07	1.37±0.023

3.3 Secondary screening of probiotic bacteria as potential goat milk yogurt starter

3.3.1 Antimicrobial activity

After screening for acid production, seven potential isolates were identified. This number was reduced to two based on the results of an antimicrobial activity test. During the evaluation, priority was given to isolates that showed a high inhibition rate against three indicator strains: *E. coli*, *S. aureus*, and *Salmonella* spp. Pictures of sample inhibition zones are given in Fig. 2. The inhibition zones obtained from the 7 selected isolates are given in Table 5 and to explain briefly, the larger the inhibition zone, the higher the antimicrobial activity is. A variety of studies are available on the isolation of *Lactobacillus* spp. from naturally fermented goat milk yogurt and investigation of their antimicrobial activities against different pathogens including *E. coli* and *S. aureus* and *Salmonella* spp. Among selected Isolates can be separate into 2 groups under morphology, the isolates that exhibited morphology of rod including R-01, R-12 and R15, the isolated R-01 and R-12 showed antimicrobial activity against 3 pathogen strains. The isolates that exhibited morphology of cocci including R-02, R-03, R-10, and R-13. Considering, R-03, R-10, and R-13 showed antimicrobial activity against 3 pathogen strains. These findings are similar to the results obtained by Yerlikaya *et al*. [24] and Celik *et al.* [18] in studies involving the isolation of bacteria from commercial yogurts and the evaluation of their antimicrobial activity against selected pathogens. However, the highest antimicrobial activity against pathogen strains was observed in the R-03 isolates, which exhibited cocci morphology. The R-12 isolate, which exhibited rod morphology, was also selected. Therefore, both were chosen for further study.

Table 5. Inhibition zone of the selected isolates against indicator strains

Isolates	Inhibition zone diameters (cm)		
	E. coli	*S. aureus*	*Salmonella* sp.
R-01	0.7±0.1	1.05±0.45	0.5±0.1
R-02	0.95±0.05	Nd	nd
R-03	1.05±0.15	1.25±0.25	0.5±0.0
R-10	0.95±0.05	1.4±0.3	0.25±0.05
R-12	0.95±0.05	0.9±0.1	0.3±0.1
R-13	0.45±0.05	0.40±0.1	0.55±0.05
R-15	nd	0.95±0.15	0.6±0.0

Remark: nd means not detected

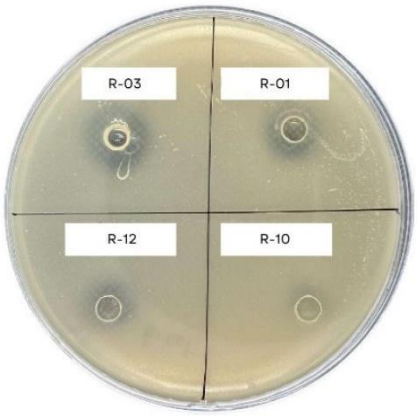

Fig. 2. A sample plate of inhibition zones obtained representing the antimicrobial activity of the isolates.

3.3.2 Acid tolerance

A key criterion for probiotics is their resistance to acidic environments. Effective probiotics endure low pH levels (as low as pH 2.0) and demonstrate tolerance to the high acid levels found in the human stomach [25]. The selected potential LAB strains were tested for their ability to tolerate acidic conditions in MRS broth with the presence of pepsin enzyme, with the pH adjusted to 2.5. It was found that all both strains (R-03 and R-12) had a survival rate more than 80%. Among strains, R-03 showed the highest survival rate of 86.05 ± 0.43 % at 4 h of incubation time (Fig. 3).

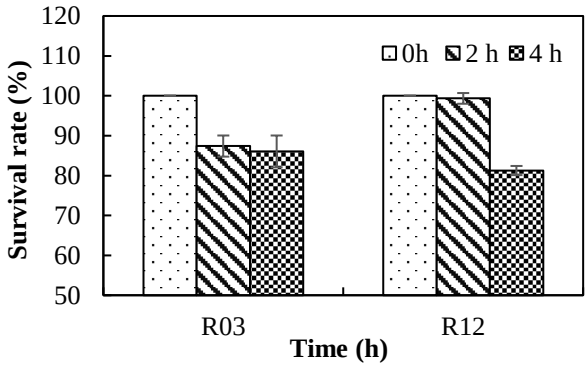

Fig. 3. Acid tolerance of selected strains at pH 2.5.

3.3.3 Bile salt tolerance

Resistance to bile salts is a crucial characteristic of probiotics, as bile salts can dissolve membrane lipids, causing cell leakage and death [26]. In this study, bile salt tolerance was tested with selected LAB strains, revealing that LAB from goat milk could grow and conjugate the bile salts. Fig. 4. illustrates the growth and survival of

these LAB strains in the presence of bile salts, among strains test, R-03 had the highest survival rate more than 90% after 4 h of incubation time.

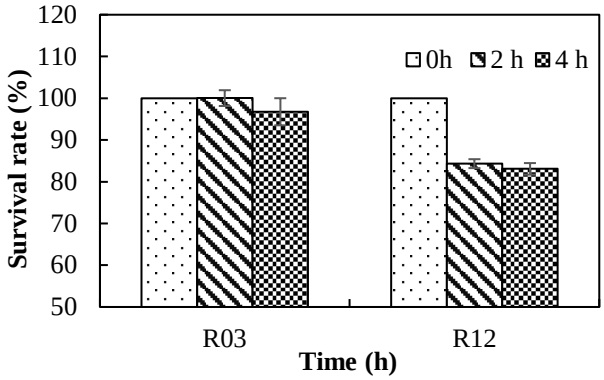

Fig. 4. Bile salt tolerance of selected strains at pH 2.5.

3.3.4 Haemolytic activity

It is well known that LAB has a long history of safe use in human food. To be classified as probiotics, bacteria must be non-hemolytic, unlike pathogenic bacteria [27]. In this study, all the examined strains were γ-hemolytic (non-hemolytic) when grown on blood agar (Table 6), and none of the strains exhibited hemolysis of blood cells. According to FAO/WHO guidelines [28], the absence of hemolysis confirms the safety of probiotics.

Table 6. Haemolytic activity test

Isolates	Haemolytic test		
	α-hemolysis	β-hemolysis	γ-hemolysis
R-03	-	-	+
R-12	-	-	+

4. CONCLUSION

The present study explored the technological potential of selected LAB strains isolated from naturally fermented goat milk for use as starters in yogurt preparation. Based on the overall evaluation of the results, the selected strains exhibited high acidifying activity. Among the 13 strains, 7 isolates showed a high acidification rate (>2.00%), and only two strains demonstrated significant antimicrobial activity, tolerance to acid and bile salts, and did not exhibit hemolysis of blood cells. They will be used to prepare goat milk yogurt in a further study.

5. REFERENCES

[1] V. Baskaran, R. Velkennedy, S. Murugan, G. Theerumalai, Modeling and prediction of the achievement level with related goals for SDG 11: Sustainable Cities and Communities, Int. Exch. Innov. Conf. Eng. Sci. 8 (2022) 19–24.

[2] S. Noor, H. Ashraf, M. Sultan, T. Miyazaki, M.H. Mahmood, Z.M. Khan, Investigation of Direct and Indirect Evaporative Cooling Options for Greenhouse Air Conditioning in Multan (Pakistan), Int. Exch. Innov. Conf. Eng. Sci. 6 (2020) 110–115.

[3] M. Pal, T.P. Dudhrejiya, S. Pinto, Goat milk products and their significance, Beverage Food World 44 (2017) 21–25.

[4] E.M. Idamokoro, The significance of goat milk in enhancing nutrition security: a scientometric evaluation of research studies from 1966 to 2020, Agriculture Food Secur. 12 (2023) 34.

[5] M. Hovjecki, Z. Miloradovic, V. Rac, P. Pudja, J. Miocinovic, Influence of heat treatment of goat milk on casein micelle size, rheological and textural properties of acid gels and set type yoghurts, J. Texture Stud. 51 (2020) 680–687.

[6] Y. Li, Y. Wang, B. Li, B. Hou, W. Hung, J. He, C. Man, *Streptococcus thermophilus* JM905—Strain carbon source utilization and its fermented milk metabolic profile at different fermentation stages, Foods 12 (2023) 3690.

[7] Y. Jiang, N. Li, Q. Wang, Z. Liu, Y.K. Lee, X. Liu, J. Zhao, H. Zhang, W. Chen, Microbial diversity and volatile profile of traditional fermented yak milk, J. Dairy Sci. 103 (2020) 87–97.

[8] F. Yıldız, Development and manufacture of yogurt and other functional dairy products, CRC Press, Boca Raton, FL, 2016.

[9] W. Liu, J. Yu, Z. Sun, Y. Song, X. Wang, H. Wang, Z. Heping, Relationships between functional genes in *Lactobacillus delbrueckii* ssp. *bulgaricus* isolates and phenotypic characteristics associated with fermentation time and flavor production in yogurt elucidated using multilocus sequence typing, J. Dairy Sci. 99 (2016) 89–103.

[10] H. Ispirli, E. Dertli, Isolation and identification of exopolysaccharide producer lactic acid bacteria from Turkish yogurt, J. Food Process. Preserv. 42 (2018) e13351.

[11] Z. Ustunol, Dairy starter cultures, in: B. Ozer, G. Akdemir-Evrendilek (Eds.), Dairy Microbiology and Biochemistry, CRC Press, Boca Raton, 2014, pp. 53–81.

[12] R.H. Farimani, M.B.H. Najafi, B.S.F. Bazzaz, M.R. Edalatian, A.R. Bahrami, A.B. Florez, Identification, typing and functional characterization of dominant lactic acid bacteria strains from Iranian traditional yoghurt, Eur. Food Res. Technol. 242 (2016) 517–526.

[13] E.O.O. Silva, C.R. Nespolo, C.P. Sehn, F.C. Pinheiro, L.M. Stefani, Lactic acid bacteria with antimicrobial, proteolytic and lipolytic activities isolated from ovine dairy products, Food Sci. Technol. 40 (2019) 293–299.

[14] F. Sonbol, A.A. Abdel, T. El-Banna, O. Al-Fakhrany, Antimicrobial activity of bacteriocins produced by *Enterococcus* isolates recovered from Egyptian homemade dairy products against some foodborne pathogens, Int. Microbiol. 23 (2020) 533–547.

[15] X. Ao, X. Zhang, L. Shi, K. Zhao, J. Yu, L. Dong, Y. Cai, Identification of lactic acid bacteria in traditional fermented yak milk and evaluation of their application in fermented milk products, J. Dairy Sci. 95 (2012) 1073–1084.

[16] Y. Widyastuti, A. Febrisiantosa, The role of lactic acid bacteria in milk fermentation, Food Nutr. Sci. 5 (2014) 1030–1037.

[17] H. Xu, W. Liu, Q. Gesudu, Z. Sun, J. Zhang, Z. Guo, H. Zhang, Assessment of the bacterial and fungal diversity in home-made yoghurts of Xinjiang, China by pyrosequencing, J. Sci. Food Agric. 95 (2015) 2007–2015.

[18] O.F. Celik, A.H. Con, H. Saygin, N. Şahin, H. Temiz, Isolation and identification of lactobacilli from traditional yogurts as potential starter cultures, LWT 148 (2021) 111774.

[19] A. Fugl, T. Berhe, A. Kiran, S. Hussain, M.F. Laursen, M.I. Bahl, E.B. Hansen, Characterisation of

lactic acid bacteria in spontaneously fermented camel milk and selection of strains for fermentation of camel milk, Int. Dairy J. 73 (2017) 19–24.
[20] B.T. Fossi, D.E. Ekabe, L.L. Toukam, H.O.T. Pambou, A. Gagneux-Brunon, C.N. Nguefeu, B. Bongue, Probiotic lactic acid bacteria isolated from traditional Cameroonian palm wine and corn beer exhibiting cholesterol-lowering activity, Heliyon 8(11) (2022).
[21] A.A. Argyri, G. Zoumpopoulou, K.A. Karatzas, E. Tsakalidou, G.J. Nychas, E.Z. Panagou, C.C. Tassou, Selection of potential probiotic lactic acid bacteria from fermented olives by in vitro tests, Food Microbiol. (2013).
[22] M.E. Fadda, V. Mossa, M. Deplano, M.B. Pisano, S. Cosentino, In vitro screening of *Kluyveromyces* strains isolated from Fiore Sardo cheese for potential use as probiotics, LWT 75 (2017) 100-106.
[23] H.M. Østlie, J. Treimo, J.A. Narvhus, Effect of temperature on growth and metabolism of probiotic bacteria in milk, Int. Dairy J. 15 (2005) 989–997.
[24] O. Yerlikaya, D. Saygılı, A. Akpınar, Evaluation of antimicrobial activity and antibiotic susceptibility profiles of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* strains isolated from commercial yoghurt starter cultures, Food Sci. Technol. 40 (2020) 1674–1681.
[25] P. Das, S. Khowala, S. Biswas, in vitro probiotic characterization of *Lactobacillus casei* isolated from marine samples, LWT 73 (2016) 383–390.
[26] E.A. Choi, H.C. Chang, Cholesterol-lowering effects of a putative probiotic strain *Lactobacillus plantarum* EM isolated from kimchi, LWT Food Sci. Technol. 62 (2015) 210–217.
[27] S.L. Sarkar, M.I. Hossain, S.A. Monika, S.K. Sanyal, P.C. Roy, M.A. Hossain, I.K. Jahid, Probiotic potential of *Pediococcus acidilactici* and *Enterococcus faecium* isolated from indigenous yogurt and raw goat milk, Microbiol. Biotechnol. Lett. 48 (2020) 276–286.
[28] FAO/WHO, Guidelines for the Evaluation of Probiotics in Food, Report of a Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food, London, Ontario, Canada, 2002.