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Predominant Species of Lactic Acid Bacteria in Fresh Goat's Milk from the Guanzhong Area of China

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Abstract

Few studies on the lactic acid bacteria (LAB) were reported in goat's milk from different regions in China, and thus there is limited information of their diversity. In this study, conventional morphological, physiological and biochemical analysis along with 16S rRNA gene sequences analysis were used to identify the LAB isolated from goat's milk. Based upon morphological, physiological and biochemical analysis, 77 strains were isolated and preliminarily characterized as potential LAB from 71 samples of goat's milk collected from three different districts in the Guanzhong area of China. All the isolates were further validated and identified by 16S rRNA gene sequencing and phylogenetic analysis. Sixty-two of the 77 isolates were identified as *Enterococcus hirae* representing 80.5% of all isolates recorded. The remaining isolates were identified as *Enterococcus faecalis*, *Enterococcus faecium*, *Weissella cibaria*, *Lactococcus lactis* and *Lactococcus garvieae*. We conclude that *Enterococcus hirae* is the predominant species of LAB in fresh goat's milk in the Guanzhong area. By combining traditional methods with 16S rRNA gene sequences analysis, identification was achieved rapidly and reliably. This research effectively characterized the LAB from goat's milk which is an essential step in the further development of the goat's milk industry and utilization of LAB.

Keywords

Goat milk; lactic acid bacteria; 16S rRNA gene; *Enterococcus hirae*

Introduction

Goat's milk is the most nutritious and easily digested alternative mammalian milk for human consumption because it is the closest, in terms of its constituents, to human milk [1-2]. Goats and cows vary considerably in their lactation organs which lead to the differences in the compositions of their milk. For instance, alfa-s1-casein in cows' milk accounts for 38% of the total casein content, but it represents only 5.6% of the total casein content of goat's milk; there is less lactose in goat's milk compared with cows' milk [3]. The unique physical and chemical properties of goat's milk make it more nutritional than cow's milk [4], which may lead to the differences of the microbial compositions in raw milk. Due to the particular properties of goat's milk (small particles of casein, low levels of lactose, etc.), it also has poor coagulation properties for yoghurt and cheese making. More research is required to facilitate the crucial selection of high-quality lactic acid bacterial starter cultures suitable for the production of fermented products from goat's milk.

Lactic acid bacteria (LAB) are a general term for Gram-positive bacteria that produce large amounts of lactic acid from carbohydrate; these bacteria are mainly found in fermented foods (even plant surfaces, soil, sea etc.) [5]. LABs are used as microbial starter in production of the fermented dairy products such as cheese, yoghurt and ice cream. Fermentation by LAB can improve the taste and structure of fermented products, but also their nutritional value. Since the chemical composition and fermentation characteristics of goat's milk are different to cows' milk, the LAB typically used for fermentation of cows' milk may not be usually suitable for use with goat's milk.

Few studies of LAB from goat's milk are reported in China, thereby in this research, we use traditional physiological and biochemical methods followed by 16S rRNA gene sequence analysis to identify LAB isolated from fresh goat's milk sampled from the Guanzhong area of Shaanxi Province [6]. This allowed us to understand the differences in LAB from goat's milk and to determine which the predominant LAB species was. It also helped establish independent intellectual property rights and lay the foundations for better screening of the probiotic and LAB for use in goat's milk fermentation and related product development.

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Materials and Methods

Sample collection

Fresh goat's milk samples (n=71) were collected from three districts (Yangling, Weinan, and Baoji district) in Shaanxi Province (Table 1). At the time of collection, ambient temperature was 28 to 34°C. Samples were taken directly from the goat's udder by hand. The first three milk drops from each goat were discarded to reduce external bacterial contamination and subsequent samples were immediately placed into sterile tubes (one sample from each goat), numbered and placed in an ice box for transportation to the laboratory, analyzing the microbial compositions immediately.

Sample ID	Number of samples	Location
Y (1-21)	21	Yangling district
WA (1-10)	10	
WB (1-10)	10	Weinan district
WD (1-10)	10	
B (1-10)	10	Baoji district

Table 1. Locations from where goat's milk samples were collected

WA (1-10), WB (1-10), WC (1-10) and WD (1-10) are the reference codes for samples collected from goats grazing in four different farms in the Weinan district (n=10 from each farm)

Counts of LAB and total bacteria in fresh goat's milk

Counts from each sample were made using the plate pouring method of Ortolani et al. [33]. From each sample, a 0.5 mL aliquot was homogenized with 4.5 mL of 0.85% (w/v) sterile physiological saline to make an initial dilution (10 fold). Serial dilutions were made for each sample and 1 mL of original sample and each dilution one (10, 100 and 1000 fold) was spread onto plates (three replicates per dilution) of sterile selective media of MRS agar for LAB enumeration [7]. and plate count agar (PCA) for total number of the bacteria [8]. Plates were incubated for 48 h at 30°C under anaerobic conditions for MRS agar and aerobic conditions for PCA prior to enumeration of the number of LAB and total bacteria, recorded as colony forming units (CFU/mL) in each sample. Statistical analysis of the data was done using ANOVA in the software package Data Processing System (DPS) 6.55. Results were expressed as 'averages ± standard deviation'.

Separation and purification of LAB

Aliquots (200 µL) from each sample of diluted goat's milk were pipetted and spread uniformly over plates containing either sterile MRS agar or M17 agar (OXOID CM0785). Culture media were incubated for 48-72 h at 30 °C under anaerobic conditions [9]. The MRS agar was mainly used for the isolation of bacilli and the M17 agar was for isolation of cocci [10]. When colony formation was observed the morphology (including the color, size, and surface smoothness) was recorded and each colony numbered. Individual colonies with different morphology were picked from the plates and streaked onto sterile MRS or M17 agar and incubated for a further 48 h at 30 °C under anaerobic conditions. This process was repeated 2-3 times, until good growth of pure single isolates was evident. Gram staining was used to determine whether the isolates were Gram positive or negative. After microscopic examination Gram positive pure cultures of each isolate were subcultured on to corresponding MRS or M17 agar and a catalase test made following the methods of [33]. The isolates with Gram-positive, catalase-negative non-spore forming and either spherical (coccoid) or rod-shaped (lactobacilli) were preliminarily considered to be potential LAB. Frozen stocks of the purified isolates were made in 30% glycerol and stored at −80 °C prior to further molecular evaluation.

Extraction and purity determination of total DNA

Genomic DNA from each LAB isolate was extracted with a bacterial genome reagent kit following the manufacturer's instructions (TIANGEN Bacterial genomic DNA extraction kit). The concentration and purity of the genomic DNA were determined using the spectrophotometer (NanoDrop 2000) and electrophoresis in 0.8% agarose gel containing 0.01% nucleic acid dye, visualized using a UVP BioImaging System.

PCR amplification of 16S rRNA gene sequences

Bacterial 16S rRNA gene sequences were amplified using the universal bacterial primers 27F and 1492R [10]. and the PCR reaction system (Thermo Electron Corporation, USA). The reaction mixture included 2.5 µL of 10 × PCR buffer (Mg2+), 1.5 mM MgCl2, 0.2 mM 2.0 µL of 10.0 mmol/L dNTP mixture, 0.3 µL of 5 U/µL Taq DNA polymerase, 1.0 µL of 10 pmol/µL primer 27F, 1.0 µL of 10 pmol/µL primer 1492R, 1 µL of 100 ng/µL template DNA and double distilled water to achieve a final volume of 25 µL. The PCR amplification procedure followed the method of Yu et al. (2015): 94°C for 5 min, 94 °C for 1 min, 58 °C for 1 min, 72 °C for 2 min, 30 cycles, and then 72 °C for 10 min, 4 °C insulation. Amplified PCR products were evaluated by electrophoresis in 1.0% agarose gels. If there were clear bands of about 1500 bp and no obvious non-specific amplification, the PCR was considered successful. The 16S rRNA gene was sequenced by Shanghai Biological Engineering Company.

Homology analysis of 16S rRNA gene sequences

16S rRNA gene consensus sequences weremade using DNA star software (v 7.1.0), obtaining 1300bp-1500 bp of effective sequence. The nucleotide sequences of the 16S rRNA

gene of all the isolates were analyzed using the BLAST program on the NCBI website. Splicing sequences were uploaded to the Gen Bank database and compared with existing type isolates using homology analysis. If there was 99% or more similarity between the 16S rRNA sequences we found and the type isolates, then we could conclude that they belonged to the same species. Phylogenetic trees were constructed using Neighbor-Joining method in Mega 5.05 software [11]

Nucleotide sequence accession numbers

16S rRNA gene sequences reported in this research were deposited in GenBank. Accession numbers assigned for the seventy-seven sequences are successive numbers from KX752822 to KX752898.

Results

Count results of LAB and total bacteria in fresh goat's milk

LAB and total bacteria were counted to preliminarily detect the amount of LAB in the sample. Overall LAB counts in goat's milk varied between 2.09 and 5.45 lg CFU/mL (Table 2) in MRS agar. The highest LAB counts came from sample Y15 which reached 105 CFU/mL; counts from WA2, WA3, WB4, WB9, WB10, WC3, WC7, WC8 and WD4 were only around 102 CFU/mL. LAB counts in goat's milk from the Yangling district varied between 3.16 and 5.45 lg CFU/mL; mean count of LAB was 6.46 times higher than that in milk from the Weinan district, and 3.09 times higher than that in milk from the Baoji district.

Total counts of all bacteria in goat's milk varied between 3.08 and 5.93 lg CFU/mL (Table 2) in PCA. Bacterial counts in Y2, Y8, Y15, Y19, Y20, Y21 and WA1 were the highest reaching 105 CFU/mL. LAB accounted for between 3.9% and 97.5% of the total bacteria counts with a mean of 61.5%. Consequently isolation of LAB from the goat's milk was feasible

Physiological and biochemical characteristics of LAB in fresh goat's milk

The individual LAB isolated from fresh goat's milk formed mostly white or transparent colonies that had neat edges, smooth surfaces and were convex at the center. There were a few brown colonies with toothed edges and without smooth surfaces. More colonies were large, white and with rough edges on the MRS medium, while more of the white colonies were small and had smooth surfaces on the M17 medium. These colonies could be potential LAB based on the morphology; differences in color, shape, size, and surface smoothness could be the fundamental for selection of variant LAB in isolation.

Seventy-seven of the 125 isolates from the 71 samples of fresh goat's milk were preliminarily characterized as potential LAB by physiological and biochemical identification method using the Gram stain and the catalase test. Twenty representative potential LAB species are listed in Table 3.

Homology analysis of 16S rRNA gene sequences

Clear bands at about 1500 bp were detected in agarose gel electrophoresis with no obvious non-specific amplification indicating a successful amplification (Fig. 1). Thereby, 16S rRNA genes of the 77 isolates from 71 milk samples were sequenced. 99% homology between the 16S rRNA gene sequences of a number of isolates with type species allowed them to be identified confidently: sixty-two isolates were homologous with the type isolate *E. hirae* ss33b (accounting for 80.5% of all isolates); eight isolates were homologous with the type isolate *E. faecalis* BCB131 (accounting for 10.4% of all isolates). The rest of 9.1% strains were identified as *Lc. lactis* (2 strains) *Lc. garvieae* (1 strain), *E. faecium* (1 strain) and *W. cibaria* (3 strains). Amongst all the isolates, *E. hirae* was isolated from samples collected from all the three districts, accounting for the highest proportion of all isolates.

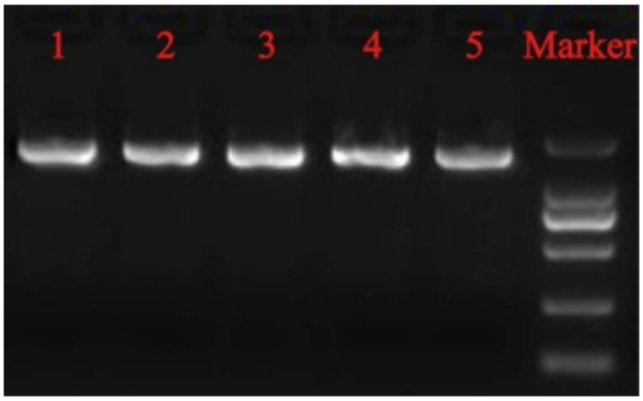

Fig 1. Electrophoresis of 16S rRNA gene PCR products from five species of LAB isolates. Samples in lanes L1-L5 were 16S rRNA gene PCR products from *Enterococcus faecalis, Enterococcus faecium, Weissella cibaria, Lactococcus lactis* and *Lactococcus garvieae* isolates respectively. Marker used in this study was DL 2000, and the fragments from top to bottom represented 2000, 1000, 750, 500, 250, and 100 bp.

Construction of a phylogenetic tree for LAB from goat's milk

The 16S rRNA gene sequences of twenty-eight representative isolates from goat's milk were selected from the 77 available to construct the phylogenetic tree (Fig. 2) and determine relationships between isolates and type strains using Neighbor-Joining method. The Neighbor-Joining tree constructed confirmed the results of the homology analysis. Eighteen isolates from goat's milk including B1, B2, and *E. hirae* ss33b had a close phylogenetic relationship with 99% homology meaning they were all *E. hirae* (Fig. 2). Five isolates including Y1, Y2, and *E. faecalis* BCB131 were located on the same branch of the tree which, combined with homology analysis confirmed they were all *E. faecalis*. Isolates WB1, WC10 and *Lc. lactis*

KLDS 4.0309 were relatively closely related and could thus be identified as *Lc. lactis*. Phylogenetic tree showed representative isolates could be clustered to three genera and five species.

Discussion

Lactic acid bacteria (LAB) are indigenous microbe in goat's milk representing a good resource for LAB isolation [12], which may be more suitable for use as starter in goat's milk fermentation. There have been few studies on the LAB in goat's milk from different regions in China especially in Guanzhongarea where they has a large-scale goat's milk production industry and claims more than 80% of the national market share in goat's milk, and thus there is limited information of their diversity. In this research, conventional morphological, physiological and biochemical analysis alongside 16S rRNA gene sequences analysis were used to identify LAB isolated from goat's milk in Guanzhong area.

The majority of LAB we collected were coccoid isolates with very few bacilli including seventy-four coccoid isolates and only three bacilli, indicating that the former was predominant in fresh goat's milk from all three districts. There were 74 isolates retrieved from the M17 culture medium (74 coccoid strains), but 3 isolates retrieved using the MRS culture medium (three bacilli strains). Coccoid isolated in this study were allocated to *Lactococcus* and *Enterococcus* representing 96.1% of all isolates recorded. The results were similar to study of researchers who isolated mainly genus of *Lactococcus* and *Enterococcus* from the goat milk [13-16].

In this research, coccoid isolates were only retrieved using the M17 medium, although coccoid colonies could be characterized in MRS agar, to avoid duplicate isolation of coccoid in same sample. Amount of the LAB isolated from raw

Sample number	Microbial composition (lg CFU/mL)		Proportion of LAB (%)	Sample number	Microbial composition (lg CFU/mL)		Proportion of LAB (%)
	Mean LAB count	Mean Bacteria counts			Mean LAB count	Mean Bacteria counts	
Y1	3.97±0.02	4.37±0.07	39.8	WB6	3.35±0.06	3.41±0.04	76.0
Y2	4.14±0.08	5.93±0.09	16.2	WB7	3.91±0.03	4.08±0.03	95.8
Y3	3.55±0.03	4.96±0.02	3.9	WB8	3.16±0.05	3.30±0.01	95.8
Y4	3.70±0.01	4.59±0.03	12.9	WB9	2.39±0.01	3.48±0.02	68.7
Y5	3.70±0.01	3.78±0.01	89.1	WB10	2.94±0.01	3.08±0.01	95.5
Y6	3.73±0.05	4.97±0.06	5.9	WC1	3.16±0.05	4.02±0.05	78.6
Y7	3.74±0.02	4.19±0.04	41.7	WC2	3.26±0.04	4.43±0.06	73.6
Y8	3.96±0.04	5.21±0.05	5.6	WC3	2.98±0.01	3.20±0.05	93.1
Y9	3.94±0.02	3.97±0.02	93.3	WC4	3.58±0.02	4.54±0.03	78.9
Y10	3.88±0.06	4.40±0.07	30.2	WC5	3.63±0.05	3.74±0.02	97.1
Y11	3.52±0.05	3.54±0.02	95.5	WC6	3.37±0.03	3.48±0.01	96.8
Y12	3.79±0.08	4.95±0.04	69.2	WC7	2.58±0.01	4.79±0.02	74.7
Y13	3.16±0.07	4.36±0.05	63.1	WC8	2.28±0.03	3.18±0.05	71.7
Y14	3.22±0.01	3.70±0.01	33.1	WC9	3.34±0.05	3.56±0.06	93.8
Y15	5.45±0.09	5.86±0.08	38.9	WC10	3.05±0.06	3.30±0.03	92.4
Y16	3.58±0.06	4.93±0.07	44.7	WD1	3.29±0.02	4.70±0.05	70.0
Y17	3.86±0.02	3.98±0.02	75.9	WD2	3.30±0.07	3.57±0.06	92.4
Y18	3.27±0.03	3.30±0.05	93.3	WD3	3.88±0.04	3.98±0.02	97.5
Y19	4.38±0.02	5.37±0.07	10.2	WD4	2.09±0.01	3.09±0.01	67.6
Y20	4.30±0.05	5.38±0.02	8.3	WD5	3.60±0.06	3.95±0.05	91.1
Y21	4.67±0.09	5.30±0.05	23.4	WD6	3.75±0.08	3.98±0.07	94.2
WA1	4.74±0.02	5.67±0.09	11.7	WD7	3.03±0.05	4.03±0.09	75.2
WA2	2.88±0.01	3.74±0.02	13.8	WD8	3.12±0.01	3.92±0.03	79.6
WA3	2.81±0.03	3.38±0.01	26.9	WD9	3.58±0.07	3.94±0.06	90.9
WA4	3.04±0.05	3.31±0.03	53.7	WD10	3.48±0.08	4.05±0.06	85.9
WA5	3.41±0.02	3.94±0.05	43.0	B1	3.96±0.01	4.36±0.04	39.8
WA6	3.15±0.01	3.41±0.02	55.0	B2	3.37±0.02	3.97±0.01	25.1
WA7	3.78±0.06	4.15±0.01	42.7	B3	3.51±0.04	3.81±0.03	50.1
WA8	3.27±0.07	3.78±0.06	30.9	B4	3.30±0.07	3.56±0.05	55.0
WA9	3.45±0.01	3.67±0.07	60.3	B5	4.37±0.08	4.47±0.03	79.4
WA10	3.30±0.06	3.45±0.01	70.8	B6	3.55±0.06	3.96±0.02	38.9
WB1	3.90±0.02	4.30±0.06	39.8	B7	3.40±0.02	4.40±0.05	10.0
WB2	3.61±0.04	4.12±0.02	30.9	B8	3.42±0.03	4.02±0.06	25.1
WB3	3.18±0.03	3.61±0.04	37.2	B9	3.40±0.01	3.98±0.03	26.3
WB4	2.88±0.07	3.18±0.03	50.1	B10	3.66±0.04	3.94±0.04	52.5
WB5	3.21±0.02	3.88±0.07	21.4				

Table 2. Viable counts of LAB and the total bacteria in fresh goat's milk

Isolate	M17		MRS		LAB Identification
	Gram stain test	Catalase test	Gram stain test	Catalase test	
WA1	+	-			+
WA1a	-	-			-
WA2	+	-			+
WA2a			-	+	-
WA8			+	-	+
WB1a	+	+			-
WB2a			-	+	-
WB3	+	-			+
WB4a			+	-	+
WC2	+	-			+
WC2a			-	-	-
WC8a			+	-	+
WD1	+	-			+
WD2	+	-			+
WD3	+	-			+
WD3a	+	+			-
Y1	+	-			+
Y3	+	-			+
Y3a	+	+			-
B1	+	-			+
B1a	-	-			-
B2	+	-			+
B2a			-	-	-

Table 3. Physiological and biochemical characteristic of the isolates

a: Indicates different size or shape of the colony on the same agar plate
+: Positive -:Negative

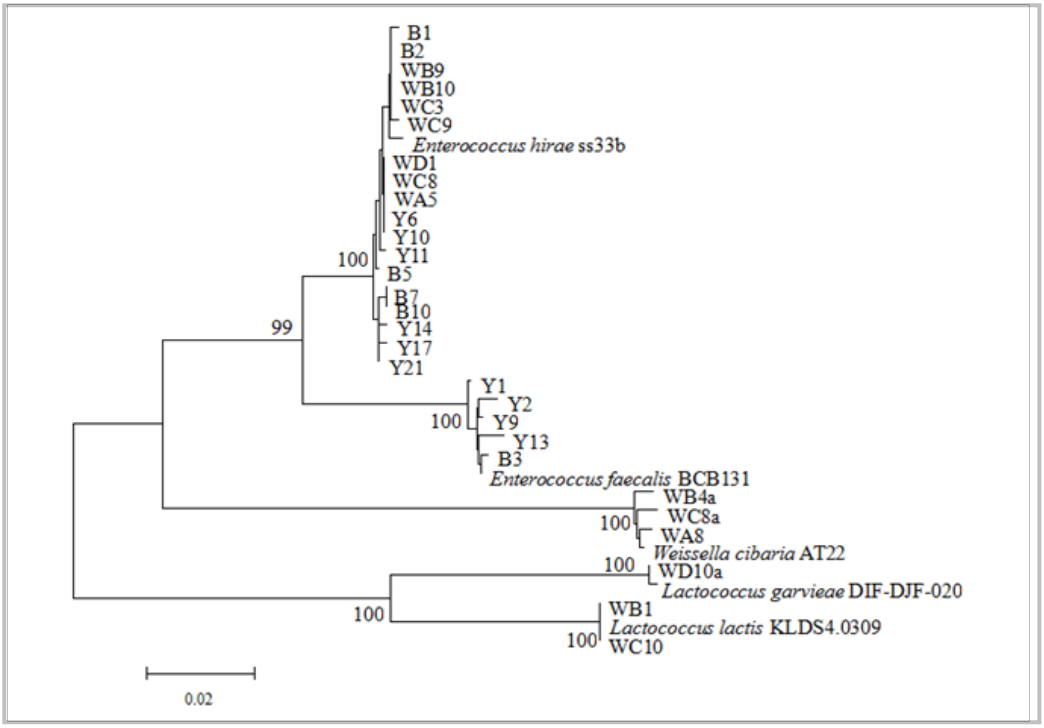

Fig 2. The phylogenetic tree of LAB isolated from fresh goat's milk and type isolates based on 16S rRNA gene.

milk were relative lower than from naturally fermented milk products, in which 3 to 5 strains per sample could be isolated[9].

In fact, the first three milk drops from goat were abandoned to reduce a large amount of contaminated bacteria from the external environment. This sampling procedure ensures the isolates could well represent the diversity of LAB inhabited the goat's milk, which is able to indicate a long term adaptation of these microflora to the goat's milk, or original existence in goats. However, this may lead to dilution of the Lactobacillus strains, even no single colony could be detected in MRS agar. The samples could also be collected from pooled milk by milking of each herd [17], but this will fail to represent the microflora in different single goats. Goat's milk samples could also incubate at different temperature to raise viable counts of the microflora prior to the isolation.

Predominance of coccoid LAB were found in goat's milk, particularly *Lactococcus* and *Enterococcus* species and species of *E. hirae* was common in goat's milk [12, 13, 15, 18]. *Lactococcus* and *Enterococcus* species were also found in the fresh milk of other mammals, for example in cows' milk [19] and camels' milk [20], but with no isolation of *E. hirae* reported in the research. The differences of predominance of LAB in raw milk may be influenced by the regions and the compositions of the milk [17]. Moreover, compositions of LAB in milk are different to studies on LAB found in fermented milk products which are predominantly lactobacilli [6, 21, 35]. This is because fatty acids compositions of the membrane and related genes could promote survival of lactobacilli in acid environment and therefore associated with milk that has undergone fermentation [22].

Seventy seven isolates of LAB from goat's milk could be allocated to six species (Table 4). In particular, *E. faecium* was isolated only from the Yangling district; *W. cibaria*, *Lc. lactis* and *Lc. garvieae* were isolated only from the Weinan district. *E. hirae* was the predominant species found in fresh goat's milk in all three districts. Overall, 94.8% of the isolates were identified as *Enterococcus* amongst which *E. hirae* was also the predominant species accounting for 80.5%. This result is similar to [23] who analyzed the microbial composition of fresh sheeps' milk and found *Enterococcus* species predominated, with no other species of LAB detected including Lactobacilli.

E.hirae has not been previously isolated from cows' milk. Therefore, it is possible that *E. hirae* is a species specifically associated with fresh goat's milk, and this may be related to its metabolic attributes. [24] found *E. hirae* was phylogenetically close (98.8%) to *E. faecium* analyzed by 16S rRNA gene sequence. From the evolutionary point of view, *E. hirae* has a similar effect on human physiology as *E. faecium* [25]. Both species can improve lipid metabolism and are involved in intestinal cholesterol absorption and accelerated rates of faecal excretion. This activity reduces the level of cholesterol and for this reason both species have been used as probiotics for the treatment of gastroenteritis and other diseases in humans and animals [26, 27]. [28] firstly found *E. hirae* could well inhibit the growth of *Listeria monocytogenes* and other *Listeria* subsp. for production of bacteriocin by thespecies; the similar research studied the inhibition effect of a food-originated strain *E. hirae* LD3 on a broad range of pathogen, which is safe for probiotic and clinical use [29]. *E. hirae* was also proved as a new resource of functional food for oxidative damage prevention [30]. In addition, *E. hirae* has good fermentation characteristics in many foods. The yoghurt fermented by single *E. hirae* I-2 or basic starter culture (*L. bulgaricus* and *S. thermophilus*) coupled with *E. hirae* I-2 showed good fermentation characteristics (low fermentation time,high viscosity, good decomposition effect on protein, et al.) and got high score in sensory evaluation [31]

Isolates	Weinan district	Yangling district	Baoji district	Total
Enterococcus hirae	38a/37b	14/14	10/9	62/60
Enterococcus faecalis	-	7/7	1/1	8/8
Enterococcus faecium	-	1/1	-	1/1
Weissella cibaria	3/3	-	-	3/3
Lactococcus lactis	2/2	-	-	2/2
Lactococcus garvieae	1/1	-	-	1/1
Total	44/40	22/21	11/10	77/71

Table 4. LAB species in fresh goat milk in relation to different districts

a: Number of isolates
b: Number of samples

Except for yoghurt application, [32] found the Moringa leaves based beetroot (MLBBR) beverage fermented with *E. hirae* CFR 3011 and *L. plantarum* could lower the content of raffinose (around 60% reduction) which could lead to flatulence; [9] reported that *E. hirae* was capable of transforming the isoflavone daidzein into O-desmethylangolensin which has more benefits to human, applied in deep processing of soybean for value-added products. Accordingly, *E. hirae* could be used in functional or fermented foods on the basis of its good probiotic properties and fermentation characteristics.

As the advantages of probiotics in human health are more widely applied, more attention has been focused on the usage and safety of these beneficial isolates. The first step in this process is to characterize and accurately classify any potentially probiotic isolates. The conventional classification methods based on morphology, physiology, nutritional requirements, growth conditions and biochemical tests are time-consuming and do not always provide a definitive identification due to the increasing numbers of species that vary in some of these characters. By combining traditional methods with 16S rRNA gene sequences

analysis identification can be achieved rapidly and reliably. The results effectively characterized the biodiversity of LAB from goat's milk. Detailed information and accurate identification is essential for further development of the goat's milk industry and the utilization of LAB. Our results provide raw data and are a source of isolates for further studies on selection of probiotic isolates and starter culture design for the industrialization of traditional fermented milk production.

Conclusions

To evaluate the microflora of goat's milk is important to determine whether any potential probiotics are present. Here we isolated and identified LAB in fresh goat's milk from different districts in the Guanzhong area of Shaanxi Province, China and the results indicated that fresh goat's milk was rich in LAB and that there were significant differences in the counts of bacteria from different sampling districts, which may be related to the breed of dairy goat, dietary condition, production areas, the lactation period, environment etc. In our study, 71 samples were collected from three districts. Seventy-seven strains were isolated using traditional pure culture methods and were identified by 16S rRNA gene sequences analysis. The 77 isolates of LAB could be classified into six species, and *E. hirae* was the dominant LAB in goat's milk which would be potential probiotic applying in fermented products and functional foods.

Acknowledgements

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