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Abstract

The study was undertaken to isolate and character lactic acid bacteria (LAB) from fermented camel milk and to screen their ability as a probiotic agent. In this research, 12 camel milk samples were collected and a total of 40 isolates were isolated on a MRS and M17 selective Medias. From MRS media, two genera belonging to Lactobacillus and Pediococcus were isolated while M17 media, Lactococcus and Leuconostoc were isolated based on their morphological and biochemical characteristics. Lactobacillus and Lactococcus were divided into two (group A and B) and three (group A, B and C) respectively on the basis of their sugar fermentation pattern of fructose, galactose, glucose, maltose, sorbitol, sucrose and D-xylose. All of LAB isolates were further screened for their antimicrobial activity against E. coli and S.aureus. Based on the zone of inhibition, 9 isolates were selected for further identification using Biolog system: Enterococcus haemoperoxidus and Lactoccus garvieae species were found as LABs. Hence, from these findings the LAB isolate can be used as biological agent against some pathogens. Further studies should be conducted to characterize probiotic properties of LAB and molecular and phenotypic techniques should be employed for species identification of LABs.

Keywords	Antimicrobial activity; Biolog; Camel; Lactic acid bacteria; Lactobacillus
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cover letter

4/12/2018

Yisehak Tsegaye Redda
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Dear Editorial board,

We wish to submit an original research article entitled "**Isolation and Screening of Lactic Acid Bacteria from Fermented Camel Milk for their Antimicrobil Activity against Common Pathogens**" for consideration by Brazilian Journal of Microbiology.

We confirm that this work is original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere.

In this paper, we report on the antimicrobial activity Lactic acid bacteria. We believe that this manuscript is appropriate for publication by Brazilian Journal of Microbiology because it is aligned with the **journal's Aims & Scope**

We have no conflicts of interest to disclose.
Thank you for your consideration of this manuscript.

Sincerely,

Yisehak Tsegaye Redda.

[redacted]

Title: Isolation and Screening of Lactic Acid Bacteria from Fermented Camel Milk for their Antimicrobil Activity against Common Pathogens

Running Title: Isolation of Lactic Acid Bacteria from Fermented Camel Milk

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ABSTRACT

The study was undertaken to isolate and character lactic acid bacteria (LAB) from fermented camel milk and to screen their ability as a probiotic agent. In this research, 12 camel milk samples were collected and a total of 40 isolates were isolated on a MRS and M17 selective Medias. From MRS media, two genera belonging to *Lactobacillus* and *Pediococcus* were isolated while M17 media, *Lactococcus* and *Leuconostoc* were isolated based on their morphological and biochemical characteristics. *Lactobacillus* and *Lactococcus* were divided into two (group A and B) and three (group A, B and C) respectively on the basis of their sugar fermentation pattern of fructose, galactose, glucose, maltose, sorbitol, sucrose and D-xylose. All of LAB isolates were further screened for their antimicrobial activity against *E. coli* and *S.aureus*. Based on the zone of inhibition, 9 isolates were selected for further identification using Biolog system: *Enterococcus haemoperoxidus* and *Lactoccus garvieae* species were found as LABs. Hence, from these findings the LAB isolate

can be used as biological agent against some pathogens. Further studies should be conducted to characterize probiotic properties of LAB and molecular and phenotypic techniques should be employed for species identification of LABs.

Keywords: *Antimicrobial activity; Biolog; Camel; Lactic acid bacteria; Lactobacillus*

1. INTRODUCTION

Pastoralists in Eastern Ethiopia prepare traditional fermented camel milk by placing fresh camel milk in a clean/smoked container, wrapping the container with a piece of cloth and keeping it in a warm (ambient temperature 25-30 °C) place for about 12-24 h to allow spontaneous fermentation to take place [1,2] stated that a lactic acid bacterium (LAB) is one of the microorganisms that dominate fermented food.

LABs are Gram positive, non-spore forming bacteria, grow under anaerobic condition, cocci or rods which produce lactic acid as the major end product during the fermentation of carbohydrate.[3] (Yam *et al.*, 2015). Many LABs have similar nutritional and growth requirements, biochemical-based methodologies for identification are not conclusive in many cases. Therefore, automated techniques such as Biolog GEN III can be help to achieve accurate identification.[4] According to Millette et al. [5], LABs possess inhibition activity towards spoilage and pathogenic bacteria such as *Escherichia coli* and *Staphylococcus aureus* by producing antimicrobial substance such as organic acids and hydrogen peroxide in the culture medium.[6,7]

Despite the camel milk considerable contribution to food security in semi dry and dry zones and its being a major component of the agropastoral systems in vast pastoral areas in Ethiopia Somali regional state. Studies on characterization of LAB from traditional camel milk are limited. Therefore the present study was aimed to isolate LAB from fermented camel milk and screen their antimicrobial activity against some pathogens.

2. MATERIAL AND METHODS

2.1 Sample Collection

Twelve samples of raw camel`s milk were collected from a lactating Arabian camel (*Camelus dromedaries*) from pastoral households of Jigjiga area in Eastern Ethiopia.

Samples were collected using sterile bottles and delivered to Molecular Biology laboratory of Mekelle University, College of Veterinary Medicine in ice box for analysis. The samples were allowed to ferment at room temperature (25-30C^0) for 1 week spontaneously as described by Yateem et al. [8] .

2.2 Isolation and Characterization of Lactic Acid Bacteria

LABs were isolated from the traditional fermented camel milk using *de Man, Rogosa and Sharpe* (MRS) and M17 agars. To favor better growth of bacteria, approximately 1 ml was inoculated into peptone water and homogenized for 2 minutes. Then, 10 fold serial dilutions was taken and inoculated into a test tube containing 5 ml MRS broth and M17 broth and incubated for 3 days and streaked into MRS agar and M17 agar plates and incubated anaerobically at 37 °C for 48 hours. Individual isolates from countable MRS and M17 agar plates were randomly-picked and sub-cultured 3 times to get pure colonies on appropriate agar medium as described by Savadogo et al. [9] . After obtaining pure colonies, bacterial isolates were characterized for Gram-staining and catalase test followed by microscopic examination to observe cell arrangement and morphological characteristics.

2.3 Antimicrobial Activity of LAB against *S.aureus* and *E.coli*

The antimicrobial property of all isolated LABs against pathogenic bacteria was tested by the agar well diffusion method Al-Otaibi et al.[10]. In this study, *Escherichia coli* and *Staphylococcus aureus* were used as indicator bacteria. Supernatants of LAB isolated were obtained by centrifugation (6000rpm, 15 minutes) of cultures grown in broths of MRS and M17 anaerobically at 37^{o}C for 48 hrs. *E. coli and S. aureus* suspension were inoculated on Mueller Hinton agar plates. Once the plates were dried aseptically, Wells of 6 mm diameter were made and 100µl of each concentration of LAB isolates were filled into well. Then the plates were incubated at 37°C for 24 hrs and antimicrobial activity was determined by measuring the clear zone around the well. The test was conducted in duplicate and the results were recorded and presented as means ± S.D and of both readings.

2.4 Identification of LAB

Isolates of LAB that showed the general characteristics of LAB and had different cellular morphology were selected and subjected to biochemical test; gas production from glucose and sugar fermentation as described by [4]. Isolates were characterized and categorized into different group within genera based on their surge fermentations patterns and morphological characteristics[11] .

Biolog rapid automated identification system: Seven (7) isolates from MRS (RA4, RE4, RA2, RF1, RB2, RB3 and RC1) and Two (2) isolates from M17 (MA1 and ME1) were selected based on the highest zone of inhibition against *E. coli* and *S. aureus* for further identification using Biolog's Identification Systems (Biolog, Hay Ward, USA) [4]. The identification process carried out as per GEN III MicroPlate™ Instructions manual; A single cell colony of each isolates was sub-cultured on BUG+ B Agar for 36-48 hrs. Pure isolate cultured bacteria were suspended in anaerobic Inoculation Fluid C. The turbidity of the suspension was measured using Biolog turbidity meter until reaching 65% of transmittance. 100 µl of the suspension was poured into each of the 94 holes of the Biolog Micro Plate™.The plate was incubated for 24-48 hrs at 37 °C under anaerobic conditions. The identification process was carried out using Omnilog Biolog software.

3. RESULTS

3.1 Isolation and Identification of Lactic Acid Bacteria

From 12 fermented camel milk samples, 40 pure colonies were selected, of which 22 and 18 colonies were obtained from MRS and M17 agar medium respectively. Of those 22 isolates picked from MRS, 8 (36%) belong to *Lactobacillus* group A, 12 (55%) to *Lactobacillus* group B and 2 (9%) to *Pediococcus* as shown in Fig.1. On the other hand, 18 isolates picked from M17, 8 (44%) belong to *Lactococcus* group A, 3 (16.6%) to *Lactococcus* group B, 3 (17%) *Lactococcus* group C and 4 (22%) to *Leuconostoc* (Fig.2). *Lactobacillus* and *Lactococcus* isolates showed variations in sugar fermentation hence divided into two (group A and B) and three (group A, B and C) respectively on the basis of their sugar fermentation profiles as shown in Table 1.

Table 1: Morphological and sugar fermentation characteristics of LAB isolates

Lactic Acid Bacteria *isolated on MRS agar*				**Lactic Acid Bacteria *isolated on* M17 *agar***			
Characteristics	RA1, RA3, RA4, RC2, RC3, RD1, RD3, RE1.	RA2, RB1, RB2, RB4,RC1, RE2, RE3, RE4, RF1, RF2, , RF3, RF4.	RB3, RD2	MA1, MA2, MA3, MD1	MB1, MB2, MB3, MC2, MD2, ME1, MF1, MF2.	MC1, MC3, MF3.	ME2, ME3, MD3.

Colony morphology	Long, blue purple, chains of 3-4 cells Pairs.	long, blue purle rounded ended, pairs	Spherical, pairs and tetrads, small and grayish-white.	diplococci in oval chain	Round, medium, Pairs and short chains.	Spherical, chains, blue purple colonies	Pairs and short chains, smooth, creamy colonies
Gram stain	G+ve, Rods	G+ve, Rods	G+ve, Cocci	G+ve, cocci	G+ve, cocci	G+ve, cocci	G+ve, cocci
Catalase Test	-	-	-	-	-	-	-
CO2 from glucose	+	+	-	+	-	-	-
Fructose	+	+	+	+	+	_	_
Galactose	+	+	_	+	+	+	+
Glucose	+	+	+	+	+	+	+
Maltose	+	+	+	+	_	_	+
Sorbitol	_	+	_	_	_	_	+
Sucrose	+	+	+	_	_	+	+
D-Xylose	_	_	_	_	_	_	_
Identified genera	*Lactobacillus* group A (n=8)	*Lactobacillus* group B (n=12)	*Pediococcus (n=2)*	*Leuconostoc* (n=4)	*Lactococcus* group A (n=8)	*Lactococcus* group B (n=3)	*Lactococcus* group C (n=3)

3.2 Biolog Identification

From Biolog approach, isolates were identified to the genus and/or species level. The results obtained were automatically read and analyzed using Biolog software. *Lactoccus garvieae* (n=1), *Paenibacillus azoreducens* (n=1) and *Actinomyces hordeovulneris* (2) were identified- from MRS while two isolates were not identified to the genus/species level. From M17, *Enterococcus haemoperoxidus* (n=2) were found as shown in Table 2.+ g

Table 2: Biolog identification of isolates into genus/species level

MRS isolates	**Genus and/or species**	**NO.**	**M17 isolates**	**Genus and/or species**	**NO.**
RC1	*Lactoccus garvieae*	1	MA1 and ME1	*Enterococcus haemoperoxidus*	2
RB3 and RA2	*Actinomyces hordeovulneris*	2			
RB2 and RE4	*Paenibacillus azoreducens*	2			
RA4 and RF1	*Unidentified*	2			
Total		7			2

3.3 Antimicrobial Activity

In this study, the 40 isolated LABs were examined for their inhibitory activity against *Escherichia coli* and *Staphylococcus aureus*. MRS isolates indicated average inhibition zone of 11.73 and 11.82 mm against *E. coli* and *S. aureus* respectively, while those M17 shown 9.98 and 10.17 mm against *E. coli* and *S. aureus* respectively. Except the supernatant of ME2 and MD3 (*Lactococcus* group C), all LABs isolated in this study inhibited the growth of *E. coli* and *S. aureus*. The strongest antimicrobial effect against *E. coli* (17.6mm inhibition zone diameter) and *S. aureus* (17.0mm inhibition zone diameter) was shown by RA4 (*Lactobacillus* group A*)* while antimicrobial effect of

other LAB was almost comparable. Zone of inhibition against *E. coli* and *S. aureus* induced by the different LAB in mm were recorded and summarized in Table 3.

Table 3: Antimicrobial activity of selected LABs against E. coli and S. aureus

MRS Isolates	***E.coli***	***S. aureus***	**M17 Isolates**	***E.coli***	***S. aureus***
RA1	11.6 ± 1.0	12.7± 1.98	MB1	10.8 ± 1.13	11.7 ± 1.27
RA3	10.8 ± 1.6	11.3 ± 0.71	MB2	11.3 ± 1.13	10.6 ± 1.13
RA4	17.0 ± 3.4	17.6 ± 1.70	MB3	8.5 ± 0.14	9.2 ± 0.57
RC2	9.6 ± 1.4	8.4 ± 1.13	MC2	9.3 ± 0.99	9.8 ± 0.71
RC3	15.0 ± 2.1	16.2 ± 2.26	MD2	10.8 ± 0.85	12.9 ± 1.41
RD1	11.5 ± 3.4	12.6± 1.27	ME1	13.6 ± 1.27	14.2 ± 0.57
RD3	9.3 ± 1.6	8.3 ± 0.71	MF1	8.5 ± 0.99	8.7 ± 0.57
RE1	10.7± 1.3	10.9 ± 0.85	MF2	10.6 ± 0.85	12.3 ± 0.71
RA2	13.5 ± 1.0	14.4 ± 2.55	MA1	13.7 ± 1.27	12.1 ± 0.85
RB1	8.8 ± 1.8	9.6 ± 1.41	MA2	13.7 ± 1.56	8.7 ± 0.85
RB2	10.4 ± 2.3	11.2 ± 1.13	MA3	12.3 ± 0.71	12.6 ± 1.13
RB4	9.9 ± 4.1	12.9 ± 0.99	MD1	12.4 ± 1.27	13.4 ± 1.13
RC1	11.7 ± 2.7	10.3 ± 0.99	MC1	9.8 ± 0.57	10.3 ± 0.14
RE2	13.4 ± 0.8	13.5 ± 2.55	MC3	11.7 ± 0.71	11.8 ± 0.57
RE3	12.5 ± 1.8	11.7 ± 1.56	MF3	10.9 ± 4.1	15.2 ± 0.85
RE4	14.2 ± 2.0	14.4 ± 2.55	ME2	0.0 ± 0.00	0.0 ± 0.00
RF1	13.5 ± 1.7	8.7 ± 1.27	ME3	8.4 ± 1.27	9.6 ± 0.57
RF2	9.6 ± 1.3	10.4 ± 1.13	MD3	0.0 ± 0.00	0.0 ± 0.00
RF3	10.8 ± 0.6	10.8 ± 1.13			
RF4	8.9 ± 1.8	11.5 ± 1.56			
RB3	8.3 ± 0.6	9.4 ± 0.28			
RD2	8.8 ± 0.8	13.3 ± 1.27			
Mean ± S.D	11.35 ± 2.30	11.82 ± 2.44	Mean ± S.D	9.97 ± 3.95	10.17 ± 4.14
Maximum	17.0	17.6	Maximum	14.3	15.2
Minimum	8.3	8.3	Minimum	0	0

4. DISCUSSION

In the present paper, LABs were isolated from traditionally fermented camel milk and identified to genus level. The identification and grouping of the isolates was based on morphological characteristics, gas production from glucose and sugar fermentation profiles. [1,12,13]

Lactobacillus and *Lactococcus* isolates were divided into two (group A and B) and three (group A, B and C) respectively on the basis of their sugar fermentation profiles. *Lactobacillus* was divided into two groups (group A and B). Group A included 8 isolates that produced acid from fructose, galactose, glucose, maltose and but did not produce acid from sucrose, sorbitol and D-xylose. Group B included 12 isolates that don't produce acid from D-xylose. *Lactococcus* were also divided into three groups (A, B and C). Group A included 8 isolates that produced acid from galactose, glucose and fructose but not maltose, Sorbital, Sucrose and D-Xylose. Group B included 3 isolates that ferment galactose, glucose and Sucrose but not fructose, maltose, Sorbital and D-Xylose. Group C included 3 isolates that don't ferment to Fructose and D-Xylose. According to Muyanja et al. [14] morphological and biochemical characterization based on sugar fermentation pattern is a useful tool for presumptive classification, but may not always provide sufficient basis for the reliable identification of lactic acid bacteria. Therefore, available systems should be combined with other techniques which allowed more accurate identification.

In this study, 20 isolates from MRS was identified as *Lactobacillus* representing the dominant flora from the total lactic acid bacteria .They were bacilli with long/ short and rounded ends, pairs or single cells and able to produce gas from glucose. This finding is consistent with the findings of Seifu et al.[1] who found 85 *Lactobacillus* from *Ititu*, traditional Ethiopian fermented camel milk from 154 LABs from MRS agar plates. and his identification of strains was based on biochemical tests only.

Fourteen isolates picked from M17 agar plates were found to belong to the genus *Lactococcus* representing second most dominant from the total LABs and divided into group A (8 isolates), group B (3 isolates) and group C (3 isolates) on the basis of sugar fermentation. They were gram-positive cocci form grouped in pairs or small chains and they were unable to produce gas from

glucose. Similar observation was reported by Khedid et al.[13] who indicated that *Lactococcus* isolates were unable to produce gas from glucose. Similar results indicating that *Lactococcus* strains as second most dominant accounting (33.3%) from traditional sudanese fermented camel's milk (*Gariss*) has been reported by Ashmaig et al.[12]

Seven isolates (10%) picked from M17 agar plates were found to belong to the genus *Leuconostoc*. They were diplococci in oval chain and produced gas from glucose[10]. The same observation was mentioned by Akhmetsadykova and Baubekova [15] who isolated a reduced number of *leuconostocs* (7 out of 44 strains) from raw and fermented camel milk. The small proportion of these species among our isolates can be due probably to their incapacity for competition with the other lactic acid bacteria.

From this finding, *Pediococcus* picked from MRS agar plates represented the smallest proportion of (2 (~9%)) isolates from the LABs. They were in pairs and tetrads and they were unable to produce gas from glucose. The present observation is in agreement with the findings of Yam [3] who reported 8% *Pediococcus* isolates out of 94 LABs from fermented camel milk.

It is usually expected potential probiotic lactic acid bacterial would be capable of inhibiting the growth of *Escherichia coli* and *Staphylococcus aureus* pathogens.[8, 16, 17] In this study, LAB isolates indicated average inhibition zone of 11.73 and 11.82 mm against *E. coli* and *S. aureus* respectively.

The highest antimicrobial activity against *E.coli* and *S. aureus* was demonstrated by RA4 that representing *Lactobacillus* group A with 17.6mm in diameter inhibition zone against *E.coli* and 17.0mm in diameter inhibition zone against *S. aureus* and ME2 and MD3 isolates of *Lactococcus* group C propagated in M17 broth presented no inhibition against both pathogens. This finding is in line with the finding of Al-Otaibi eta al.[10] who reported maximum inhibition of 20mm against in both *E.coli* and *S. aureus* by *Lactobacillus* genus from camel milk. But, opposite to Muna and Adel [17] who indicated no inhibition of *Lactobacillus* against *E.coli* isolated from camel milk and propagated in MRS broth. The difference may be because of the wide spectrum of antimicrobial activities of LAB isolates which varies according to the serotype[9] .

The inhibitory action of LAB bacteria is mainly due to the accumulation of main primary metabolites such as lactic and acetic acids, ethanol and carbon dioxide. Additionally, LAB are also capable of producing antimicrobial compounds such as formic and benzoic acids, hydrogen peroxide**,** diacetyl, acetoin and bacteriocins. The production levels and the proportions among those compounds depend on the strain, medium compounds and physical parameters.[18] .

Finally, Biolog identification was applied to 9 isolates that presented highest zone of inhibition. The two isolates from M17 were identified as *Enterococcus haemoperoxidus* while only *Lactoccus garvieae* was found in MRS isolates as LAB species. The finding is in agreements with Khedid et al. [13] who reported *Enterococcus haemoperoxidus* and *Lactoccus garvieae* from raw camel milk in Morocco using carbohydrates fermentations.

Paenibacillus azoreducens and *Actinomyces hordeovulneris* were encountered during Biolog identification system and none of these species were reported as lactic acid bacteria by literatures reviewed up to date.[4] stated that phenotypic tests like Biolog test have limitations such as their poor reproducibility, a result of the plasticity of bacterial growth, the logistic difficulties for large-scale applications and their sometimes poor discriminatory power.

In this paper, RC1 was initially characterized as Lactobacillus spp due to cell morphology and gas production from glucose. In contrast to that Biolog identified as Lactoccus garvieae. According to Moraes et al. [20], different methods of evaluation provided different patterns of genera and species identification for the LAB isolates. Regarding his results, sequencing of 16S rDNA gene and species-specific PCR, the most frequent genera observed was *Enterococcus* spp., whereas API50CHL mostly identified *Lactobacillus* spp. and BioLog mostly identified *Enterococcus* spp. The reason is because many LABs have similar nutritional and growth requirements and a reliability rate varies as well [20] .

The LABs isolated and characterized showed a capacity to inhibit *E.coli* and *S. aureus* however at different inhibition levels. These two undesirable strains are known like contaminant of the

acidified dairy products. Therefore, antimicrobial activity reflects LAB`s bio-preservation value against spoilage and pathogenic microorganisms.

Declarations

Consent for publication

"Not applicable"

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YT, NA and GG conceived and designed the study. AMA and YT performed the experiments. YT, NA, GG, HT, GMC, conducted the systematic literature search, analyzed the data and wrote the manuscript. All authors have read and approved the final version of this manuscript.

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Reference

1. Seifu E, Abraham A, Kurtu M, Yilma Z. Isolation and characterization of lactic acid bacteria from Ititu : Ethiopian traditional fermented camel milk. *J Camelid Sci*. 2012;5(1):82-98. https://www.researchgate.net/profile/Zelalem_Yilma/publication/264398395_Isolation_and_characterization_of_lactic_acid_bacteria_from_Ititu_Ethiopian_traditional_fermented_camel_milk/links/543817a80cf204cab1d541bc.pdf. Accessed April 9, 2018.

2. Scott R, Sullivan WC. Ecology of Fermented Foods Introduction : *Hum Ecol*. 2008;15(1):25-31. http://www.jstor.org/stable/24707481?casa_token=tvW3aIH3dh4AAAAA:tFVxGhzSkYCx70ZpaGom5Xf6l3i3ar_rLK8F4809D2xSrZ7MFvlgkdnRKQxN6Qz4Ia8knq6oXXDtd73XL-JKZpd2_7127LUBujR7NFBll_z81ec62KGB. Accessed April 9, 2018.

3. Yam BZ. Isolation and Identification of Yeasts and Lactic Acid Bacteria from Local Traditional Fermented Camel Milk, Chal. *J Food Process Technol*. 2015;06(07):1. doi:10.4172/2157-7110.1000460

4. Mohania D, Nagpal R, Kumar M, et al. Molecular approaches for identification and characterization of lactic acid bacteria. *J Dig Dis*. 2008;9(4):190-198. doi:10.1111/j.1751-2980.2008.00345.x

5. Millette M, Cornut G, Dupont C, Shareck F, Archambault D, Lacroix M. Capacity of human nisin- and pediocin-producing lactic acid bacteria to reduce intestinal colonization by vancomycin-resistant enterococci. *Appl Environ Microbiol*. 2008;74(7):1997-2003. doi:10.1128/AEM.02150-07

6. Ito A, Sato Y, Kudo S, Sato S, Nakajima H, Toba T. The screening of hydrogen peroxide-producing lactic acid bacteria and their application to inactivating psychrotrophic food-borne pathogens. *Curr Microbiol*. 2003;47(3):231-236. doi:10.1007/s00284-002-3993-1

7. Alegría Á, Delgado S, Roces C, López B, Mayo B. Bacteriocins produced by wild Lactococcus lactis strains isolated from traditional, starter-free cheeses made of raw milk. *Int J Food Microbiol*. 2010;143(1-2):61-66. https://www.sciencedirect.com/science/article/pii/S0168160510004022. Accessed April 9, 2018.

8. Yateem A, Balba MT, Ai-Surrayai T, Ai-Mutairi B, Al-Daher R. isolation of lactic

acid bacteria with probiotic potential from camel milk. *Int J dairy Sci*. 2008;3:194-199. doi:http://dx.doi.org/10.3923/ijds.2008.194.199

9. Savadogo A, Ouattara C, Bassole I. Antimicrobial activities of lactic acid bacteria strains isolated from Burkina Faso fermented milk. *Pakistan J Nutr*. 2004;3(3):174-179. http://www.docsdrive.com/pdfs/ansinet/pjn/2004/174-179.pdf. Accessed April 9, 2018.

10. Al-Otaibi MM, Al-Zoreky NS, El-Dermerdash HA. Camel's Milk as a Natural Source for Probiotics. *Res J Microbiol*. 2013;8(2):70-80. doi:10.3923/jm.2013.70.80

11. Seifu E. Handling, preservation and utilization of camel milk and camel milk products in Shinile and Jijiga Zones, eastern Ethiopia. *Livest Res Rural Dev*. 2007;19(6).

12. Ashmaig A, Hasan A, Gaali E El. Identification of lactic acid bacteria isolated from traditional Sudanese fermented camel ' s milk (Gariss). *African J Microbiol Reserach*. 2009;3(8):451-457. http://www.academicjournals.org/journal/AJMR/article-abstract/0103B5E13715. Accessed April 9, 2018.

13. Khedid K, Faid M, Mokhtari A, Soulaymani A, Zinedine A. Characterization of lactic acid bacteria isolated from the one humped camel milk produced in Morocco. *Microbiol Res*. 2009:81-91. https://www.sciencedirect.com/science/article/pii/S0944501306001327. Accessed April 9, 2018.

14. Muyanja CMBK, Narvhus JA, Treimo J, Langsrud T. Isolation, characterisation and identification of lactic acid bacteria from bushera : a Ugandan traditional fermen ted beverage. *Int J Food Microbiol*. 2003;80(3):201 – 210. https://www.sciencedirect.com/science/article/pii/S0168160502001484. Accessed April 9, 2018.

15. Akhmetsadykova SH, Baubekova A. Lactic acid bacteria biodiversity in raw and fermented camel milk. *African J Food Sci Technol*. 2015;6(3):2141-5455. doi:10.14303/ajfst.2015.026

16. Rushdy AA, Gomaa EZ. Antimicrobial compounds produced by probiotic Lactobacillus brevis isolated from dairy products. *Ann Microbiol*. 2013;63(1):81-90. https://link.springer.com/article/10.1007/s13213-012-0447-2. Accessed April 9, 2018.

17. Muna MA, Adel MM. Isolation of Lactobacillus strains with probiotic potential from camels milk. *African J Microbiol Res*. 2014;8(15):1645-1655.

doi:10.5897/AJMR2013.6598

18. Trabelsi I, Slima S Ben, Chaabane H, Riadh BS. Purification and characterization of a novel exopolysaccharides produced by Lactobacillus sp. Ca6. *Int J Biol Macromol*. 2015;74:541-546. doi:10.1016/j.ijbiomac.2014.12.045

19. Liu S, Han Y, Zhou Z. Lactic acid bacteria in traditional fermented Chinese foods. *Food Res Int*. 2011;44(3):643-651. doi:10.1016/J.FOODRES.2010.12.034

20. Moraes PM, Martins Perin L, Silva Júnior A, Nero LA. Comparison of phenotypic and molecular tests to identify lactic acid bacteria. *Brazilian J Microbiol*. 2013;44(1):109-112. doi:10.1590/S1517-83822013000100015

Declarations

Competing interests

The authors declare that they have no competing interests.

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Table 1: Morphological and sugar fermentation characteristics of LAB isolates

Lactic Acid Bacteria *isolated on MRS agar*				**Lactic Acid Bacteria *isolated on* M17 *agar***			
Characteristics	RA1, RA3, RA4, RC2, RC3, RD1, RD3, RE1.	RA2, RB1, RB2, RB4,RC1, RE2, RE3, RE4, RF1, RF2, , RF3, RF4.	RB3, RD2	MA1, MA2, MA3, MD1	MB1, MB2, MB3, MC2, MD2, ME1, MF1, MF2.	MC1, MC3, MF3.	ME2, ME3, MD3.
Colony morphology	Long, blue purple, chains of 3-4 cells Pairs.	long, blue purle rounded ended, pairs	Spherical, pairs and tetrads, small and grayish-white.	diplococci in oval chain	Round, medium, Pairs and short chains.	Spherical, chains, blue purple colonies	Pairs and short chains, smooth, creamy colonies
Gram stain	G+ve, Rods	G+ve, Rods	G+ve, Cocci	G+ve, cocci	G+ve, cocci	G+ve, cocci	G+ve, cocci
Catalase	-	-	-	-	-	-	-
CO2 from glucose	+	+	-	+	-	-	-
Fructose	+	+	+	+	+	_	_
Galactose	+	+	_	+	+	+	+
Glucose	+	+	+	+	+	+	+
Maltose	+	+	+	+	_	_	+
Sorbitol	_	+	_	_	_	_	+
Sucrose	+	+	+	_	_	+	+
D-Xylose	_	_	_	_	_	_	_
Identified genera	*Lactobacillus* group A (n=8)	*Lactobacillus* group B (n=12)	*Pediococcus (n=2)*	*Leuconostoc* (n=4)	*Lactococcus* group A (n=8)	*Lactococcus* group B (n=3)	*Lactococcus* group C (n=3)

Table 2: Biolog identification of isolates into genus/species level

MRS isolates	Genus and/or species	NO.	M17 isolates	Genus and/or species	NO.
RC1	*Lactoccus garvieae*	1	MA1 and ME1	*Enterococcus haemoperoxidus*	2
RB3 and RA2	*Actinomyces hordeovulneris*	2			
RB2 and RE4	*Paenibacillus azoreducens*	2			
RA4 and RF1	*Unidentified*	2			
Total		7			2

Table 3: Antimicrobial activity of selected LABs against E. coli and S. aureus

MRS Isolates	*E.coli*	*S. aureus*	M17 Isolates	*E.coli*	*S. aureus*
RA1	11.6 ± 1.0	12.7± 1.98	MB1	10.8 ± 1.13	11.7 ± 1.27
RA3	10.8 ± 1.6	11.3 ± 0.71	MB2	11.3 ± 1.13	10.6 ± 1.13
RA4	17.0 ± 3.4	17.6 ± 1.70	MB3	8.5 ± 0.14	9.2 ± 0.57
RC2	9.6 ± 1.4	8.4 ± 1.13	MC2	9.3 ± 0.99	9.8 ± 0.71
RC3	15.0 ± 2.1	16.2 ± 2.26	MD2	10.8 ± 0.85	12.9 ± 1.41
RD1	11.5 ± 3.4	12.6± 1.27	ME1	13.6 ± 1.27	14.2 ± 0.57

RD3	9.3 ± 1.6	8.3 ± 0.71	MF1	8.5 ± 0.99	8.7 ± 0.57
RE1	10.7± 1.3	10.9 ± 0.85	MF2	10.6 ± 0.85	12.3 ± 0.71
RA2	13.5 ± 1.0	14.4 ± 2.55	MA1	13.7 ± 1.27	12.1 ± 0.85
RB1	8.8 ± 1.8	9.6 ± 1.41	MA2	13.7 ± 1.56	8.7 ± 0.85
RB2	10.4 ± 2.3	11.2 ± 1.13	MA3	12.3 ± 0.71	12.6 ± 1.13
RB4	9.9 ± 4.1	12.9 ± 0.99	MD1	12.4 ± 1.27	13.4 ± 1.13
RC1	11.7 ± 2.7	10.3 ± 0.99	MC1	9.8 ± 0.57	10.3 ± 0.14
RE2	13.4 ± 0.8	13.5 ± 2.55	MC3	11.7 ± 0.71	11.8 ± 0.57
RE3	12.5 ± 1.8	11.7 ± 1.56	MF3	10.9 ± 4.1	15.2 ± 0.85
RE4	14.2 ± 2.0	14.4 ± 2.55	ME2	0.0 ± 0.00	0.0 ± 0.00
RF1	13.5 ± 1.7	8.7 ± 1.27	ME3	8.4 ± 1.27	9.6 ± 0.57
RF2	9.6 ± 1.3	10.4 ± 1.13	MD3	0.0 ± 0.00	0.0 ± 0.00
RF3	10.8 ± 0.6	10.8 ± 1.13			
RF4	8.9 ± 1.8	11.5 ± 1.56			
RB3	8.3 ± 0.6	9.4 ± 0.28			
RD2	8.8 ± 0.8	13.3 ± 1.27			
Mean ± S.D	11.35 ± 2.30	11.82 ± 2.44	Mean ± S.D	9.97 ± 3.95	10.17 ± 4.14
Maximum	17.0	17.6	Maximum	14.3	15.2
Minimum	8.3	8.3	Minimum	0	0