IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 9, Issue 2 (Mar. – Apr. 2023), PP 30-37
www.iosrjournals.org

Isolation and Identification of Lactic acid Bacteria of *Berkeep*, a Traditionally Fermented Milk Product of Western Sudan

Zawahir A. Musa[1] Omer I. Ahmed[1] Warda S. Abdelgadir[2]
[1] *Sudan University of Science and Technology, Faculty of Animal Production Science and Technology*
[2] *Federal Ministry of Agriculture, National Food Research Centre, Khartoum North, Sudan*
Corresponding Author: Zawahir Abuelbashar Musa

Abstract:
Background:
The bacteria involved in lactic acid fermentation belong to two families' Lactobacillaceae and Streptococcaceae and four genera Lactobacillus, Leuconostoc, Pediococcus and Streptococcus. Members of LAB share the property of being Gram-positive bacteria that ferment carbohydrates into energy and lactic acid and provide formation of the curd. Furthermore, they show proteolytic activity and also they play a role in the production of aroma compounds and antimicrobial substances.
Materials & Methods*: This study was conducted during the period January 2019– January 2022, a total of 75 samples of Berkeep were randomly collected from traditional production sites in Kass, Elkoumah and Manwashy, Darfur State, Western Sudan. Samples were analyzed for isolation & identification of LAB & the determination of bacteria counts (log_{10}cfu $mL^{-1)}$ on plate count agar, MRS and M17 for the total load of aerobic mesophilic bacteria, lactobacilli and lactococci, respectively, at the Department of Botany and Plant Biotechnology, Faculty of Agriculture, University of Khartoum. DNA extraction was carried out at the research laboratory, Department of Parasitology, Faculty of Veterinary Medicine, Sudan University of Science and Technology. The RAPD-PCR analysis was conducted at the Central laboratory, Department of Molecular Biology, Ministry of Higher Education and Scientific Research (Sudan).*
Results*: Similar counts were observed in the three production sites for the above-mentioned bacteria. The pH of Berkeep samples was significantly higher (P < 0.01) in Manwashy (4.06) and lower in Elkoumah (3.57), whereas the titratable acidity was significantly higher (P < 0.01) in Elkoumah (2.51±0.30) than in Kass (1.70± 0.37) and Manwashy (1.88 ±0.28). A total of 306 bacterial isolates were obtained from Berkeep samples on MRS and M17, all were Gram +ve and catalase -ve rods and cocci. Based on growth morphology, gas production from glucose, lipolytic activity and other biochemical tests, four genera of LAB were identified as Lactobacillus, Enterococcus, Pediococcus and Leuconostoc. Isolated LAB was dominated lactobacillus spp. (62.42%) followed by Enterococcus (22.22%) then Pediococcus (9.15%) and Leuconostoc (6.21%).*
Conclusion*: The present study revealed that Berkeep, a traditionally fermented milk product of Western Sudan contained four genera of LAB dominated by Lactobacillus and including Enterococcus, Pediococcus and Leuconostoc. Extra work is recommended to further characterize these bacteria to species and strain levels based on 16S rRNA sequencing and plasmid profiling, respectively.*
Key words*: Lactic acid bacteria, Berkeep, Fermented milk, RAPD-PCR*

Date of Submission: 24-03-2023 Date of Acceptance: 06-04-2023

I. Introduction

Fermented dairy products are foods abundantly consumed worldwide and they have shown a significant consumption increase in recent years.

The consumer interest in fermented dairy products due to the nutritional and health-giving properties offered by these products because their effect on the intestinal microflora that contributes to a healthy life and to increase life anticipation [1].

Fermentation processes generally enhance the nutritional value of many foods and increase the bioavailability of nutrients. There is an increment of lactic acid, galactose and other nutrients concentrations as a result of the fermentation action by lactic acid bacteria, hence giving chances to Lactose-intolerant individuals tolerate lactose when it is consumed in fermented products better than milk [2].

In Sudan, accurate statistics of the annual milk production are kept and available in 2016 to 2020. [3] gave an estimate of annual milk production of 4.66 million tons of which 3.0 million (64.7%) are cow's milk. Goat's milk made 1.2 million tons, sheep 0.417 million tons and camel's milk only 0.062 million tons. The bulk of milk in the country is produced by cattle herds owned by nomads and these produce a plenty of milk supply in the rainy season which extends in central Sudan for 3–5 months. The surplus milk thus produced is fermented by souring into one or other of certain dairy products some of which are spread wide in the country whereas others are confined to certain geographical areas [4].

Berkeep is a dairy product which has been produced since ancient times by rural breeders and farmers in Darfur State, Western Sudan. It is made mainly from cow's milk, goat's milk or their mixture. On house hold level, berkeep is traditionally fermented in two ways, one of which is natural fermentation where the milk surplus to the consumption of the family is collected in a container. The container could either be *Kas* (the lower half of a cut gourd) made from the dried fruit of the plant *Lagenaria peucantha*, plastic, metal containers or tanned goat's skin (Fig. 1a&b). The milk is boiled, then covered and left to cool to ambient temperature (25-30°C) and to spontaneously ferment for 2-3 days. In the other way, the milk is boiled, cooled to about 37°C, then inoculated with a small amount of the previously produced *Berkeep* as starter and incubated for 1-2 days at room temperature without adding any flavoring material. The product is known to keep its quality for more than 2 weeks. The end product is known to have a unique pleasant flavor along with a smooth texture.

To the best of our knowledge, this is the first study to be done for this traditional product. Hence, the main objective of this research is to isolate and identify the predominant lactic acid bacteria responsible for the fermentation of the traditional fermented milk *Berkeep* as produced in different villages in Darfur State (Western Sudan).

II. Materials and Methods

Sampling

A total of 75 samples of *Berkeep* were randomly collected from traditional markets of Kass, Elkoumah, and Manwashy (25 samples from each) in Darfur State. Samples were collected aseptically in sterile screw-cap containers and transported immediately in an ice box to the microbiology laboratory at the Faculty of Agriculture, University of Khartoum and were analyzed microbiologically upon arrival and their pH was recorded.

Microbiological analysis

For all samples, 10 mL were homogenized in 90 mL sterile diluent (1% peptone (Difco, Detroit, Michigan, USA), 0.9% NaCl, pH 7.0) by use of a stomacher (Lab Blender, Model 400, Seward Medical, London, England) for 30 s, normal speed. Hundred mL from ten-fold dilutions of the samples were inoculated De Man, Rogosa and Sharpe (MRS) agar (Difco) for lactobacilli, M17 agar (Difco) for lactococci. The plates were incubated at 30°C for 2–3 d. using the anaerobic jars and the AnaeroGen C system (Oxoid, Basingstoke, UK) anaerobic system envelopes (Becton Dickinson, Cockeysville, USA). Then, at the appropriate dilution, colonies were counted using a colony counter and the results were expressed as $\log_{10}$ cfu/ml. Representative colonies on MRS and M17 were tested for their Gram reaction and catalase activity. Only Gram-positive bacteria with catalase negative reactions were observed and the representative isolates were purified by successive streaking onto the same agar substrate. For the Gram-positive, catalase negative cocci and rods, hetero- and homofermentative activity (using MRS and M17 broth with inverted Durham tubes), Oxidase activity, growth and viability at 6.5% NaCl [5] lipolytic activity, and fermentation test (O/F), motility and acid and CO_2 production from Glucose [6] were carried out.

a) Kas

b) Si'in

Fig. 1: Containers for *Berkeep* fermentation

Randomly amplified Polymorphic DNA (RAPD-PCR)

All Gram positive, catalase negative bacteria (presumptive LAB) were screened into groups using RAPD-PCR. Genomic DNA was extracted by the procedure described by [7]. RAPD analysis was carried out using the primers R2 5´-GGCGACCACTAG 3´ and M13 5´ GAGGGTGGCGGTTCT-3´ and cluster analysis was carried out as described previously [8] (Bonomo *et al.*, 2008). Maxime PCR Premix Kit (i-Tag) for 20 µl run was used to achieve the PCR process with slight modification of extra addition of 2.5 µl of $MgCl_2$. The PCR cocktails (20 µl) consisted of 1 µl of the primers, 5 µl of DNA, 2.5 Ui-tag DNA polymerase, 2.5 mM of each dNTP, 1X reaction buffer. 1X gel loading buffer and 2.5 µl of $MgCl_2$. Amplification condition were initial denaturation at 94° C for 5 minutes, 40 cycles of 94°C for 1 minute, annealing at 38°C for R2 and 40°C for M13 for 45 second. And elongation at 72°C for 1 min, followed by a final elongation at 72°C for 10 minutes. The PCR products were visualized by running in 1.5% agarose gel electrophoreses with 100 bp DNA ladder (Sigma, Saint Louis, USA). The electrophoresis conditions were 100 V, 60 Am, for 30 min with 1X TAE as running buffer.

III. Results

The results of the analysis of seventy-five *Berkeep* samples collected from three production sites in Darfur, Western Sudan are shown in Table 1. The samples analysed immediately after collection from the three production sites contained 8.63±0.86, 8.87±.0.70 and 8.65±0.93 $\log_{10}$cfu/ml lactobacilli respectively. Lactococci in Elkoumah is 8.06±0.50 $\log_{10}$cfu/ml similar to that in Manwashy but both are a little bit higher than those contained in Kass 7.80±0.75 $\log_{10}$cfu, although not significant increment. The pH of the samples collected from Elkoumah is significantly lower ($p<0.01$) than those of Manwashy and Kass, whereas the titratable acidity is higher ($p<0.01$). Aerobic mesophilic bacteria count on PCA were in the same range, 7.10±0.35, 6.87±0.70 and 7.06±.56 in Elkoumah, Manwashy and Kass respectively. These bacteria were not studied in any details but catalase positive Gram positive and Gram-negative bacteria were seen in addition to lactic acid bacteria.

In this study, the identification of LAB was performed through morphological characteristics and biochemical tests to the genus level. Genotypic tests were carried out to confirm the obtained results. For all samples, the bacteria on MRS and M17 were predominantly Gram positive, catalase and oxidase negative, non-motile, non-endospore forming, grew at 6.5% NaCl, and they were all facultative anaerobes rods and cocci, respectively. From 191 rods, 37 isolates have lipolytic activity, whereas thirty-one M17 isolates from a total of 115 were found to be lipolytic, as well. The majority of isolates on MRS and M17 were homofermentative producing only lactic acid from glucose fermentation (Table 2&3).

Table no. 1: Bacterial counts $\log_{10}$cfu/mL[1] and pH of *Berkeep* from different production sites

Source of sample	*Lactobacillus* spp.	*Lactococcus* spp.	Aerobic mesophilic bacteria	pH	Titratable acidity
Elkoumah	8.63±0.86	8.06±0.50	7.10±.35	3.59±0.07^{c}	2.51±0.30^{a}
Manwashy	8.87±.0.70	8.09±0.51	6.87±70	4.06±0.45^{a}	1.88±0.28^{b}
Kass	8.65±0.93	7.80±0.75	7.06±.56	3.78±0.19^{b}	1.70±0.37^{b}
Sig.	(0.220) NS	(0.508) NS	(0.286) NS	(0.000) **	(0.002) **

Sig. Significant different at ($p<0.05$).

** highly significant different at ($p<0.01$)

a, b and c: within the same column followed by different superscript are significantly different at ($p<0.05$).

NS: Not significant different at ($p>0.05$).

Under the microscope, the bacteria on M17 are either ovoid in chains or arranged in pairs, short chains or in tetrads. According to the differences in morphology, fermentation type and lipolytic activity, LAB isolates (306 isolates) were screened into 4 genera based on [9] Cowan and Steel manual (1993). The rod-shaped isolates on MRS (191 isolates) constituted 62.42% of the total number were assigned as lactobacillus subspecies. Lactococci on M17 showed three genera including enterococcus spp. (68 isolates) arranged in pairs or short chains, Pediococcus spp. (28 isolates) found pairs or tetrads and Leuconostoc (19 isolates) which were ovoid in shape appeared single or in chains (Table 2). Following preliminarily phenotypic characterization the isolates were genotypically grouped by RAPD-PCR and subsequent cluster analysis. Cluster analysis showed a clear separation into 4 clusters (Fig. $2_{a, b, c}$ & Table 4).

IV. Discussion:

Species of lactic acid bacteria (LAB) belong to numerous genera under the family of Lactobacillaceae. They are considered as potential microorganisms and have been widely used in food fermentation due to their important status as generally recognized as safe (GRAS) microorganisms. They are also known for their fermentative ability and thus, improving organoleptic attributes, imparting enriched nutrients and enhancing food safety, hence increasing health benefits [10, 11]. These diverse microorganisms have a common defining

characteristic that they produce lactic acid as the end product in the process of carbohydrates fermentation [12, 13]. Traditionally, they are closely linked to the fermentation of human foods especially in dairy products [14] (He *et al*., 2020). According to the results obtained in this study, the majority of the LAB isolated from berkeep from the three different production areas was *Lactobacillus* spp. (62.42%). *Lactococci* in Berkeep constituted 37.58% of Enterococcus spp. was (22.22%), *Pediococcus* spp. (9.15%). and *Leuconostoc* spp was (6.21%). [15] isolated and identified five different genera of LAB including *Lactobacillus* (24.38%), *Lactococcus* (21.94%), *Streptococcus* (19.51%), *Leuconostoc* (14.64%), *Bifidobacteria* (12.19%), and *Pediococcus* (7.31%) from raw cow's milk, cheese and yogurt of North Western Ethiopia. *Lactobacillus*, *Enterococcus* and *Leuconostoc* spp. were isolated by [16] from different types of fermented foods including dairy product of Burkina Faso. With the dominance of *Enterococci.* Genera of *Leuconostoc*, *Lactobacillus*, *Weissella* and *Pediococcus* were identified by [17] in One Humped Camel raw milk from the Golestan Province of Iran. It was stated by [18] that LAB dominated the microflora of the camel fermented milk Garris, and the major genera were *Lactobacillus* (74%), followed by *Lactococcus* (12%), *Enterococcus* (10%) and *Leuconostoc* (4%). A complex LAB composition was present in traditional fermented yak milk products in the Sichuan Province of China, and all the isolates belonged to six genera. The distribution of the isolates by genus was as follows: *Leuconostoc* (40.8%), *Lactobacillus* (39.0%), Streptococcus (13.2%), Lactococcus (5.6%), Enterococcus (0.94%), and *Weissella* (0.46%) [19].

Table no 2: Phenotypic properties of LAB isolated from *Berkeep*

Area	Isolates	Isolates number	Cellular shape and arrangement	Lipolysis	Gas from glucose	Acid from glucose	LAB spp.
Elkoumah	G1	38	Rod	+/-	+/-	+	Lactobacillus
	G2	34	Pairs/short chain	+/-	-	+	Enterococcus
	G3	10	Cocci in pairs or tetrads	+/-	-	+	Pediococcus
	G4	7	Ovoid (single/ chain)	+/-	+	+	Leuconostoc
Manwashy	G1	103	Rod	+/-	+/-	+	Lactobacillus
	G2	11	Short chain cocci	-	-	+	Enterococcus
	G3	9	Tetrads	-	-	+	Pediococcus
	G4	5	Ovoid (single/chain)	+/-	+	+	Leuconostoc
Kass	G1	50	Rod	+/-	+/-	+	Lactobacillus
	G2	23	Short chain cocci	-	-	+	Enterococcus
	G3	9	Cocci in pairs or tetrads	+/-	-	+	Pediococcus
	G4	7	Ovoid in chain	+/-	+	+	Leuconostoc

G1 to G5: Group of isolates, (+): Positive reaction, (-): Negative reaction, +/-: Different reaction**s**

Table no 3: Numbers of lipolytic and gas producing LAB isolated from *Berkeep*

***Lactobacillus* spp.**					
Production site	Number of Isolates	Lipolytic activity		Gas from glucose	
		+ve	-ve	+ve	-ve
Elkoumah	38	6	32	12	26
Manwashy	103	21	82	38	65
Kass	50	10	40	15	34
Total	191	37	154	66	125
***Lactococcus* ssp.**					
Elkoumah	51	18	33	7	44
Manwashy	25	4	21	5	20
Kass	39	9	30	7	33
Total	115	31	84	19	97

Table no 4: RAPD-PCR identification of Lab isolated from Berkeep

RAPD-PCR group	No. of isolates	RAPD-PCR size (bp)	Identification spp.
A	45	210, 250,390, 500,700,900	Enterococcus
B	9	190,290, 400	Enterococcus
C	14	180, 350, 700	Enterococcus
D	19	600, 900, 1000, 1400	Leuconostoc
E	28	190, 280,310, 600, 700, 800	Pediococcus
F	59	140, 150, 190,310, 390, 400	Lactobacillus
G	95	310, 490, 610, 700	Lactobacillus
H	20	200, 310,490, 500, 610, 700	Lactobacillus
I	9	210, 450	Lactobacillus
J	8	290, 350, 600, 700, 800,1300	Lactobacillus

RAPD-PCR Random Amplified Polymorphic DNA-Polymerase Chain Reaction

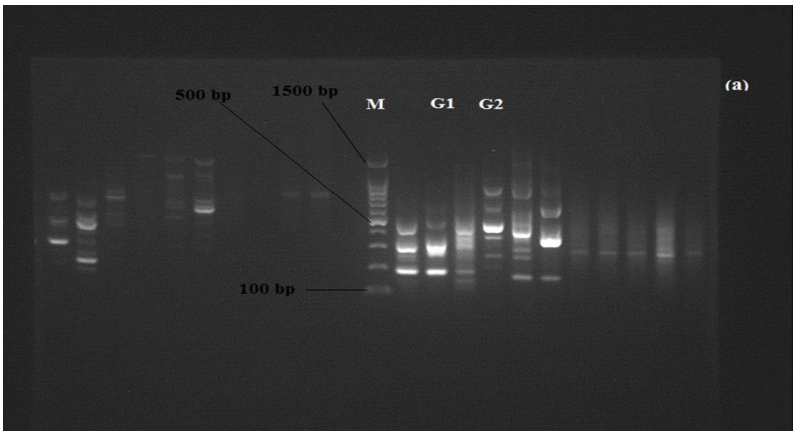

Fig. 2a: Cluster analysis using RAPD-PCR and the primers R2 and M13, G1 and G2, only a limited sub-sample of representative isolates is shown

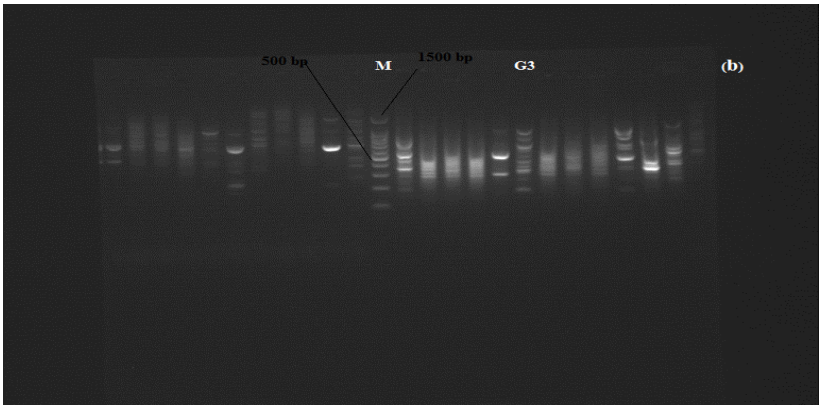

Fig. 2b: Cluster analysis using RAPD-PCR and the primers R2 and M13, G3, only a limited sub-sample of representative isolates is shown

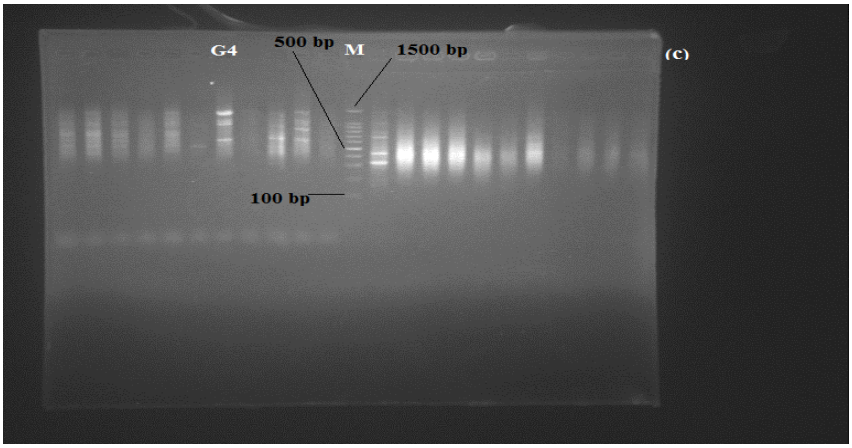

Fig. 2$_C$: Cluster analysis using RAPD-PCR and the primers R2 and M13, G4, only a limited sub-sample of representative isolates is shown.

Lactobacillus spp. were found to dominate the LAB responsible for the fermentation of Berkeep. The dominance of *Lactobacillus* (30%) followed by *Leuconostoc* (26%), *Enterococcus* (20%), *Streptococcus* (18%) and *Aerococcus* (6%) was presented by [20] in rayb, a traditional fermented dairy product made in Egypt from buffalo milk. [21] identified *Lactobacillus* as dominant and *Leuconostoc*, *Lactococcus*, *Enterococcus*, and *Streptococcus* in Nunu, a spontaneously fermented yoghurt-like milk product consumed as a staple food commodity in parts of the Saharan West Africa. In Ethiopian fermented milk, Ergo and Ititu, [22] identified the genera, *Lactobacillus, Lactococcus, Leuconostoc*, *Streptococcus*.

The dominance of *Lactobacillus* spp. in most of these products might be attributed to their ability to survive highly acidic environment of pH=4 to 5 or even lower, hence it could be responsible for final stages of fermentation in Berkeep i.e. low pH conditions favour the growth of *lactobacillus* spp. [23, 24]. Hundred and seventy-one strains of lactic acid bacteria were isolated by [25] from 44 traditional fermented milk samples from different region of Tibet, China. In these samples, the concentrations of lactic acid bacteria ranged from 10^5 to 10^9 cfu/mL. *Lactobacillus* was considered to be the major population with 71.3% higher than *Lactococcus* which constituted 28.7% of the total number of isolates. [26] studied different samples of Tarag, a naturally fermented milk product of Mongolia and China. Fourteen genera were found to be common in all Tarag samples from 4 different sampling locations (Mongolia, and Gansu, Sichuan, and Inner Mongolia provinces, *Lactobacillus* (67.22, 52.51, 49.91, and 43.70%), *Streptococcus* (12.07, 33.55, 29.51, and 0.71%), *Lactococcus* (3.66, 0.17, 2.26, and 21.86%), *Leuconostoc* (1.92, 0.02, 1.60, and 0.16%), indicating the dominance of *Lactobacillus* as the major fermenters in Tarag samples of Mongolia.

Chigee, a fermented yak milk, and mare milk were studied by [27] An *et al*. (2004). The authors found that chigee microbiota consisted mainly of *Lactobacillus* and *Lactococcus* spp. The isolates from mare milk consisted of *Leuconostoc*., *Lactococcus, Streptococcus and Enterococcus* spp.

Species of *Lactococcus* and *Leuconostoc* isolated from raw milk by [27, 19] when dealing with yak fermented milk and milk products- indicated that *Lactococcus* and *Leuconostoc* spp. could have been obtained from the raw milk in the fermentation process. They were possibly able to grow during the early stage of fermentation, but could not survive in the final products because of their low acid tolerance [28] (Miyamoto *et al*., 2010). Therefore, our result of the relatively low percentage of *Pediococcus and Leuconostoc* spp. compared with *Lactobacillus, Enterococcus* spp. could most likely be explained.

Berkeeb is known for its unique pleasant favor and smooth texture. The lactic acid fermentation determines the physical characteristics and the flavor of the fermented milk by the accumulation of volatile products with a small number of carbon atoms. These products accumulate more when species capable of metabolizing citric acid intervene [29]. [30] stated that citrate metabolism plays an important role in many food fermentations involving lactic acid bacteria. Since citrate is a highly oxidized substrate, no reducing equivalents are produced during its degradation, resulting in the formation of metabolic end products other than lactic acid. Some of these end products, such as diacetyl and acetaldehyde, have very distinct aroma properties and contribute significantly to the quality of the fermented foods. *Enterococcus* strains have the metabolic potential to metabolize citrate into acetate, formate and ethanol and to convert lactose into lactate, therefore to actively contribute to the flavor development of fermented dairy products [31].

V. Conclusion

The present study reported that LAB species isolated from Berkeep, a traditionally fermented milk product of Western Sudan were divided into four genera including *Lactobacillus*, *Enterococcus*, *Pediococcus* and

Leuconostoc. *Lactobacillus* spp. were found to dominate LAB isolated from *Berkeep* samples collected from the three production sites, Elkoumah, Manwashy and Kass followed by *Enterococcus* spp. Further work is recommended to identify and characterize these bacteria to species and strain levels based on 16S rRNA sequencing and plasmid profiling, respectively.

Acknowledgements

The authors are very grateful to the Deutscher Akademischer Austauschienst German Academic Exchange Service (DAAD) for the offered grant. They also express their thanks and gratitude to Mrs. Swhair Ramadan, Department of parasitology, Faculty of Veterinary Medicine, Sudan University of Science and Technology, and Mr. Taj eldein Mustafa, Department of Plant and Plant Biotechnology, Faculty of Agriculture, University of Khartoum, for their utmost and genuine technical support.

References

[1]. Bourrie, B.C.T., Willing, B.P. and Cotter, P.D., The microbiota and health promoting characteristics of the fermented beverage kefir, Frontiers in Microbiology, 2016, 7:1-17.
[2]. Shiby, V.K. and Mishra, H.N., Fermented milks and milk products as functional foods-A review. Critical Reviews in Food Science and Nutrition, 2013, 53(5): 482–496.
[3]. Ministry of Animal Resources, Department of Statistics and Information, Annual report for Animal and Animal products Statistics, Statistical Bulletin, 2020, No. 29-30.
[4]. Abdelgadir, W. S., Ahmed, T. K. and Dirar, H. A., The traditional fermented milk products of the Sudan, International Journal of Food Microbiology, 1998, 44:1-13.
[5]. Pal. V., Jamuna, M. and Jeevaratnam, K., Isolation and characterization of bacteriocin producing lactic acid bacteria from a South Indian special dosa (appam) batter, J. Cult. Collect., 2005, 4:53-60.
[6]. Harrigan, W.F., Laboratory Methods in Food Microbiology. 3rd Edn., Academic Press, San Digo, 1998, Pages: 532.
[7]. Doyle, J.J. and Doyle, J.L., A Rapid DNA Isolation Procedure for Small Quantities of Fresh Leaf Tissue, Phytochemical Bulletin, 1987, 19:11-15.
[8]. Bonomo, M.G., Ricciardi, A., Zotta, T., Parente, E. and Salzano, G., Molecular and technological characterization of lactic acid bacteria from traditional fermented sausages of Basilicata region (Southern Italy), Meat Science, 2008, 80:1238-1248.
[9]. Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd edn. Ed GI Barrow, RKA Feltham. (Pp 331) Cambridge University Press.
[10]. Panesar, P.S., Fermented Dairy Products: Starter Cultures and Potential Nutritional Benefits, Food and Nutrition Sciences, 2011, 2(1):47-51.
[11]. Liu, S-N., Han, Y. and Z.-J. Zhou, Z-J., Lactic Acid Bacteria in Traditional Fermented Chinese Foods, Food Research International, 2011, 44(3):643-651.
[12]. Levit, R.; Savoy de Giori, G.; Moreno de LeBlanc, A.; LeBlanc, J.G., Recent update on lactic acid bacteria producing riboflavin and folates: Application for food fortification and treatment of intestinal inflammation, J. Appl. Microbiol., 2021, 130:1412–1424.
[13]. Börner, R.A.; Kandasamy, V.; Axelsen, A.M.; Nielsen, A.T.; Bosma, E.F., Genome editing of lactic acid bacteria: Opportunities for food, feed, pharma and biotech, FEMS Microbiol. Lett., 2019, 366(1):fny291.
[14]. He, L.; Li, J.; Sun, Z., More critical consideration on enhancing micronutrient bioavailability of phytate rich foods by phytase producing lactic acid bacteria, Trends Food Sci. Technol. 2020, 102:37-38.
[15]. Taye, Y., Degu, T., Fesseha, H. and Mathewos, M., Isolation and Identification of Lactic Acid Bacteria from Cow Milk and Milk Products, The Scientific World Journal, 2021, 2021:1-6.
[16]. Cissé , H., Kagambèga , B., Adama Sawadogo , A., Tankoano, A., Sangaré , G., Yves Traoré , Ouoba , L.I.I. and Aly Savadogo, A., Molecular characterization of Bacillus, lactic acid bacteria and yeast as potential probiotic isolated from fermented food, Scientific Africa, 2019, 6: e00175.
[17]. Davati, N., Yazdi, F.T., Zibaee, S., Shahidi, F. and Edalatian, M.R., Study of Lactic Acid Bacteria Community from Raw Milk of Iranian One Humped Camel and Evaluation of Their Probiotic Properties, Jundishapur J. Microbiol., 2015, 8(5): e16750.
[18]. Sulieman, A.E., Ilayan, A.A. and El Faki, A.E., Chemical and microbiological quality of Garris, Sudanese fermented camel's milk product, International Journal of Food Science and Technology, 2006, 41:321–328.
[19]. Bao, Q., Yu, J., Liu, W., Qing, M., Wang,W., Chen, X., Wang,F., Li, M., Wang, H., Lv, Q. and Zhang, H., Predominant lactic acid bacteria in traditional fermented yak milk products in the Sichuan Province of China, Dairy Sci. Technol., 2012, 92:309–319.
[20]. Abd El Gawad, I.A., Abd El Fatah, A.M. and Al Rubayyi, K.A., Identification and characterization of dominant lactic acid bacteria isolated from traditional rayeb milk in Egypt, Journal of American Science, 2010, 6(10):728-735.
[21]. Akabanda1 F., Owusu-Kwarteng, J., Glover, R.L.K. and Tano-Debrah, K., Microbiological Characteristics of Ghanaian Traditional Fermented Milk Product, *Nunu*, Nature and Science, 2010, 8(9):178-187.
[22]. Gonfa, A, Alemu, F., Urga, K., and Abegaz, B., Microbiological aspects of Ergo (Ititu) fermentation, SINET: Ethiopian Journal of Science, 1999, 22(2):283-290.
[23]. El-Shafei, K., Ibrahim, G.A., Tawfik, N.F., Beneficial uses of locally isolated lactic acid bacteria, Egypt, J. Dairy Sci., 2002, 30:15–25.
[24]. Soomro, A. H. and Masud, T., Protein Pattern and Plasmid profile of Lactic Acid Bacteria Isolated from Dahi, A Tradition Fermented Milk product of Pakistan, Food Technol. Biotechnol., 2007, 45(4):447-453.
[25]. Airidengcaicike, Chen, X., Du, X., Wang, W., Zhang, J., Sun, Z., Liu, W., Li, L., Sun, T., Zhang, H., Isolation and identification of cultivable lactic acid bacteria in traditional fermented milk of Tibet in China, Int. J. Dairy Technol.,2010, 63:437–444.
[26]. Sun, Z., Wenjun Liu, W., Bao, Q., Zhang, J., Hou, Q., Kwok, L., Sun, T., and Zhang, H., Investigation of bacterial and fungal diversity in tarag using high-throughput sequencing, J. Dairy Sci., 2014, 97:6085-6096.
[27]. An, Y., Adachi, Y. and Ogawa, Y., Classification of lactic acid bacteria isolated from chigee and mare milk collected in Inner Mongolia, Anim. Sci. J., 2004,75:245-252.
[28]. Miyamoto, M., Seto, Y., Nakajima, H., Burenjargal, S., Gombojav, A., Demberel, S. and Miyamoto. T., Denaturing gradient gel electrophoresis analysis of lactic acid bacteria and yeasts in traditional Mongolian fermented milk, Food Sci. Technol. Res., 2010, 16:319-326.

[29]. Bottazzi, V., Other fermented dairy products. In: Biotechnology, Vol. 5, Food and feed production with microorganisms, ed. Reed, G., 1983, 5:315-363. Weinheim: Verlag Chemie.
[30]. Hugenholtz, J., Citrate metabolism in lactic acid bacteria, FEMS Microbiology Reviews, 1993, 12(1-3):165-178.
[31]. Sarantinopoulos, P., Kalantzopoulos, G. and Tsakalidou, E., Citrate metabolism by *Enterococcus feacalis* FAIR-E 229, Applied and Environmental Microbiology, 2002, 67(12):5482-5487.