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Isolation and Identification of Lactic Acid Bacteria and Yeast from Raw Milk in Khartoum State (Sudan)

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Abstract: Fifty four raw milk samples were collected from cows (farms and venders), goats, ewes and camels of different areas of Khartoum state and microbiologically analyzed. Enumeration and isolation were carried out anaerobically at 37°C on MRS and M17 for lactococci and lactobacilli, respectively, aerobically on PDA at 25°C for yeasts and on nutrient agar for total viable count at 37°C. Sixty three MRS and M17 isolates and six PDA Isolates were purified and kept at 4°C for further identification. The presence of Lactic Acid Bacteria (LAB) and yeast was confirmed by colonial morphology, microscopy in addition to other biochemical tests. The ability of streptococci to ferment and assimilate sugars was carried out using the API kits. The results obtained showed that the milk samples contained lactobacilli and lactococci in the range of 3.50-6.30 and 3.48-6.21 log mL⁻¹, respectively, yeast in the range of 2.00-3.95 log mL⁻¹ and the total viable count in the range of 3.48-7.98 log mL⁻¹. Lactic acid bacteria isolated from milk samples belonged to lactobacillus and streptococcus genera. The homofermentative lactobacilli from cows and camel milk were tentatively identified as *Lactobacillus plantarum* and *Lb. acidophilus*, whereas the hetero fermentative ones from cows, goats and ewes milk were found to be *Lb. fermentum*. The homofermentative streptococci isolated from all milk samples were tentatively identified as *Streptococcus cremoris* and *Streptococcus lactis*, whereas the only heterofermentative strain from camel milk was found to be *Leuconostoc lactis*. Yeasts which were only isolated from cow's milk, were identified as *Debaryomyces hansenii* (4 strains), *Kluveromyces lactis* (one strain) and *Saccharomyces rouxii* (one strain).

Key words: Raw milk, different animal species, lactic acid bacteria, yeast

INTRODUCTION

Milk is the natural secretion of the mammary gland of mammals. It contains a large variety of nutrients more than food stuffs. It is a natural food for young and adults especially that of cows and goats. Other animals that produce milk for human consumption are water buffalo, sheep and camels. The milk of different animal species contains the same constituents, but varied in composition. (Eckles *et al.*, 1951; Walstra *et al.*, 1999).

In Sudan milk is considered one of the oldest kind of food and so many people depend on its products. Cow's milk is predominant, but some people depend on goat milk where the goats are kept by the families and camel milk by nomadic people. Internationally, sheep are reared mainly for their meat and hide and not for milk. Whereas in Sudan only small sector of people used to drink sheep milk and in most cases they mix it with other types of milks especially goats and cows.

Milk is sterile during secretion from healthy animals but the components that are foreign to it, enter the milk in the udder or during or after milking as well as any changes occurring in the milk are often detrimental to its quality (Walstra *et al.*, 1999). Lactic acid bacteria are normally present in milk and as they are able to ferment lactose, the major milk sugar, they are used as starter cultures in the production of cultured dairy products such as yoghurt.

Microbiological investigation of fresh goat's milk by Muddathir (1996) revealed the presence of different species of lactic acid bacteria and yeasts in addition to some other contaminants. Abdel Gadir *et al.* (2001) studied the microbiology of local cow's milk and revealed the presence of high counts of lactococci and lactobacilli in addition to a considerable numbers of yeasts.

This study was conducted against the background of the key role that milk plays in the nutrition of almost all of the Sudanese people and the probability of ingesting even small numbers of beneficial microorganism.

The objective of this study is to isolate and identify the predominant microbial groups in milk of different animal species obtained from farms and vendors in plastic and metal containers.

MATERIALS AND METHODS

Fifty four milk samples were collected from different animal species, cows (farms and vendors), camels, ewes and goats from different areas in Khartoum state (Khartoum, Khartoum North and Omdurman), collection was done in sterile screw capped bottles and from plastic and metal containers. Samples were transported in cooler boxes to the laboratory at the Faculty of Agriculture, University of Khartoum and analyzed upon arrival.

Isolation of Microorganism

Ten milliliters of each sample was pipetted aseptically into 90 mL of sterile peptone physiological saline solution (1% peptone (Oxoid), 0.9% NaCl, pH 7.0). Serial dilutions were made for each sample and 0.1 mL of the appropriate dilution was spread plated on universal and selective media. MRS and M17 (Difco) were used for enumeration and isolation of Lactobacilli and lactococci, respectively and Plates were incubated aerobically at 37°C using the jars together with gas generating kits (Difco).

Yeasts were enumerated and isolated on Potato Dextrose Agar at 25°C for 2-3 days. For the total viable count, 1 mL portion of the appropriate dilutions were pour plated on Nutrient agar (Oxoid) and incubated at 37°C.

Isolations were obtained randomly from the countable plates of MRS, M17 and PDA and purity was checked by successive streaking on the same media and stored on slants (of the same media) at 4°C for further investigation.

Identification of Lactic Acid Bacteria to the Genus Level

The bacteria were characterized by microscopic examination and by conventional biochemical and physiological tests. The cultures were examined for colony and cell morphology, motility, Gram stain and production of acid from glucose (Harrigan and McCance, 1976) in addition to the oxidation and fermentation test according to Hugh and Leifson (1953).

Identification of Streptococci Group

Streptococci were further identified by their production of carbon dioxide from glucose using Gibson litmus milk, production of acid from sugars and production of ammonia from arginine (Harrigan and McCance, 1976). The salt tolerance test was done using M17 broth containing 4% (w/v) NaCl with anaerobic incubation. Growth at pH 9.2 and on 40°C was determined in M17 broth.

API 20 streptococci (Bio Mérieux) were used to test the carbohydrate assimilation and fermentation pattern.

Identification of Lactobacilli

After their microscopic examination, Gram +ve and catalase -ve lactobacilli were tested for their sugar fermentation pattern, production of ammonia from arginine in addition to their ability of growth at 15 and 45°C according to Harrigan and McCance (1976).

Identification of Yeast

Pure isolates of yeasts were identified according to Lodder (1970), with additional standard taxonomical methods e.g., the use of API 20 C AUX diagnostic kits (Bio Mérieux) assisted by comparison with the table of Lodder (1970).

The biochemical tests included the assimilation pattern among glucose, sucrose, galactose, melibiose, lactose and raffinose, fermentation of glucose, growth at 37°C and in 50% glucose. The ability of yeast to form and hydrolyze starch was also tested. The formation of mycelium and pseudohyphae was examined by microscopy and the ascospore formation was tested on YMA and the cell morphology on YM broth culture.

RESULTS AND DISCUSSION

Fifty four milk samples were collected from dairy farms and vendors at Khartoum, Khartoum North and Omdurman towns (Khartoum state). Milk samples were collected from Cows, goats, camels and ewes. Sixty three isolate obtained from MRS and M17 plates were grouped as lactic acid bacteria according to the fact that they are Gram +ve, catalase -ve and Oxidase -ve rods and cocci. Six isolates from PDA were identified as yeast according to their microscopic appearance and cultural characteristics. The dominant lactic acid bacteria in milk of different animal species were found to be *Streptococcus lactis* (34.9%), *Lactobacillus fermentum* (20.6%), *Lb. plantarum* (19.1%), *Strep. cremoris* (14.3%), *Lb. acidophilus* (9.5%) and *Leuconostoc lactis* (1.6%) and the yeasts *Debaryomyces hansenii* (66.6%), *Kluyveromyces lactis* (16.7%) and *Saccharomyces rouxii* (16.7%).

The results obtained showed that, the milk samples contained lactobacilli and lactococci in the range of 3.50-6.30 and 3.48-6.31 log mL⁻¹, respectively, yeasts in the range of 2.00-3.95 log mL⁻¹ and the total viable count in the range of 3.48-7.98 log mL⁻¹. High LAB and yeasts counts were reported in local cow's milk by Abdel Gadir *et al.* (2001) and the situation was similar to that prevail in some other African countries (Mutukumira, 1996).

Figure 1 shows the microbial mean counts in milk of different animal species. It was noticed that the microbial counts of the vendor cows milk in both containers (plastic and metal) were higher than the count obtained from other animal species. In cow's (farm and vendors) and goat's milk, the counts of streptococci on M17 were higher than those of lactobacilli, whereas the lactobacilli were higher in the camel milk. The ewe's milk contained almost the same counts of lactobacilli and streptococci.

Among the 63 lactic acid bacteria isolated 22.2% (14) isolates produced gas from glucose (heterofermentatives), whereas 77.8% (49 isolates) were homofermentatives. Of the 14 heterofermentative lactic isolates, 13 rods were arginine positive, able to grow at 45°C but not at 15°C, able to ferment raffinose, arabinose, lactose, sucrose but not salicin and were tentatively identified as *Lactobacillus fermentum*. Although *Lb. fermentum* was isolated by Abdel Gadir *et al.* (2001) from cows milk, but it is mostly reported in cereal based fermented doughs (Halm *et al.*, 1993; Hamad *et al.* (1997). The heterofermentative arginine negative lactic coccus failed to grow at 4°C, 4% NaCl and at pH 9.2 but produce acetoin in litmus milk and tentatively identified as *Leuconostoc lactis* (Table 1, 2).

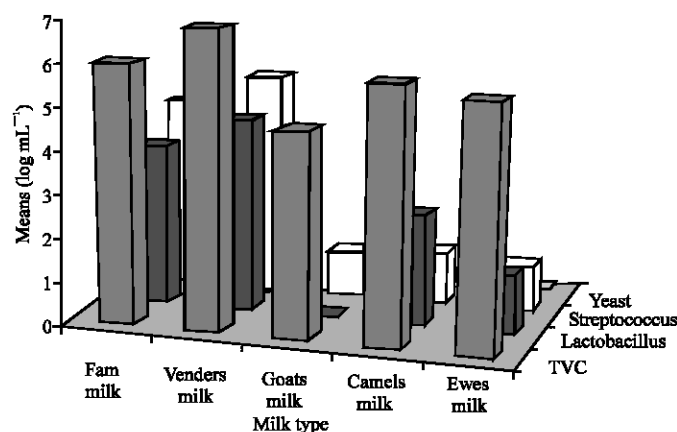


Fig. 1: Mean values of different milk types

Table 1: Identification of lactobacilli from different types of milk

Identification	Fermentation	Sun.	Sali.	Lact.	Ara.	Mel.	Raf.	Growth at		NH ₃ from arginine	Gas from glucose	No. of isolates	Group No.
								45°C	15°C				
<i>Lb. fermentum</i>	Hetero.	+	-	+	+	+	+	+	-	+	+	13	1
<i>Lb. acidophilus</i>	Homo.	+	+	+	-	-	-	-	-	-	-	6	2
<i>Lb. plantarum</i>	Homo.	+	+	+	+	+	+	-	+	-	-	12	3

Raf. = Raffinose, Mel = Melebiose, Ara. = Arabinose, Sal. = Salicin, Sun = Sucrose, Homo. = Homofermentative, Hetero = Heterofermentative, +ve = Positive, -ve = Negative

Table 2: Identification of streptococci in different types of milk

Identification	Fermentation	Growth at			NH ₃ from arginine	Acetoin in litmus milk	No. of isolates	Group No.
		pH 9.2	4% NaCl	4°C				
<i>Strep. cremoris</i>	Homo.	-	-	-	-	+	9	4
<i>Strep. lactis</i>	Homo.	+	+	+	+	+	22	5
<i>Leuconostoc lactis</i>	Homo.	-	-	-	-	+	1	6

Homo. = Homofermentative, Hetero. = Heterofermentative, +ve = Positive, -ve = Negative

From the 49 homofermentative lactic isolates, 31 isolates produced Acetoin in litmus milk. Twenty two isolates (from the 31 isolates) were arginine positive and were able to grow at 4°C, 4% NaCl and at pH 9.2 and tentatively identified as *Strep. lactis* and nine isolates failed to grow in the above conditions were tentatively identified as *Strep. cremoris* (Table 2). The remaining 18 homo. isolates were rod in shape, arginine +ve, able to ferment lactose Salicin and sucrose, six of them failed to ferment arabinose, melebiose and raffinose and failed to grow at 15°C, tentatively identified as *Lactobacillus acidophilus*. *Lb. acidophilus* is used in the production of different types of fermented dairy products, which are used to correct lactose maldigestion an intolerance (Wardlow, 1999) and is used as probiotic which upon ingestion exert health benefits beyond inherent basic nutrition (Wood, 1992).

The remaining 12 isolates which were able to ferment the above mentioned sugars, were identified as *Lactobacillus plantarum* (Table 1). Miyamoto (1989) isolated *Lb. plantarum* from *Maziwa lala*, a traditional fermented milk of Kenya.

Yeast populations were isolated only from cow's milk and according to morphology and other biochemical tests they were grouped into three comprised *Debaryomyces hansenii*, *Kluyveromyces lactis* and *Saccharomyces rouxii*. Table 3 showed the distinction of yeast isolates based on characteristics and properties. High count of yeasts in local cow's milk was reported by

Table 3: Identification of yeasts

Identified yeast	Assimilation of carbon compounds						Fermentation of glucose	Nitrate	Starch hydrolysis	Starch formation	Growth at		No. of isolates	Group No.
	Glu.	Sun.	Lac.	Mel.	Raff.	Gal.					37°C	Glucose 50%		
<i>Debaryomyces hansenii</i>	+	+	+	+	+	+	+	-	+	-	+	W	4	1
<i>Kluyveromyces lactis</i>	+	-	+	-	+	-	+	-	+	-	+	-	1	2
<i>Saccharomyces rouxii</i>	+	+	-	-	-	-	+	-	-	-	+	+	1	3

Glu. = Glucose, Sun. = Sucrose, Lac. = Lactose, Mel. = Melebiose, Raff. = Raffinose, Gal = Galactose, +ve = Positive, -ve = Negative

Abdel Gadir *et al.* (2001), whereas Sulieman and Dirar (2005) isolated neither yeast nor moulds from raw milk produced by smallholder farmers in Gezira (Central Sudan). *Kluyveromyces* sp. and *Saccharomyces* sp. usually cause milk spoilage being lactose fermenters. *Debaryomyces hansenii*, *Kluyveromyces lactis* and *Saccharomyces rouxii* are closely associated with the successful development of all mould-ripened cheese and other types of cheeses.

The above identification results were confirmed by the carbohydrates fermentation and assimilation profile obtained by API kits in correlation with Bergey's Manual (Teuber *et al.*, 1986).

Data from this study suggest a possible interaction between LAB and yeasts in milk and since there is no previous study to estimate the standards of LAB in milk, the parameters in this study can be recommended as foundation for further studies of the mean values for fresh milk under Sudan conditions.

REFERENCES

- Abdel Gadir, S.W., S.H. Hamad, P.L. Moller and M. Jakobsen, 2001. Characterization of the dominant microbiota of Sudanese ferment milk Rob. Int. Dairy J., 11: 63-70.
- Eckles, C.H., W.B. Coms and H. Macy, 1951. Milk and Milk Products. 4th Edn., New York, pp: 95-98.
- Halm, M., A. Lillie, A.K. Sorensen and M. Jakobsen, 1993. Microbiological and aromatic characterization of fermented maize dough for Kinkey production in Ghana. Int. J. Food Microbiol., 19: 134-143.
- Hamad, S.H., M.C. Dieng, M.N. Ehrmann and R.F. Vogel, 1997. Characterization of the bacteria flora of Sudanese sorghum flour and sorghum sour dough. J. Applied Microbiol., 83: 764-770.
- Harrigan, W.F. and M.E. McCane, 1976. Laboratory Methods in Food and Dairy Microbiol. London.
- Hugh, R. and E. Leifson, 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram-negative bacteria. J. Bact., 66: 24.
- Loddor, J., 1970. The Yeast Taxonomic Study. 2nd Edn., North Holland Publ. Corp. Amsterdam, Holland.
- Miyamoto, T., S.G.G. Gichuru, T. Akimoto and T. Nakae, 1989. Identification and properties of lactic acid bacteria isolated from traditional fermented beverages in East Africa. Jap. J. Zootech. Sci., 57: 256-276.
- Muddathir, A., 1996. Studies on the microbiology of fresh goats milk. UK. J. Agric. Sci., 4: 140-149.
- Mutukumira, A.N., 1996. Investigation of some aspects for the development of starter cultures for industrial production of traditional fermented milk in Zimbabwe. Ph.D Thesis, Agric. Univ. Norway.

- Sulieman, A.E. and H.A. Dirar, 2005. Chemical and microbiological characterization of raw milk produced by smallholder farmer in gezira (Sudan). *UK. J. Agric. Sci.*, 13: 367-377.
- Teuber, M., A. Geis and H. Neve, 1986. Genus *Lactobacillus*. In: *Bergey's Manual of Systemic Bacteriology*. 8th Edn., Waverly Press Inc., Baltimore, M., USA.
- Walstra, P., T.J. Geurts, A. Noomen, A. Jellema and M.A.S. Van Bookel, 1999. Composition, structure and properties. In: *Dairy Technology: Principles of Milk Properties and Processes*. Marcel Dekker, New York.
- Wardlaw, G.M., 1999. *Perspectives in Nutrition*. McGraw-Hill, Boston.
- Wood, B.J., 1992. *The Lactic Acid Bacteria in Health and Disease*. Elsevier Applied Science, London.