PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 3 (2): 134-139, 2004

Microorganisms Involved in Fulani Traditional Fermented Milk in Burkina Faso

Aly Savadogo[1]*, C.A.T. Ouattara[1], P.W. Savadogo[2], A.S. Ouattara[1], N. Barro[1] and A.S. Traore[1]
[1]Laboratoire de Microbiologie et de Biotechnologie - Centre de Recherche en Sciences Biologiques, Alimentaires et Nutritionnelles (CRSBAN)-Département de Biochimie-Microbiologie (DBM)-Unité de Formation et de Recherche en Sciences de la Vie et de la Terre (UFR/SVT), Université de Ouagadougou, 03BP 7131 Burkina Faso
[2]Institut de l'Environnement et de la Recherche Agricole (INERA), Ouaga, Burkina Faso
E-mail: aly.savadogo@univ-ouaga.bf

Abstract: Thirty samples of traditional fermented milk were collected in northern Burkina from Fulani individual household. Microbial analysis was done by standard methods and with API 50 CH identification system. The predominant microflora was lactic acid bacteria, belonging to the genus *Lactobacillus* (32%), following by *Leuconostoc* (30%), *Lactococcus* (20%), *Leuconostoc/ß-bacterium* (10%), *Streptococcus* (6%) *and Enteroccoccus* (2%) genus. Yeasts, molds and Enterobacteria were also isolated. Twenty representative lactic acid bacteria strains were identified to species level belonging to species *Lactococcus lactis ssp. lactis*, *Lactococcus lactis subsp. lactis biovar. diacetylactis*, *Lactobacillus confusus*, *Lactobacillus delbrueckii subsp. lactis*, *Lactobacillus plantarum*, *Leuconostoc citreum*, *Leuconostoc lactis*.

Key words: Traditional fermented milk, Fulani, lactic acid bacteria, Burkina Faso

Introduction

One of people development factor is the ability to produce and store large amount of foods. For many peoples engaged in stock farming, cultured dairy products are important as foods. Milk has been preserved since early times by fermentation. Many traditional fermented milk products were made in Asia, Africa, the Middle East, and northern and eastern Europe. The microbiological characteristics of several fermented milk have been studied in Indonesia (Yodoamijoyo *et al*., 1983; Hosono *et al*., 1989), in Zimbabwe (Feresu and Muzondo, 1990), in South Africa (Keller and Jordan, 1990; Beukes *et al*., 2001), in Morocco (Hamama, 1992), in Tanzania (Isono *et al*., 1994). Some of the major fermentation processes are based on the use of lactic acid bacteria, which produce organic acids. The presence of fermentative lactic acid bacteria is crucial to the intrinsic properties of fermented food products (Thomas, 1985; Tamine and Robinson, 1988; Mckay and Baldwin, 1990; Soomro *et al*., 2002; Ehrmann *et al*., 2002).

The nature of fermented products is different from one region to another. Thus is depending on the local indigenous microflora, which in turn reflected the climatic conditions of the area. Thus traditional fermented milk in regions with a cold temperature climate contained mesophilic bacteria such as *Lactococcus* and *Leuconostoc spp*., whilst thermophilic bacteria, which include mostly *Lactobacillus* and *Streptococcus*, prevailed in regions with a hot, subtropical or tropical climate (Thomas, 1985; Tamine and Robinson, 1988; Kurmann, 1994).

Burkina Faso milk production was estimated to 37 392 tons of commercialized milk in 1998 (Réseau Documentaire d'Elevage, 1998) . The Fulani of Burkina Faso, ferment their milk in calabashes, gourds, clay pots. These containers need to be seeded with a natural microbial inoculum before it could be use for the production of fermented milk. Containers filled with milk fresh milk are covered and placed in house. The milk are coagulated, the whey and proteins were homogenized. Their fermented milk contained important lactic acid bacteria (e.g. exopolysaccharides producing bacteria) (Savadogo *et al*., 2001).

In Burkina Faso the rural population still produce unpasteurized fermented milk by traditional methods, since such milk product still enjoy loyal following in rural communities. Calabashes and gourds filled with fresh milk were covered and placed in the hut or house. The art of making traditional fermented milk will handed down from one generation to the next.

To our knowledge, few information exists on the traditional fermentative microflora that Fulani tribe used to produce fermented milk in Burkina Faso. The objectives of this study were to collect traditional fermented milk samples in rural areas, to determine the predominant microbial groups.

This paper deals with isolation and identification of microorganisms from Fulani fermented milk.

Materials and Methods

Fermented milk sampling: Fermented milk from thirty goat's and cow's were collected from individual households of rural areas in northern Burkina from July 1999 to January 2000. Samples were collected in sterile small bottles and stored in laboratory under refrigeration

at 5°C until they were used in experiments.

Enumeration and isolation of microorganisms: Ten milliliters of each sample were aseptically added into 90 ml of sterile 0.9% NaCl solution and mixed thoroughly. Serial dilutions (10^{-1} to 10^{-9}) were performed and 1 ml aliquots of the appropriate dilutions were directly inoculated in triplicate on the following media:
(a) Plate count agar (Fluka Biochemika 70152) incubated at 30°C for 72 h for enumeration of total aerobic mesophilic bacteria. (b) MRS agar (De Man *et al.*, 1960) (Fluka Biochemika 69966) incubated anaerobically at 42°C for 48 h for enumeration of thermophilic *Lactobacilli* and *Streptococci*. MRS agar plates were also incubated anaerobically at 35°C for 48 h for enumeration of mesophilic *Lactobacilli* and *Leuconostoc*. (C) M17 agar (Therzaghi and Sandine, 1975) (Difco) incubated aerobically at 30°C for 48 h for enumeration of *Lactococci*. (d) Rogosa agar (Rogosa *et al.*, 1951) incubated anaerobically at 35°C for 48 h for enumeration of *Lactobacilli*. (e) Violet Red Bile agar (VRBG) (Fluka Biochemika 70189) incubated aerobically at 37°C for 24 h for enumeration of enterobacteria. (f) YGC agar (Fluka Biochemika 95765) incubated aerobically at 25°C for 96 h for enumeration of yeast plus moulds. (g) *Salmonella* and *Shigella* agar (SS) (Fluka Biochemika 85640) was used for *Salmonella* and *Shigella* detection. The plates were incubated at 37°C for 48 h. Anaerobic jars (Biolab) with gas generating kits (Genbox CO_2 Biomerieux 96126) were used for incubation under anaerobic conditions.
Twenty-five colonies were picked randomly from plates of MRS (35°C), MRS (42°C), M17 (30°C), Rogosa (35°C). Isolates (100) were cultivated in MRS broth. Purity was checked by streaking on MRS agar. These pure isolates were cultivated in MRS broth at 30°C for 18 h. Cells were harvested by centrifugation at 3000 x g for 15 min and washed twice with a sterile 0.9% NaCl solution. The washed whole cells were lyophilized and kept in refrigerator until use.

Identification of the lactic acid bacteria to genus level: Gram-positive, catalase-negative, isolates from MRS agar, Rogosa agar, and M17 agar were assigned to a genus on basis of key characteristics and tests of table 1 (Harrigan and McCance, 1976; Garvie, 1984; Garvie, 1986; Hammes *et al.*, 1992; Holzapfel and Schillinger, 1992; Teuber *et al.*, 1992; Weiss, 1992; Axellsson, 1993; Dicks *et al.*, 1993). Morphological and arrangement of cells were examined according to the standard method. Growth at 10, 15 and 45°C in broth was determined by visual turbidity after 72 h incubation. Gas production from glucose was assessed in sugar basal medium (SBM) broth containing 2% (w/v) glucose dispensed in test tubes containing inverted Durham tubes. The inoculated tubes were examinated for the production of gas after 3 days's incubation.
Indole production from tryptone was assessed in 10 ml medium containing per litre 10 g tryptone and 5 g NaCl. After inoculation and incubation for 3 days, the cultures were tested for the presence of indole by method 2 of Cowan (Cowan ,1974).
Arginine deamination was detected in SBM supplemented with 1% (w/v) arginine monochloride, 0.3% (w/v) Bacto-agar and 0.01% (w/v) phenol red, pH 7.2. After inoculation the medium was incubated in anaerobic jars for 3 days. Arginine hydrolysis was observed by the culture turning yellow.
The salt tolerance test was done using MRS broth, containing 6.5 (w/v) NaCl with incubation time of 4 days at 37°C.

Identification of the lactic acid bacteria to the species level: Twenty representative isolates of MRS (35°C, 42°C), M17 (30°C) and Rogosa (35°C) were selected for identification to species level using the API 50 CH galleries and API 50 CHL medium (bio Mérieux Sa).
Tests were performed according to the manufacturer's instructions. The APILAB PLUS database (bio Mérieux Sa) was used to interpret the result.

Results

pH of the samples: The pH of the 30 samples ranged from 4.00 to 5.86 with an average of 4.70.

Enumeration of microorganisms: The Table 2 summarizes the microbial counts obtained from traditional fermented milks from Northern of Burkina.
The total microflora and specific group of organisms were enumerated by using eight (8) different culture media (Table 2).
Mean counts on MRS agar (35°C) and M17 agar were 7.80×10^7 and 7.75×10^7 cfu ml^{-1} respectively, and were higher than the mean total plate count (6.71×10^7 cfu ml^{-1}), indicating the predominance of lactic acid bacteria. These mean counts also exceeded counts obtained on Rogosa agar (24.44×10^6 cfu ml^{-1}).
The mean count of thermophilic bacteria (42°C) on MRS agar 8.04×10^5 cfu ml^{-1} was less than the mean mesophilic count (35°C) 7.80×10^7 cfu ml^{-1}.
The mean count of coliforms was 0.98×10^4 cfu ml^{-1} for 25 samples.
The mean count on YGC agar were 2.6×10^4 cfu ml^{-1} for 25 samples, this mean count is lower than the count on MRS (35 and 42°C), plate count agar, Rogosa agar, M17 agar and VRBGA agar.

Characteristics and Identification of lactic acid bacteria: The greatest part of the number of isolates from MRS (35 and 42°C), Rogosa, M17 was Gram-positive and catalase –negative. One hundred (100) isolates could be identified (according to the

Table 1: Characteristics of lactic acid bacteria (Harrigan and McCance, 1976; Garvie, 1984; Garvie, 1986; Hammes *et al.*, 1992; Holzapfel and Schillinger, 1992; Teuber *et al.*, 1992; Weiss, 1992; Axellsson, 1993; Dicks *et al.*, 1993)

Characteristics	*Leuconostocs*	*Streptococci*			*Enterococci*	*Pediococci*	*Lactobacilli*		
		Pyogenes	*Viridans*	*Lactic*			*Strepto*	*Thermo*	*Beta*
Cell form	Spherical but often lenticular	Spherical or ovoid			Spherical	Spherical	Rods/Cocobacilli		
Cellular arrangement	Pairs and chains	Chain and pairs			Mainly in pairs, short chains	Pairs,tetrads, clusters, single cells are rare, no chains	Chain formation common		
Growth									
At 10°C	+	-	-	+	+	±	ND	ND	ND
At 45°C	-	-	+	-	+	+	±	+	+
At 15°C	+						+		+
NH from arginine	-	+	-	±	+	+	-	+	-
Gas from glucose	+	-	-	-	-	-	-	-	-
Growth in 6.5% NaC	±	-	-	-	+	±	±	±	±
Reaction in litmus milk	Comparatively inactive. Few strains capable of producing acid. Very few strains capable of clotting the milk. No strains giving reduction	No Reduction of litmus Before Clotting of milk	No Reduction of litmus Before Clotting of milk	ARC	Comparatively Inactive. Rarely Produce sufficient acid to cause clotting		Various reactions depending on the species		

+ = positive, - = negative, ± = response varies between species, ARC = acid reduction clot, ND = no data

Table 2: Microbiological profile of the traditional fermented milks from northern Burkina

Medium	Ranges of counts (CFU ml^{-1}) for all samples	Mean Counts (CFU ml-1) For all samples
Total plate count agar (aerobic mesophilic bacteria)	$8.12x10^5$-$3.6x10^8$	$6.71x10^7$
MRS agar (42°C) (*Thermophilic Lactobacilli* and *Streptococci*)	$2.4x10^4$-$5.3x10^6$	$8.04x10^5$
MRS agar (35°C)(Mesophilic *Lactobacilli* and *Leuconostoc*)	$4.23x10^5$-$3.9x10^8$	$7.80x10^6$
Rogosa agar *(Lactobacilli)*	$0.30x10^6$-$1x10^8$	$24.44x10^6$
M17 *(Lactococci)*	$3.72x10^5$-$2.51x10^7$	$7.75x10^7$
VRBG (violet red bile agar) (*Enterobacteria*)	$0.25x10^2$-$3.5x10^4$ n=18	$0.98x10^4$
YGC (*Yeast and moulds*)	$1.83x10^3$-$3.7x10^6$ n=25	$2.6x10^4$ n=25

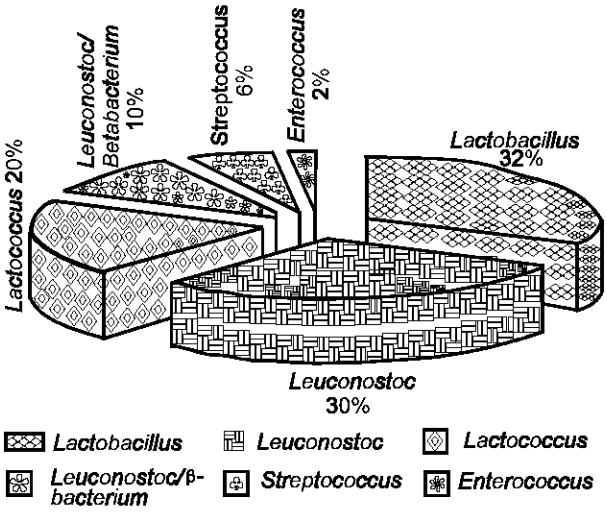

Fig. 1: Percentage distribution of the 100 isolates (MRS, Rogosa, M17) from traditional fermented milks of northern Burkina (Dori)

characteristics of Table 1) and were divided into six genera: *Leuconostoc, Lactococcus, Lactobacillus, Enterococcus*, *Leuconostoc/ß- betabacterium* and *Streptococcus* (Fig. 1).

Ten isolates were assigned to the *Leuconostoc/ß-betabacterium* group, which could either belong to the genus *Leuconostoc* or *Lactobacillus*.

Thirty-two isolates were rod-shaped and could be identified as *Streptobacterium* (22 isolates) or *Betabacterium* (10 isolates) (Harrigan and McCance, 1976).

The ability to produce gas was an important characteristic for distinguishing the *Leuconostoc* (Garvie, 1984), thirty isolates belonged to the genus *Leuconostoc*, twenty isolates to the genus *Lactococcus*, 6 isolates to *Streptococcus* and 2 isolates to *Enterococcus*.

Identification of lactic acid bacteria to species level: From the 20 isolates of lactic acid bacteria identified with API 50 CH identification system, six belonged to the species *Lactococcus lactis ssp. lactis*, four belonged to the species *Lactococcus lactis subsp. lactis biovar. diacetylactis*, two belonged to *Lactobacillus confusus*, three belonged to *Lactobacillus delbrueckii subsp.*, two belonged to *Lacctobacillus plantarum*, one belonged to *Leuconostoc citreum*, and two belonged to *Leuconostoc lactis*.

Pathogens detection: Little numbers of *Salmonella* and *Shigella* species were detected in two samples (5-20 cfu ml^{-1}).

Discussion

Lactic acid bacteria predominated the total microflora; their numbers between $2.4x10^4$ and $3.9x10^8$ cfu ml^{-1} were recorded with mean values on MRS (35°C), M17 and Rogosa agar. Similar results were reported in Tanzania (Isono *et al.*, 1994), in Zimbabwe (Feresu and Muzondo, 1990), in South Africa (Beukes *et al.*, 2001). Earliest investigation [3,8,13] has reported that traditional fermented milks in regions with a cold climate containing mesophilic bacteria such as *Lactococcus* and *Leuconostoc spp.* Whereas, in warm regions, thermophilic bacteria like *Lactobacillus* and *Streptococcus* prevailed. The dominance of mesophilic bacteria in our samples could be explained by the fact that our samples were collected in the cooler month in Burkina (December, January, February) and the ambient temperature at which the natural fermentation took place probably. This result supports the theory that traditionally fermented milks depend on the microorganisms found in a particular climatic region. The distribution of lactic acid bacteria depend of nature of fermented milk or fermented food.

The Enterobacteria, yeasts and molds formed the minority groups, these population may not be essential microorganisms in Burkina fermented milk samples, and the level of sample's pH cannot favorise their growth. The presence of yeasts may be influenced by the age of our samples as well as the containers and processing methods used. Also several species of yeasts are used in kefir and koumiss, and contribute to the characteristic aromas and tastes .

The number of coliforms in some samples was high. Similar results have been reported with Moroccan traditional fermented dairy products like «Lben» and «Jben» which showed high number of indicator microorganisms (coliforms and *Enterococci)* and pathogens such as *Salmonella spp.*, *Yersinia enterocolitica, Listeria monocytogenes* and enterotoxigenic *Staphylococcus aureus* (Hamama, 1992). All these results can be explained by the fact that the methods of production of the various traditional foods are usually primitive (Isono *et al.*, 1994; Dirar, 1997) and the major risk enhancing factors are the use of contaminated raw materials, lack of pasteurization, use of poorly controlled natural fermentations and inadequate storage and maturation conditions (Nout, 1994).

The number of yeast (2.6×10^4cfu ml^{-1}) was lower than mean count for lactic acid bacteria. Other researchers investigating on fermented milk product reported highest number (1.1×10^7 cfu ml^{-1}) (Hosono *et al.*, 1989).

The fifteen (15) isolates from YGC, were identified as *Saccharomyces spp.* on a base of morphological and biochemical characteristics (multilateral budding, formed pseudohyphae and asci containing one to four globose ascopores, fermented glucose, galactose and maltose, did not assimilate lactose and nitrate; and their cells were globose to subglobose or ellipsoide to cylindrical).

Generally, the species identified in the present study, were in good agreement with other studies. *Lactobacillus plantarum, Lactococcus lactis ssp. lactis, Lactobacillus delbrueckii subsp lactis, Leuconostoc lactis* and *Leuconostoc citreum* were identified in South African traditional fermented milks (Beukes *et al.*, 2001). *Lactococccus lactis ssp. lactis, Lactobacillus plantarum* and *Lactobacillus delbrueckii subsp. lactis* were identified in Zimbabwean fermented milk (Feresu and Muzondo, 1990). *Lactobacillus plantarum*, *Lactococcus lactis ssp. lactis* and *Lactobacillus confus* were identified in Masaï fermented milk in Northern Tanzania (Isono *et al.*, 1994). All these species cited were identified in Fulani fermented milk. This fact explain the diversity of lactic acid bacteria species in Fulani fermented milk.

It is known that aseptically drawn milk contains no Lactobacilli when it leaves the udder, but contamination with these organisms rapidly occurs from dairy utensils, dust and feedstuffs (Sharpe, 1981). Since unpasteurized milk was used for traditional fermentation in this study, it can be assumed that the isolates originated from such contamination.

All these species identified can contribute to the quality of Fulani traditional fermented milk by acid, flavour and aroma production.

Conclusion: One hundred strains of lactic acid bacteria were isolated from the following fermented milks. They were biochemically characterized and classified into six genera. The distribution of dominant lactic acid bacteria identified were investigated in the present study. These researches are all vital in the sense that functional properties in lactic acid bacteria improve preservative effect and add flavor and taste (Daly and Davis, 1998; Soomro *et al.,* 2002).

Lactic acid bacteria have an essential role in most food and beverage fermentation processes, one of the earliest known food preservation of fermented foods and beverage belong to the following genera: *Lactococcus, Lactobacillus*, *Leuconostoc*, *Pediococcus, Streptococcus and Bifidobacterium.* The main role of lactic acid bacteria in food manufacturing is to acidify raw materials by producing of lactic acid from energy sources (carbon hydrates).

This study on traditional fermented milk from northern Burkina (Dori) showed that lactic acid bacteria are the dominant microflora. The microbiological composition of lactic acid bacteria found in our samples coincided with that of commercial mesophilic starter cultures. Identifies species from Fulani fermented milk can be use as staters for our small scale dairy industries. The further studies will be focus the molecular characterization of bacteria species.

References

Axellsson, L.T., 1993. Lactic acid bacteria: classification and physiology. In: Salminen S., A. Von Wright (Eds.). Lactic acid bacteria. Marcel Dekker, New York, p: 1-64.

Beukes, E.M., B.H. Bester and J.F. Mostert, 2001. The microbiology of South African traditional fermented milk. Int. J. Food Microbiol., 63 : 189-197.

Cowan, S.T., 1974. Cowan and Steel's Manual for the Identification of Medical Bacteria. 2nd ed., Cambridge University press, Cambridge.

Daly, C. and R. Davis, 1998. The biotechnology of lactic acid bacteria with emphasis on applications in food safety and human health. Agri. Food Sci. in Finland, 7: 251-264.

De Man, J.C., M. Rogosa and M.E. Sharpe, 1960. A medium for the cultivation of lactobacilli. J. Appl. Bacteriol., 23: 130-135.

Dicks, L.M.T., L. Fantuzzi, F.C. Gonzales, M. Du Toit and F. Dellaglio, 1993. *Leuconostoc argentinum sp.nov* isolated from argentine raw milk. Int. J. Syst. Bacteriol., 43: 347-351.

Dirar, H.A., 1997. Introduction. In: Dirar H.A. (Ed.). Food processing technology for Africa. United Nations Industrial Development Organization, Vienna, p: 1-10.

Ehrmann, M.A., P. Kurzak, J. Bauerr and R.F. Vogel, 2002. Characterization of lactobacilli to wards their use as probiotic adjuncts in poultry. J. Appl. Microbiol., 92: 966-975.

Feresu, S.B. and M.I. Muzondo, 1990. Identification of some lactic acid bacteria from two Zimbabwean fermented milk products. Word J. Microbiol. and Biotech., 6: 178-186.

Garvie, E.I., 1984. Separation of species of the genus *Leuconostoc* and differentiation of the *Leuconostocs* from other lactic acid bacteria. In: Bergan T. (Ed.). Methods in microbiology. Academic press, London, p: 147-178.

Garvie, E.I., 1986. Genus *Leuconostoc*. In: Sneath, P. H. A., N. S. Mair, M. E. Sharpe, J. G. Holt (Ed.). Bergey's Manual of Systematic Bacteriology. Williams and Wilkins, Baltimore, p: 1071-1075.

Hamama, A., 1992. Moroccan traditional fermented dairy products. In: Ruskin, F.R. (Ed.). Applications of biotechnology to traditional fermented foods. National Academy press, Washington DC, p: 75-79.

Hammes, W.P., N. Weiss and W.H. Holzapfel, 1992. The genera *Lactobacillus* and *Carnobacterium.* In: Barlows A., H. G. Trüper, M. Dworkin, W. Harder, K.-H. Schleifer (Ed.). The prokaryotes. Springer, Berlin, p: 1534-1593.

Harrigan, W.F. and M.E. McCance, 1976. Laboratory methods in Food and Dairy Microbiology, Academic press, London.

Holzapfel, W.H. and V. Schillinger, 1992. The genus *Leuconostoc*. In: Barlows A., H. G. Trüper, M. Dworkin, W. Harder and K.-H. Schleifer (Eds.). The prokaryotes. Springer, Berlin, p: 1509-1534.

Hosono, A., R. Wardojo and H. Otani, 1989. Microbial flora in «*Dadih*», a traditional fermented milk in Indonesia. Lebensm Wiss Tec., 22: 20-24.

Isono, Y., I. Shingu and S. Shimizu, 1994. Identification and Characteristics of lactic acid bacteria isolated from Masaï fermented milk in Northern Tanzania. Biosci. Biotech. Biochem., 58: 660-664.

Keller, J.J. and I. Jordan, 1990. Fermented milks for the South African market. S. Afr. J. Dairy Sci., 22: 47-49.

Kurmann, J.A., 1994. The production of fermented milk in the world: aspects of the production of fermented milks. Int. Dairy Federation Bull., 179: 16-26.

Mckay, L.L. and K.A. Baldwin, 1990. Application for biotechnology: present and future improvements in lactic acid bacteria. FEMS Microbiol. Rev., 87: 3-14.

Nout M.J.R., 1994. Fermented foods and food safety. Food Res. Int., 27: 291-298.

Réseau Documentaire d'Elevage au Burkina Faso, (Juillet, 1997–décembre, 1998). Revue de presse sur l'élevage au Burkina Faso, Syfia Bull. de presse, Paris.

Rogosa, M., J.A. Mitchell and R.F. Wiseman, 1951. A selective medium for the isolation of oral and fecal lactobacilli. J. Bacteriol., 62: 132-133.

Savadogo, A., C.A.T. Ouattara, A.S. Ouattara and A.S. Traoré, 2001. Isolement et Caractérisation de bactéries lactiques productrices d' exopolysaccharides à partir de laits du Burkina Faso. Revue Sci. et Tech., Séries Sci. Nat. et Agro., 25: 75-85.

Sharpe, M. E.,1981. The genus *Lactobacillus*. In: Starr M.P., H. Stolp, H.G. Truper, A. Balows, H.G. Schlegel (Eds.). The Prokaryotes: A Handbook on Habitats, Isolation and Identification of Bacteria. Springer, New York, p: 1653-1679.

Soomro, A.H., T. Masud and K. Anwaar, 2002. Role of lactic acid bacteria (LAB) in food preservation and Human health. Pak. J. Nutr., 1: 20-24.

Tamine, A.Y. and R.K. Robinson, 1988. Fermented milks and their future trends: technological aspects. J. Dairy Res., 55: 281-307.

Teuber, N., A. Geis and H. Neve, 1992. The genus *Lactococcus*. In: Barlows, A., H. G. Trüper, M. Dworkin, W. Harder and K.-H. Schleifer (Eds.). The prokaryotes. Springer, Berlin, p: 148-150.

Therzaghi, B.E. and W.E. Sandine, 1975. Improved medium for lactic streptococci and their bacteriophages. Appl. Microbiol., 29: 807-813.

Thomas, T.D., 1985. Role of lactic acid bacteria and their improvement for production of better fermented animal products. New Zealand J. Dairy Sci. Tec., 20: 1-10.

Weiss, N., 1992. The genera *Pediococcus* and *Aerococcus*. In: Barlows A., H. G. Trüper, M. Dworkin, W. Harder, K.-H. Schleifer (Eds.). The prokaryotes. Springer, Berlin, p: 1502-1507.

Yodoamijoyo, R.M., Z. Tirza, S.R. Herastuti, A. Tomomatsu, A. Matsuyama and A. Hosono, 1983. Microbiological aspects of Dadih in Indonesia. J. Dairy Food Sci., 32: 7-14.