Identification and Characterization of Dominant Lactic Acid Bacteria Isolated from Traditional Rayeb Milk in Egypt

Abd El Gawad, I.A.; Abd El Fatah, A.M. and Al Rubayyi, K.A*

Department of Dairy Science and Technology, Faculty of Agric., Cairo University, Giza, Egypt
*Kahalid308@hotmail.com

Abstract: A total of 170 strains were isolated from 40 traditional Rayeb milk samples that were collected from different areas in Egypt. The Lactic acid bacteria (LAB) dominated the microbial population of Rayeb milk and were identified on basis of their morphological, physiological and biochemical (API) characteristics. Among the isolates, the Lactobacilli were dominant. The distribution of the isolates by genus was as fallows: Lactobacilli (30%), Leuconostoc (26%), Enterococcus (20%), Streptococcus (18%) and Aerococcus (6%). Thirty eight representative LAB strains were identified to species level belonging to species *Str. thermophilus*, *L. bulgaricus*, *L. helviticus*, *L .acidophilus*, *L. delbuerkii, Leu. cremoris, Ent. faecium, Str. durans, Str. acidomonas* and *Aer. viridans*. The identified strains were then evaluated for some technological properties. Most strains of lactobacilli produced EPS and two strains only had antagonistic properties against *E. coli* and *S. aureus*. [Journal of American Science 2010;6(10):728-735]. (ISSN: 1545-1003).

Key words: Traditional Egyption Rayeb milk, LAB, API technique.

1. Introduction:

Dairy products made from locally produced raw milk are still a very important part of the daily diet; the nature of these products is different from one region to another depending on the local indigenous microflora, which in turn reflects the climatic conditions of the area. These products have one feature in common: fermentation by lactic acid bacteria (LAB) is an integral part of their manufacture.

Rayeb milk is a traditional fermented milk product popular in rural areas of Egypt; Rayeb milk is traditionally made from raw buffalo milk by spontaneously fermentation. The raw milk is left to sour spontaneously at room temperature until it coagulate; it contains a mixed culture of lactic acid bacteria and other fermentative organisms.

Lactic acid bacterial (LAB) have a long history of safe use in fermented foods. Today, LAB still play an essential role in the majority of food fermentations and one of the most important contributions of these microorganisms is the extended shelf life of fermented products. However, they also have beneficial influence on nutritional and sensory characteristics as well as on the standardization of end products (De vuyst and Leroy, 2007 and Olaoye and Onilude, 2008)

Nowadays, consumers demand large variations in flavour of dairy products besides consistency in overall quality. Therefore, the dairy industry is keen on exploring new possibilities for expanding the diversity of its product range. Accordingly, there is great interest in searching for potential starter organisms from the pool of wild LAB recoverable from raw milk or raw milk products (Wouters et al., 2002). The need for new products requires the use of new microbial strains with novel properties. This has led to a request for novel strains for the innovation and diversification of dairy products. This novel strain can be achieved either by exploring the biodiversity within natural from various ecological niches or by genetic modification of known production strains. The wild lactic acid bacterial flora represents a natural reservoir for cultures that were not exposed to any industrial selection. Some interesting characteristics of these microorganism are their ability to produce acid at a high and predictable rate, proteolytic activity, synthesize EPS and produce antimicrobial compounds which is essential in fermented milk starter strains (El Soda et al ;2003).

Therefore, the isolation and identification of new strains from the Egyptian traditional Rayeb milk is necessary in order to bring novel strains to the industry. Phenotypic methods relying on physiological and biochemical criteria have been widely used for LAB identification. The use of phenotypic mean of identification of LAB from different sources has been reported (Guessas and kihal, 2004 and Nair and surendran,2005).

The objective of this study was to characterize and identify dominant LAB that occur naturally in the Egyptian traditional Rayeb milk by using both physiological and biochemical methods and to determine their technological properties .

2. Materials and Methods

1- Collection of samples:

Forty samples of Rayeb milk made by traditional method were collected aseptically from local producers from Giza, Mnofeya, Sharkeya, Mansoura and Fayoum governorates (eight samples from each governorate). Samples were brought to the laboratory at 4-5 ° C by using of an icebox and stored in laboratory under refrigeration at 4° C until used.

2- Microbial population counting:

2.1- Lactic acid bacterial count:

Lactic acid bacterial count (LAB) was estimated using tomato juice agar medium as described by Oxoid Manual (1982).

2.2- Coliform Count:

Coliforms were counted using Violet Red Bile Agar medium as reported by American public Health Association (APHA,1992).

2.3- Staphylococci Count :

Staphylococci count were determined using Baird Parker Base medium ,according to Niskanen and Aalto (1978).

2.4- Mould and yeasts count

Moulds and yeasts count were determined using Malt-Extract Agar medium as suggested by Harrigan and Mc Conce (1976).

3- Isolation of lactic acid bacteria:

Ten milliliters of each Rayeb milk samples were aseptically added into 90 ml of sterile 0.9% NaCl solution and mixed thoroughly. Serial dilutions (10^{-1} to 10^{-7}) performed and 1 ml aliquots of appropriate dilution were directly inoculated in triplicate on the following media:

(a) MRS agar (Oxoide, UK), incubated anaerobically in an Anaerobic Gas-Pack system (Biomerieux, France) for 48 h at 37° C for isolation of lactobacilli(De Man et al .,1960);

(b) M17 agar (Oxoide), incubated aerobically for 48 h at 37°C for isolation of lactobacilli, enterococci and *Streptococcus thermophilus* (Therzaghi and Sandine,1975);

(c) MRS agar containing 30 µgml^{-1} vancomycin (Sigma, USA) incubated anaerobically for 72 h at 25 ° C for isolation of leuconostoc (Florez et al.,2006).

After incubation, all colonies from plates representing 15-20 colonies were further purified by successive streaking on the corresponding agar.

One hundred and seventy purified isolates were obtained from the above mentioned samples. These isolates were preserved in sterile skim milk supplemented with 15% (v/v) glycerol as cryoprotective agent and stored at -20 °C until further tests.

4- Identification of lactic acid bacteria

4.1. Preliminary characterization of isolates:

All isolates were microscopically examined for Gram stain reaction, cell morphology and cellular arrangement(Gerhardt etal.,1981 and Sneath et al.,1986). Catalase activity and production of CO_2 from glucose (Harrigan and McCance,1976) were also determined to identify the isolates at the genus level. Only Gram-positive and Catalase negative isolates were identified at species level.

4.1.1. Catalase test:

A drop of 3 percent hydrogen peroxide was placed on a clean microscopic slide. A visible amount of bacterial growth was added with the inoculating loop. Both were mixed and observed for gas bubble production.

4.1.2. CO_2 production from glucose:

Fifty µl of overnight cultures were transferred into the 8 ml of MRS and M17 broth media with inverted Durham tube. After incubation for 5 days at 30 °C, gas accumulation in Durham tubes was taken as the evidence for CO_2 production from glucose.

4.2. API Systems:

The API 50 CHL test strips (Biomerieux, France) was used for the identification of lactobacilli, lactococci, leuconostoc, pediococcus and *S. thermophilus* strains while API 20 STREP (Biomerieux, France) was used for the enzymatic and carbohydrate fermentation patterns of enterococci strains. The API system was preformed according to the manufacture's instructions. The API LAB PLUS database (Bio Merieux, France) was used for the interpretation of the result.

5- Performance tests:

5-1 Acid production:

Acid production ability was assayed by inoculating 10% skim milk with 24 h old cultures at 1% level and incubation at 30 C. Acidity was determined every 1 h to 7 h of incubation.

5-2 Antibacterial activity:

LAB isolates were screened for antibacterial activity by the agar well diffusion method of (Varadaraj et al., 1993). The indicator strains used included *E. coli* and *Staph. aureus*. Pure isolated strains were cultivated in broth media and were added at 0.1 ml to each well and the petri plates were kept at 4 C for 2h to facilitate diffusion of culture into the medium. The plates were then incubated at 37 C for 24h. The plates were checked for zones of inhibition surrounding the producer strain colonies (Geis et al., 1983).

5-3 Exopolysaccharides production:

The screening of EPS production was limited to the strains showing weak pellet after centrifugation. The procedure used was that described by (Prescott et al., 1996). The strain producing capsules were plated on the suitable media and incubated at the optimum growth temperature for 24hr. They were tested for slime formation using the loop.

3. Results and Discussion:

Microbial population of traditional Rayeb milk:

The mean log counts ($cfug^{-1}$) of the dominant microbial groups of the forty batches of Rayeb milk collected from five Egyptian governorates are summarized in Table (1). Counts of coliform, mould and yeasts and Staphylococci groups were high in all samples from different governorates, and ranged from 1.02×10^{-2} to 9.89×10^{-2} $cfug^{-1}$. All these populations rose from around $10^{2}cfug^{-1}$ to 10^{4} $cfug^{-1}$. These results can be explained by the fact that the methods of production of the various traditional foods are usually primitive and the major risk enhancing factors are the use of contaminated raw materials, lack of pasteurization and inadequate fermentation and storage conditions (Savadogo et al.,2004) .Despite the large number of different bacterial species of the lactic acid bacteria (LAB) group dominated the microbiota, in all samples to reach a final population of around 10^{6} to 10^{7} $cfug^{-1}$.

Table (1): Mean log counts *($cfug^{-1}$) of microbial groups found in Rayeb milk samples obtained from different Egyptian governorates.

Microbial groups	Egyptian governorates**				
	A	B	C	D	E
LAB	$2.2\times10^{6}\pm278$	$1.66\times10^{6}\pm307$	$2.2\times10^{6}\pm214$	$2.30\times10^{7}\pm312$	$2.0\times10^{6}\pm221$
Coliform	$1.99\times10^{2}\pm379$	$1.42\times10^{2}\pm387$	$4.62\times10^{2}\pm126$	$3.30\times10^{2}\pm840$	$2.33\times10^{2}\pm362$
M & Y	$1.02\times10^{2}\pm1.52$	$3.57\times10^{2}\pm3.52$	$4.87\times10^{2}\pm411$	$4.68\times10^{2}\pm952$	$6.74\times10^{2}\pm801$
Staph	$3.99\times10^{2}\pm411$	$7.87\times10^{2}\pm892$	$2.28\times10^{2}\pm389$	$9.89\times10^{2}\pm2.51$	$1.16\times10^{2}\pm2.96$

* Mean log counts±SD **A: Giza B: Mnofeya C: Sharkeya D: Mansoura E: Fayoum

Isolation and identification of LAB:

LAB was the predominant microbial group in Rayeb milk, which is important because of the key role it plays in fermentation processes and its production of lactic acid and antimicrobial substances, as well as its potential use as a starter of Rayeb milk in standardized production. For these reasons, LAB strains were isolated and identified.

One hundred and seventy LAB isolated from Rayeb milk samples were identified phenotypically. The isolates were grouped on the basis of Gram stain reaction, cell shape, cellular arrangement, production of acid from glucose and lactose ,production of gas from glucose and catalase activity (Data not shown) . Based on phenotypic characteristics, the majority of isolates were Gram-positive and catalase-negative. Table (2) summarizes the results above described and distribution of isolates from the different media as follows: 67 isolates on M17 agar, 57 isolates on MRS agar and 46 isolates on MRS+V agar. According to these results, the estimated selectivity of M17 was 80.6, 94.1 and 100% for Streptococcus, Enterococcus and Aerococcus, respectively . The selectivity of the MRS medium was 92.0% for Lactobacilli and it was <10% for other genera, while the selectivity of the MRS+V medium was 88% for Leuconostoc and it was <10% for other genera. Similar results were reported in Florez et al., 2006 who found that the estimated selectivity was 67.1, 59.3 and 93.6% for M17, MRS and MRS+V, respectively .

Table (2): Grouping of representative strains of LAB isolated from traditional Rayeb milk using different media

Genus	Culture medium*			Total
	M17	MRS	MRS+V	
Streptococci	25	4	2	31
Lactobacilli	-	46	4	50
Leuconostoc	-	5	40	45
Enterococci	32	2	-	34
Aerococcus	10	-	-	10
Total	67	57	46	170

*Culture media utilized for counting of the different lactic acid bacteria groups:
M17 for streptococci MRS for lactobacilli MRS plus vancomycin (μg ml^{-1}) for leuconostoc spp.

Fig.(1) illustrates the distribution at genus Level of 170 LAB identified from Rayeb milk . The isolates were divided into five genera: lactobacilli (30%), Leuconostoc (26%), enterococci (20%) Streptococci (18%) and aerococcus (6%) (Fig1). Similar results were reported by Savadogo et al. (2004) and Harun-ur-Rashid et al. (2007) who identified six genera from traditional fermented milk :leuconostoc, lactococcus, lactobacillus, enterococcus, streptococcus and pediococcus.

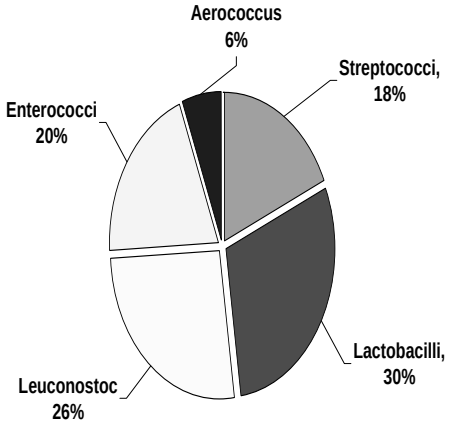

Fig (1): Distribution of LAB at genus level isolated from traditional Rayeb milk in Egypt.

The dominance of lactobacillus among the isolated strains is consistent with the finding of El-Shafei (2002), as the Rayeb milk is the heterogeneous mixture of different microorganisms. Moreover, The buffalo milk, which is commonly used for the preparation of this fermented milk , might favour the growth of these species .Lactobacillus is able to survive in highly acidic environment of pH=4 to 5 or even lower ,and due to these properties, lactobacillus is responsible for final stages of fermentation in the products . This further showed that low pH conditions favour the growth of lactobacillus(Soomro and Masud,2007).

Identification of LAB to species level:

The isolated LAB were identified on basis of their morphological characteristics, gas and acid production from glucose and lactose (Table 3).

Based on phenotypic characteristics and interpretation of the API database, thirty eight strains were satisfactorily identified, of which 22 were identified using a sugar – fermentation profile API 20 STREP and 16were identified using API 50 as shown in Table (4). These strains were tentatively identified as *Str. thermophilus, L. bulgaricus, L. helviticus, L. acidophilus, L. delbuerkii, Leu. cremoris, Ent. faecium, Str. durans, Str. acidomonas and Aer. viridans.*

Table (3): Morphological and simple physiological Characterization of LAB isolated from the traditional Rayeb milk

Species	No. of isolates	Cell shape	Gram stain reaction	Catalase activity	Acid from glucose	Acid from lactose	CO_2 from glucose	Cellular arrangement
Enterococcus faecium	11	spherical	(11) +	(11) -	(11) +	(11) +	2/9	pairs and short chains
Aerococcus viridians	7	Cocci	(7) +	(7) -	(7) +	(7) +	1/6	single and pairs
Str. Acidomonas	4	spherical	(4) +	(4) -	(4) +	(4) +	0/4	short chains
Lb. bulgaricus	4	Rods	(4) +	(4) -	(4) +	(4) +	0/4	single, pairs and short chains
Lb. acidophilus	3	Rods	(3) +	(3) -	(3) +	(3) +	0/3	single, pairs and short chains
Str. Thermophilus	3	spherical	(3) +	(3) -	(3) +	(3) +	0/3	pairs and long chains
Lb. delbuerkii	2	Rods	(2) +	(2) -	(2) +	(2) +	0/2	single, pairs and short chains
Leu. Cremoris	2	Cocci	(2) +	(2) -	(2) +	(2) -	2/0	pairs and short chains
Lb. helviticus	1	Rods	(1) +	(1) -	(1) +	(1) +	0/1	single, pairs and short chains
Str. Durans	1	Cocci	(1) +	(1)-	(1) +	(1) +	0/1	pairs and short chains

(+) all strain positive (-) all strain negative (-/-) number of positive /negative strain

Table (4): API results of isolated strains

Species identified using API system	No. of identified strains	Frequency (%) in total	Frequency (%) in genus
Enterococcus faecium	11	28.9	100
Aerococcus viridans	7	18.4	100
Str. acidomonas	4	10.5	50
Lb. bulgaricus	4	10.5	40
Lb. acidophilus	3	7.9	30
Str. thermophilus	3	7.9	37.5
Lb. delbuerkii	2	5.3	20
Leu. cremoris	2	5.3	100
Lb. helviticus	1	2.6	10
Str. durans	1	2.6	12.5

Based on API 20 STREP identification, 11 isolates (28.9%) were registered as *Ent. faecium*, 7 isolates (18.4%) as *Aer. viridans* and 4 isolates (10.5%) as *Str. acidominimus* (Table 4). The high percentage of *Ent. faecium* is an indicator of the key role played by these bacteria in the Rayeb milk fermentation process , analogous behavior has been reported in a large variety of Egyptian dairy products (EL.Soda etal.,2003). *S. acidominimus* was identified as the etiologic agent of community – acquired pneumonia, pericardits and meningitis in an adult male (Akaike et al.,1988).

As shown in Table (4), identified Lactobacillus strains (26.3%) were classified into 4 species. *L. bulgaricus* was the predominant species (10.5%) followed by *L. acidophilus* (7.9%), *L. delbuerkii* (5.3%) and *L. heliviticus* (2.6%). Similar results were obtained by (Hamza et al. (2009) who found that 33.3% of isolates from Sudanese sour milk (Rayeb) was general lactobacillus. Beukes et al. (2001) and Harun-ur-Rashid et al. (2007) reported that five genera of LAB (streptococcus, Lactobacillus, Enterococcus, Leuconostoc and Lactococcus) were found in traditionally fermented South African milk. Among the other species found in the Rayeb is the *Str.thermophilus* which represented 7.9% as identified by the API 50 CHL identification system. Two of the 38 representative isolates (5.3%) were identified as *Leu. mesenteroides subsp. cremoris*. Analysis of the representative of these isolates (38 isolates) showed that 2.6% belonged to the species *Str. durans*.

The predominance of lactobacillus genera due to these types of bacteria are commonly associated with the warm climatic condition of productive regions (Cueto et al., 2007). While Leuconostoc strains were found at low level because they are mesophilic bacteria, complex nutritional requirements and show a weak competitiveness during milk fermentation (Mathara et al., 2004).

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

Technological properties of LAB isolates:

Study of technological properties of LAB strains isolated from traditional Rayeb milk is an important criterion for selection of starter cultures to be used in the standardized production of Rayeb milk.

1- Acidifying activity:

Table (5) showed the technological activities of isolates from the traditional Rayeb milk (acid and EPS production and antibacterial activity).

The ability of LAB strains for acid production at 30 °C were performed. The obtained results revealed that the acidifying activity of Lactobacillus strains was significantly higher than the activity of the other species. The data in Table (5) shows a typical example of ten cultures with different acidifying rates. The acidity was higher at 5h especially for *L. helveticus* reach the maximum values (1.5%). All strains of *L. bulgaricus*. *L. acidophilus* and *L. delbuerkii* are considered to produce higher titratable acidity reach 0.79-0.81%. These results concur with those reported by other authors (Alonso-Calleja et al., 2002 and Badis et al., 2004a).

The acidifying capacity of the *S. thermopilus* isolates obtained was relatively homogeneous, after 5h incubation. However, the values were around 0.58% after 5h. The speed of acidification was slow by comparison with the Lactobacillus isolates. These results are in agreement with Zourari et al., 1992 and Badis et al., 2004b.

Among the other isolated strains found in the present study are *Ent. faecium, Aer. viridans, Str. acidomonas and Str. durans* which produced acidity varied between 0.52 and 0.73% after 5h of incubation. Slow acidifying activity was found in leuconostoc isolates. This genus is heterofermentative and sensitive to growth at low pH (Kihal et al., 1996).

The difference observed from one lactic acid bacteria species to another were explained by Badis et al., 2004. In fact, the acidifying activity of each strain is related to its specific capacity to break down the substances in the medium and render them capable of assimilation. On occasion, differences are also due to the presence or absence of nutrient transport systems (Albenzio et al., 2001).

From the date in Table (5), it can be noticed that, *L.helveticus* only caused coagulation of milk after 3h. However, all isolated strains except *Leu. cremoris* caused coagulation of milk after 5h with different rate of acid production. Coagulation of milk by LAB strains shows their potential as starters or adjunct cultures in the production of fermented milk products.

Table (5): Technological activities of isolates (antibacterial activity, acid and EPS production) from traditional Rayeb milk

Strains	No. of isolates	Antibacterial activity				EPS		Acidification and coagulation		
		E. coli		*S. aureus*						
		-	+	-	+	-	+	3hr	4hr	5hr
Enterococcus Faecium	11	11	-	11	-	11	-	-(0.37)	-(0.48)	+(0.71)
Aerococcus Viridians	7	7	-	7	-	7	-	-(0.38)	-(0.42)	+(0.52)
Str. Acidomonas	4	-	4	-	4	1	3	-(0.44)	+(0.65)	+(0.73)
Lb. bulgaricus	4	3	1	2	2	-	4	-(0.36)	-(0.59)	+(0.81)
Lb. acidophilus	3	3	-	3	-	3	-	-(0.35)	+(0.63)	+(0.81)
Str. Thermophilus	3	3	-	3	-	1	2	-(0.25)	-(0.50)	+(0.58)
Lb. delbuerkii	2	2	-	2	-	1	1	-(0.43)	+(0.73)	+(0.79)
Leu. Cremoris	2	1	1	2	-	2	-	-(0.19)	-(0.25)	-(0.28)
Lb. helviticus	1	1	-	1	-	-	1	+(0.65)	+(0.97)	+(1.5)
Str. Durans	1	1	-	1	-	1	-	-(0.38)	-(0.46)	+(0.65)

2- EPS production:

Microbial exopolysaccharides are used as thickeners or viscosifiers, stabilizing or emulsifying agents, and as gelling and water-binding agents or texturizers. From Table(5) showing the ability of 38 strains to produce EPS, it appears that all *L. bulgaricus* isolates, 2 of 3 *S. thermophilus*, 1 of 2 *L. delbuerkii* and *L. helviticus* isolates were able to produce EPS. These cultures will be used for their ability to improve the texture of Rayeb milk (Marshall and Rawson, 1999).

3- Antibacterial activity:

The culture of LAB, that included 38 isolates of genera of lactobacillus, streptococcus, leuconostoc, enteococcus and aerococcus isolated from traditional Rayeb milk were tested by well diffusion method to know if the antibacterial metabolites produced. From the results (Table 5), it could be noticed that none of Enterococcus, Aerococcus, *S. thermophilus, L. acidophilus, L. delbuerkii and L. helviticus* isolates showed antibacterial activity against indicator strains of *E. coli* and *S. aureus*. Among the *L. bulgaricus* strains, one strain showed antibacterial activity against *E. coli* and *S. aureus*, while the another strain showed antibacterial activity against *S. aureus* only. This suggests that antimicrobial properties of such strains can reduce the number of other undesired microorganisms in milk products as well as perform

essential roles in the preservation of product for human consumption (Mufandaedza et al., 2006).

In conclusion, this study on traditional Egyptian Rayeb milk showed that lactic acid bacteria are the dominant microflora, which have a significant effect on the overall quality of Rayeb milk. Some of the isolated and identified LAB showed outstanding performances that were similar to commercialy available cultures. The further study will be focus on the genotypic characterization of these isolates

Corresponding author
Al Rubayyi, K.A
Department of Dairy Science and Technology, Faculty of Agric., Cairo University, Giza, Egypt
Kahalid308@hotmail.com

5. References:

1. Akaike, T.; Suga, M.; Ando, M.; Ando, Y.; Araki, S. and Fujise, R. (1988). Streptococcus *acidominimus* infections in a human. Jpn. J Med., 27 (3): 317-320.
2. Albenzio, M.; Corbo, M.R.; Rehman, S.U.; Fox, P.F.; De Angelis, M.; Corsetti, A.; Sevi, A. and Gobbetti, M. (2001). Microbiological and biochemical characteristics of Canestrato Pugliese cheese made from raw milk, pasteurized milk or by heating the curd in hot whey. Int. J. Food Microbiol., 67:35-48.
3. Alonso-Calleja, C.; Carballo, J.; Capita, R.; Bernardo, A. and García-López, M.L. (2002). Comparison of acidifying activity of *Lactococcus lactis* subsp. *lactis* strains isolated from goat's milk and Valdeteja cheese. Lett. Appl. Microbiol. 34:134-138.
4. American public Health Association APHA (1992) A text book for the examination of dairy products 16 th edition,Washington D.C.,USA.
5. Badis, A.; Guetarni, D.; Moussa-Boudjem, B.; Henni, D.E.; Tornadijo, M.E. and Kihal, M. (2004-a). Identification of cultivable lactic acid bacteria isolated from Algerian raw goat's milk and evaluation of their technological properties. Food Microbiology, 21(3): 343-349.
6. Badis, A.; Guetarni, D.; Moussa Boudjema, B.; Henni, D.E., and M. Kihal, M. (2004-b). Identification and technological properties of lactic acid bacteria isolated from raw goat milk of four Algerian races. Food Microbiol. 21:579–588.
7. Beukes, E.M.; Bester, B.H. and Mostert, J. F. (2001). The microbiology of South African traditional fermented milks. International Journal Food Microbiology, 63, 189–197.
8. Cueto, C.; García, D; Garcés, F. and Cruz, J. (2007). Preliminary studies on the microbiological characterization of lactic acid bacteria in suero costeño, a Colombian traditional fermented milk product. Rev. Latinoam. Microbiol., 49 (1-2): 12-18.
9. De Man, J.C.; Rogosa, M. and Sharpe, M.E. (1960). A medium for the cultivation of lactobacilli. J. Appl. Bacteriol., 43:130-135.
10. De Vuyst, L. and Leroy, F. (2007). Bacteriocins from Lactic acid bacteria: production, purrification and food application. J. Mol Microbial. Biotechnol., 13:194-199.
11. El-Shafei,K.; Ibrahim, G.A. and Tawfik, N.F. (2002). Beneficial uses of locally isolated lactic acid bacteria, Egypt .J.Dairy sci.,30:15-25
12. ELSoda, M.; Ahmed, N.; Omran, N.; Osman, G. and Morsi, A. (2003). Isolation, identification and selection of Lactic acid bacterial cultures for cheese making. Emir. J. Agric. Sci, 15:51-71.
13. Florez, A.B.; Lopez–Diaz, T. M.; Alvarez-Martin, P. and Mayo, B. (2006). Microbial characterisation of the tradition Spanish blue-veined Cabrales cheese: identification of dominant lactic acid bacteria. Food Technol, 223:503-508.
14. Geis, E.; Singh, J. and Teuber, M. (1983). Potential of lactic Streptococci to produce bacteriocin. Appl. Environ. Microbiol. 45:205-211.
15. Gerhardt, P.; Murray, R.G.E.; Costilow, R.N; Nester, E.W.; Wood, W.A.; Krieg, N.R. and Phillips, G.B.(1981). Manual of methods for general bacteriology. American Society for Microbiology Washington DC.
16. Guessas, B. and Kihal, M. (2004). Characterization of Lactic acid bacterial isolated from Algerian arid Zone raw goats milk. Afr. J. of Biotech, 3:339-342.
17. Hamza, A.A.; El Gaali, E.I. and Mahdi, A.A. (2009). Use of the RAPD-PCR fingerprinting and API system for clustering lactic acid bacteria isolated from traditional Sudanese sour milk (Roab). African Journal of Biotechnology, 8 (15): 3399-3404.
18. Harrigan,W.F and McCance, M.E. (1976). Laboratory methods in food and dairy microbiology. Academic press, London .
19. Harun-ur-Rashid, M.; Togo, K.; Ueda, M. and Miyamoto, T. (2007). Identification and characterization of dominant lactic acid

bacteria isolated from traditional fermented milk Dahi in Bangladesh. World J. Microbiol. Biotechnol., 23:125-133.
20. Kihal, M.; Prévost, H.; Lhotte, M.E.; Huang, D.Q. and Diviès, C. (1996). Instability of plasmid-encoded citrate permease in Leuconostoc. J. Appl. Microbiol., 22:219-223.
21. Marshall,V. and Rawson, H.L. (1999). Effects of exopolysaccharide-producing strains of thermophilic lactic acid bacteria on the texture of stirred yoghurt. Internation J. of food Science and Technology, 34:137-143.
22. Mathara, J.M.; Schillinger, U.; Kutima, P.M.; Mbugua, S.K. and Holzapfel, W.H. (2004). Isolation, identification and characterisation of the dominant microorganisms of kule naoto: the Maasai traditional fermented milk in Kenya. Int J Food Microbiol. 94(3):269-278.
23. Mufandaedza, J; Viljoen, B.C.; Feresu, S.B. and Gadaga, T.H. (2006). Antimicrobial properties of lactic acid bacteria and yeast-LAB cultures isolated from traditional fermented milk against pathogenic *Escherichia coli* and *Salmonella enteritidis* strains. Int. J. Food Microbiol., 108:146-152.
24. Nair, P.S. and Surendran, P.K. (2005). Biochemical Characterization of lactic acid bacteria isolated from fish and prawn. J. of Culture Collections, 4:48-52.
25. Niskanen, A. and Alto, M. (1978). Comparison of selective media for coagulase –positive enterotoxigenic *Staphylococci aureus*. Appl. Envir. Microbiol., 35:1233-1236.
26. Olaoye, O.A. and Onilude, A.A. (2008). Identification of pediococcus ssp. from beef and evaluation of their lactic acid production in Varying concentrations of different carbon sources. Adv. in Nat. Appl. Sci.,2 (In press).
27. Oxoid Manual (1982). The oxide manual of culture media, ingredients and other laboratory services. Oxide limited, Basingstoke, Hampshire, England.
28. Prescott, L.M.; Harleyand, J.P. and Klein, D.A. (1996). The study of microbiology structure microscopy and specimen preparation. pp. 17-36. Ed. Elizabeth M. Seivers, Publishers Kerin Kane Microbiology Wm.c. Brown publishers.
29. Savadogo, A.; Ouattara, C.A.T.; Savadogo, P.W.; Ouatta, A.S.; Barro, N. and Traore, A.S. (2004).Microorganisms Involved in Fulani Tradition Fermented Milk in Burkina Faso. Pakistan Journal of Nutrition, 3(2):134-139.
30. Sneath, P.H.A.; Mair, N.S.; Sharpe, E.M. and Holt, J.G. (1986). Bergey's manual of systematic bacteriology. vol. 2. Williams &Wilkins, Baltimore.
31. Soomro, A. H. and Masud, T. (2007). Protein Pattern and Plasmid profile of Lactic Acid Bacteria Isolated from Dahi, A Tradition Fermented Milk product of Pakistan. Food Technol. Biotechnol. 45(4):447-453.
32. Therzaghi, B.E. and Sandine, W.E. (1975). Improved medium for lactic streptococci and their bacteriopage. App. Microbiol., 29:807-813.
33. Varadaraj, M.C.; Devi, N.; KeShava and Manjreker, S.P. (1993). Antimicriobial activity of neutralized extracellular cultured filtrates of lactic acid bacteria isolated from a cultured Indian milk products (dahi). Int. J. Food Microbial, 20:259-267.
34. Wouters,J.T.M.; Ayad,E.H.E.; Hugenholtz,J. and Smit.S.,(2002).Microbial from row milk for fermented dairy products Int.Dairy.J.12,91-109
35. Zourari, A.; Accolas, J. P. and Desmazeaud, M. J. (1992). Metabolism and biochemical characteristics of yogurt bacteria. *Lait, 72:*1-34.

8/22/2010