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Isolation and Identification of Lactic Acid Bacteria from Raw Cow Milk, White Cheese and Rob in Sudan

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Abstract: The cultural, physiological and biochemical of fifty two strains of Lactic Acid Bacteria (LAB) isolated from twenty samples of Sudanese fermented milk (Rob), white cheese and raw milk were investigated. Three genera were found, *Lactobacillus* (69.23%), *Lactococcus* (19.23%) and *pediococcus* (11.53%). The dominate species were *Lactobacillus xylosus* (10 strains), *Lactococcus lactic* sub. *cremoris* (10) strain from raw cow milk. *Lactobacillus delbrucckii* (8 strains) from Rob and *Pediococcus cereviasae* (6 strains) from white cheese. *Lactobacillus casei* sp. Comprise 53.3% of cheese sample and 13.3% of raw milk sample (M_2). Other isolates were, *Lacobacillus fermentum* from rob, *Lactobacillus brevis* from white cheese and unidentified *Lactobacillus* spp. from rob. This study suggests that dairy products in Sudan may be a rich source of lactic acid bacteria.

Key words: Lactic acid bacteria, raw cow milk, fermented dairy products, health benefits, Isolation, Identification

INTRODUCTION

Lactic Acid Bacteria (LAB) have played along and important role in Food technology and have a long history of use by man for food production and food preservation. LAB are Gram positive, non spore forming bacteria, grow under anaerobic condition, *cocci* or *rods* which produce lactic acid as the major end product during the fermentation of carbohydrate (Axelsson, 2004). LAB divided into two groups based on the products produced from the fermentation of glucose. Homofermentative lactic acid bacteria and heterofermentative organism. LAB is generally associated with habitat rich in nutrients such as milk, cheese, meat, beverages and vegetables. They could be also isolated from soil, lakes, intestinal tract of animals and humans (Tserovska *et al*., 2002; Chen *et al*., 2005; Schillinger and Luke, 1989). LAB are widely used as starter cultures in the manufacture of fermented products including milk products such as yoghurt, cheese, meat products, bakery products wine and vegetable (Axelsson, 2004). Many recent studies have shown the health effects of various strains of lactic acid bacteria in humans and animals and have tried to describe their action mechanism in the digestive tract. A number and a variety of potential beneficial effects have been published. Some of these effects have already been described such as the improvement of lactose digestion and the treatment of diarrheal disorders (Gillilard, 1990; Drouault and Corthier, 2001). LAB can act as cell factories for production of food additive and aroma compounds and they have a potential use for the establishing the so called functional food (Halzapfel *et al*., 2001). The samples used in this study are traditional fermented milk (Rob) which made from pasteurized milk with addition of small amount of yoghurt, left at room temperature until it tastes sour. The white cheese which is popular in the country is made from raw unpasteurized cow milk. The processing of white cheese like feta cheese, semi hard and is kept in brine (Helen *et al*., 1981). The third sample was raw cow milk. The aim of the study was the isolation of LAB from raw milk, Rob and white cheese manufactured in Sudan so as to be preserved in cultural national bank. To ower knowledge this the first study for Isolation of lactic acid bacteria from raw milk and traditional white cheese in Sudan.

MATERIALS AND METHODS

Samples: A total of ten samples of Rob (R = 4 samples) raw cow milk (M = 2 samples) and white cheese (Ch = 4 samples) were collected from the local market inside ice box. The samples were transferred immediately to the laboratory for microbiological analysis.

Isolation of lactic acid bacteria: Twenty five grams from each sample were inoculated into 1% peptone water (Oxoid) and shacked thoroughly. Serial dilutions (1 ml) of these samples were spread on sterile Petri dishes of MRS agar. Incubation was carried out anaerobically at 30°C for 48 h. Colonies with distinct morphological differences such as color, shape and size were selected and purified by streaking at least three times in MRS agar.

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Identification of bacterial strains: All strains were maintained by weakly sub culturing on MRS agar. All tests were carried out from 48 h MRS agar cultures. The morphological characteristics of isolates were examined after staining by Gram stain (Harrigan and MacCance, 1976). Growth characteristic were monitored daily at 15, 30, 45 and 50°C in tubes of MRS broth over a 7 day period. Salt tolerance was assessed after 3 days of incubation at concentration of 4% and 6.5% Nacl in MRS broth. Catalse test was carried out by transferring a drop of MRS broth culture onto a clean slide, flooded with a drop of H_2O_2 and observed for production of effervescence. CO_2 production, from glucose in Gibson semi solid medium, after 1-7 days of incubation at 37°C. Production of ammonia form arginine was detected using Nessler's reagent. Milk curdle characteristic was tested by inoculating isolates in Litmus medium (Schillinger and Luke, 1989).

Carbohydrate fermentation: All strains were grown overnight at 37°C in MRS broth, but glucose and meat extract were omitted. Solution of 1% of the test carbohydrates were sterilized by membrane filtration (0:13 mum; pore size = 0.45 µm Adventec, Tokyo) and added to sterilized medium at a final concentration of 20 gm $litre^{-1}$. Carbohydrates utilization was assessed at 24 h, 48 h and 7day after incubation of the tested isolates at 37°C.

RESULTS AND DISCUSSION

Eighty strains were isolated from raw milk, white cheese and Rob. The Gram-negative and catalase positive strains were regarded as non-LAB (Sharpe, 1979) and were not tested further. After original characterization and grouping fifty two lactic acid bacterial cultures were isolated. Generally the LAB isolated from the three samples, belonged to the genera *Lactobacillus*, *Lactococcus* (previously) *streptococcus* and *pediococcus*. The genus *lactobacillus* was dominant in all samples tested (69.23%) followed by the genus *Lactococcus* (19.23%) and the genus *pediococcus* (11.53%). Strains M1, M2, R1, R2, R4, ch1, ch3 and ch4 were referred to genus *Lactobacillus*. They are Gram positive, non spore forming rods, catalase negative. The morphological, physiological and biochemical characteristics were tested. The data are shown in Table 1 and 2.

Most strain (83.3%) isolated from sample M1 were identified as *L. Xylosus*, which has been reclassified as *L. lactic* subs *lactic* (Schleifer *et al*., 1985). Cells were coco bacilli in shape, grew at 15, 30 and 45°C. It can tolerated 4% and 6.5% Nacl and Curdle the milk. All carbohydrates tested were fermented except arabinose, salicine and sorbitol (Table 2). Strain M2, ch3 and ch4 are similar in their morphological and physiological characteristics except strain M2 which grew at all temperature tested (15, 30, 45°C) and unable to grow at 6.5 NaCl. These strains referred to *L. casie* and can be differentiated by carbohydrate fermentation (Table 2). They were identified as *L. casie* subsp. *aloctosus* and *L. casie* subsp. *Casie* respectively. Sub species of L*actobacillus casie* can be differentiated according to growth temperature and lactose fermentation (Garvie, 1986). *Lactobacillus casie sp*. comprise 53.3% of cheese samples isolates (Table 3) and 13.3% from raw milk samples (M2). Strain R1 and strain ch1 are hetero

Table 1: Morphological and physiological characteristics of the isolated strains

	Strains									
Characteristics	M1	R1	Ch1	M2	Ch2	Ch3	R2	Ch4	R3	R4
Cell morphology	Coccbacilli	Rods	Rods	Rods	Cocci tetrads	Short rod	Rod	Rod	Cocci	Rod
Gram strain	G+	G+	G+	G+	G+	G+	G+	G+	G+	G+
Spores formation	-	-	-	-	-	-	-	-	-	-
Colony morphology	1-2 mm white	1 mm white	<1 mm white	<1 mm white	1 mm white	1 mm white	1-2 mm white	1-2 mm white	2 mm white	1-2 mm white
Catalase activity	-	-	-	-	-	-	-	-	-	-
Gas production from Gibson semi solid media	-	+	+	-	-	-	-	-	-	-
Growth at different temperatures										
15°C	+	-	-	+	+	+	-	+	-	+
30°C	+	+	+	+	+	+	-	+	+	+
45°C	+	+	-	+	-	-	+	-	-	+
50°C	-	-	-	-	-	-	+	-	-	-
Growth in NaCl										
4%	+	-	-	+	+	+	+	+	-	+
6.5%	+	-	-	-	+	+	-	+	-	+
Gelatin liquefaction	-	-	-	-	+	-	-	-	-	-
Milk curd	+	+	+	+	+	+	+	+	-	+
NH_3 from Arginine	-	+	+	-	+	-	-	-	-	-

Table 2: Biochemical characteristic of the tested strains

	Strains									
Carbon source	M1	R1	Ch1	M2	Ch2	Ch3	R2	Ch4	R3	R4
Arabinose	-	-	-	-	-	-	-	-	-	
Cellobiose	-	-	-	+	+	-	+	+	-	+
Esculin	+	-	-	+	+	-	+	-	-	+
Fructose	+	+	-	+	+	-	-	-	-	-
Glactose	+	-	+	+	+	+	-	+	+	+
Glucose	+	+	+	+	+	-	+	+	+	+
Lactose	+	+	-	+	+	-	-	+	-	-
Maltose	+	-	-	+	+	-	-	-	-	-
Mannitol	+	-	-	+	-	-	-	-	-	-
Mannose	+	+	-	+	-	-	+	-	-	+
Raffinose	+	+	+	-	-	-	-	-	-	+
Ribose	+	+	-	+	+	+	-	+	+	-
Salicine	-	-	-	-	-	-	-	-	-	-
Sorbitel	-	-	-	-	-	-	-	-	-	-
Sucrose	+	-	-	+	-	+	-	-	-	+
Trehalose	+	-	-	+	+	-	-	-	-	-
Xylose	+	-	-	-	-	-	-	-	+	+

Table 3: The group identities and number of isolates

Sample	Group	Identities of isolates	Number of isolates
Milk	M1	*Lactobacillus xylosus*	10
	M2	*Lactobacillus casie subsp. Pseudoplanturum*	2
Total			12
Rob	R1	*Lactobacillus frementum*	4
	R2	*Lactobacillus delbrucckii*	8
	R3	*Lactococcus lactis subsp. Cremoris*	10
	R4	*Lactobacillus sp.*	3
Total			25
Cheese	ch1	*Lactobacillus brevis*	3
	ch2	*Pediococcus cerevisiae*	6
	ch3	*Lactobacillus casie subsp. aloctosus*	3
	ch4	*Lactobacillus casie subsp. casie*	3
Total			15
Total number of lactic acid bacteria isolates			52

fermentative, produce CO_2 from Gibson semisolid medium. They are similar in their morphological and physiological characteristics except growth temperature. Strain ch1 grew at 30°C but strain R1 grow at 30°C and 45°C. The two strains could ferment glucose and raffinose. These strains were characterized as *L. fermentum* and *L. brevis* respectively. Strain R2 which isolated from Rob, could grow at high temperature (45 and 50°C) and tolerated growth in 4% NaCl and curdle the milk. It could ferment cellobiose, Esculin, glactose and ribose.This strain was referred to *L. delbrucckii*. It comprised 32% of the total isolates of rob. Other strain isolated from rob (R4) were not identified to species level.

Cells of strains R3 were cocci in shape. They were isolated from Rob and strains ch2 isolated from white cheese, the morphological of its cells formed tetrads. They were Gram +ve, nonspore forming, non motile, catalase negative and facultative anaerobes. The morphological, physiological characteristics are shown in Table 1 and biochemical characteristic in Table 2. They were homofermentative, not producing CO_2 from Gibson semisolid medium. Strains R3 could grow only at 30°C while strains ch2 grew at 30°C and 15°C. Strain ch2 tolerate growth at 4% and 6.5% NaCl but strain R3 was not.

The two strains R3 and ch2 were different in carbohydrate fermentation (Table 2). A according to Bergey's Manual (Gravie, 1986) strain R3 considered as *Lactococcus Lactis* subsp. *Cremoris* (formerly recognized as *streptococcus cremoris* (Axelsson, 2004). Strain ch2 were referred to as *Pedicococcus cerevisiae*. Results of the microbiological analyses, obtained from this study showed that, LAB isolated from raw milk, Rob and cheese are the normal flora of these products. Axelsson (2004) reported that LAB is generally associated with habitats rich in nutnents however *Lactococcus lactis* is actually used in dairy technology. It is well known that LAB produces a variety of antimicrobial substances with potential importance for food fermentation and preservation. Topisirovic (2006) isolated lactocci and lactobacilli from home made cheese, which were bacteriocin producer. They showed antimicrobial activity against some spoilage organism.

The same species were isolated from Rob samples, which act as normal preservative for Rob and cannot spoiled easily when stored at room temperature. The lactic acid bacteria isolated from Rob sample in this study are almost like the finding of Abdelgadir *et al.* (2001) and Hamza *et al.* (2005) who were studying lactic acid bacteria of Sudan fermented milk (Rob).

Pedicoccus cerevisiae represented 60% of total isolates of white cheese; these organisms are used for conservation of meat and vegetable foods and play an essential role as components trade starter culture by Sausage production (Raccah, 1987).

In this study *Lactococcus lactis subsp. cremoris* isolated from Rob, but plants are thought to be their original ecological niche and have been recovered from many different plants (Klijn *et al.*, 1995; Salama *et al.*, 1995). All *Lactobacillus* species isolated from the samples tested are associated with dairy industry. Hegazi and Elnaga (1980) isolated *L. brevis, L. casei subsp. pseudo plantarum, L. Casei sub sp alactosus* and *L. xylosus* from *Awshari* cheese in Iraq.

The strains which are isolated from raw milk, Rob & white cheese are of industrial uses in dairy industry. *L. lactis subsp. Cremoris*, used as dairy starter culture (Klijn *et al.*, 1995). *L. fermentum* was used with other mixed culture as a raw mixed starter. It is thermophilic cheese culture, used in traditional cheese making in Switzerland (Annika and Marc, 2004). *L. casei* strains are used as probiotic bacteria (Shah, 2002).

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