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Biochemical Properties of Some Thermophilic Lactic Acid Bacteria Strains from Traditional Fermented Milk Relevant to Their Technological Performance as Starter Culture

[1]Zambou Ngoufack François, [2]Nour El Hoda, [3]Fonteh A Florence,
[4]Moundipa Fewou Paul, [1]Tchouanguep Mbiapo Félicité and [2]Morsi El Soda
[1]Department of Biochemistry, Faculty of Sciences,
University of Dschang, Cameroon P.O. Box 67 Dschang-Cameroon
[2]Laboratory of Microbial Biochemistry, Faculty of Agriculture,
University of Alexandria, Egypt
[3]Department of Animal Production, Faculty of Agronomy and Agricultural Sciences,
University of Dschang Cameroon
[4]Department of Biochemistry, Faculty of Sciences, University of Yaounde I,
Cameroon P.O. Box 812 Yaounde, Cameroon

Abstract: The aim of this study was to characterize isolates of thermophilic lactic acid bacteria from traditional fermented milk and to study some of their important technological properties. Five isolates of thermophilic lactic acid bacteria from traditionally raw cow's fermented milk were identified using phenotypic criteria and Sodium Dodecyl Sulphate-Polyacrylamide gel electrophoresis of whole cell proteins. Four isolates named 2, 8, 13 and 20 FM were identified as *Streptococcus thermophilus* while isolate 285 N was identified as *Lactobacillus delbrueckii* ssp. *bulgaricus*. All these strains exhibited good acidification activity although two strains of *Sc. thermophilus* (2FM, 20FM) and *Lb. bulgaricus* (285N) presented the best acidification rates. In addition to their fast acid production, strains 20FM and 285N produced exopolysaccharides. Based on these characteristics, strains 2FM, 20FM and 285N were selected and used as pure or mixed cultures in the manufacture of fermented milk. In mixed cultures, the *Sc. thermophilus/Lb. bulgaricus* association was positive for all combination tested. The combination of strain 285N with strain 2FM or 20FM had a significant effect on acid production by *Lb. delbrueckii ssp. bulgaricus* strain (285N). These bacterial associations also affected the rheological properties of fermented milk samples. Strains 2FM, 20FM and 285N presented interesting biotechnological profiles and may influence the quality of fermented milk if they are used in association as starters in yoghurt manufacture.

Key words: Lactic acid bacteria, acidification, exopolysaccharides, selection

INTRODUCTION

Lactic Acid Bacteria (LAB) are industrially important organisms recognized for their fermentative ability as their heath and nutritional benefits (Schmidt *et al*., 1994). In some traditional fermented products, wild lactic acid bacteria assume spontaneous and uncontrolled lactic fermentation of raw milk, which is one of the oldest techniques known for the preservation of milk. Yoghurt is a well-known and popular fermented dairy product in developing countries. Yoghurt production implies the use of mixed cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* commonly used as starter (Zourari and Desmazeaud, 1991; Zourari *et al*., 1992). In this product these benefit microorganisms play several important functions: conversion of lactose to lactic acid-degradation of casein-formation of aroma compounds-viscosifying and water binding functions (Terence *et al*., 1984; Zourari and Desmazeaud, 1991; Hassan *et al*., 1996; El Soda, 1997; Laws and Marshall, 2001; Giraffa *et al*., 2004). Viscosifying and water binding functions of starter cultures are due to the extracellular materials produced by some culture bacteria. It was demonstrated that the ropiness produced by some bacteria is related to synthesis and excretion of exopolymers (Cerning, 1995; De vuyst and Degeest, 1999; Zambou *et al*., 2004). In order to make yoghurt processing attractive and the product affordable, quite a number of process manipulation have been adopted including selection of appropriate starter cultures, which increase

Corresponding Author: Zambou Ngoufack François, Department of Biochemistry, Faculty of Sciences, University of Dschang, Cameroon P.O. Box 67 Dschang-Cameroon Tel: 00(237)781 11 29 Fax: 00(237) 345 17 35

gel thickness and reduce syneresis. Due to the widespread nature of LAB, it is then interesting to examined strains from various origins in search of effective strains with specific technological properties in relation to yoghurt technology. It is therefore necessary to classify and select strains in view of their future application. For yoghurt related strains, technological characterization should take into account, combined properties such as acidification rate, flavoring and exopolysaccharides producing activities.

In the present investigation, thermophilic lactic acid bacteria strains isolated from traditional fermented milk were identified and some of their biotechnological relevant properties were examined in order to be selected as starters in the manufacture of yoghurt.

MATERIALS AND METHODS

Traditional fermented milk samples: The fermented milk used in this study was of the traditional Egyptian type made from raw cow's milk. Five different fermented milk samples were collected from local retailers in the region of Alexandria in Egypt. The local fermented milk portion (1 mL) was aseptically sampled, into 9 mL of sterile MRS broth and ST broth and homogenized.

Isolation of lactic acid bacteria strains: Five strains comprising four cocci and one rod were investigated in this study. The cocci strains were isolated on ST media at 42°C while rod strain was isolated on MRS under anaerobic conditions using the gas pak system (GENER box anae indicator, Biomerieux) at 42°C for 48 h. The purified rod cultures were grown on MRS broth (De Man *et al*., 1960 while cocci strains, were grown on ST (*Streptococcus thermophilus*) broth at 42°C (Ronalds *et al*., 1996). The purified strains were stored at -20°C in sterile reconstituted skim milk (12.5% W/V) supplemented with 15% glycerol.

Identification of lactic acid bacteria strains: Identification of strains at genus level was carried out using morphological, phenotypic and biochemical methods. The identification of the isolates was performed according to the criteria of Bergey's Manual of determinative bacteriology and using Sharpe (1979) criteria. All strains were tested for growth at 10°C for 10 days, 45°C for 48 h and CO_2 production from glucose. For cocci strains, growth on SF broth medium and in the presence of 6.5% NaCl were also considered (Harigon and Mac Cane, 1976; Holt *et al*., 1994).

Sugar fermentation profile of isolates: The carbohydrate fermentation profiles of purified isolates were determined using API 50 CH system (Biomerieux, Marcy l'Etoile France). Interpretations of these fermentation profiles were facilitated by systematically comparing all results obtained for the isolates studied with information from the computer-aided database API LAB Plus V3.2.2.

SDS-PAGE: In order to confirm identification of isolates, four cocci strains were subjected to SDS-PAGE of the whole-cell (Pot *et al*., 1994). Identification of the isolates was performed by comparison of their protein patterns to the fingerprints of reference strains of LAB in the database of the Laboratory of Microbial Biochemistry of the University of Alexandria. Along side the LAB strains reference bacterial protein extracts (*Psychrobacter immobilis*, LMG 1125) were included in the run in order to test the reproducibility of the electrophoretic system.

Assessment of technological performance of strains: Acidifying activity: Before use, *Sc. thermophilus* and *Lb. bulgaricus* cultures were propagated twice in ST broth and MRS broth, respectively and incubated at 42°C for 16 h. Seventy milliliter of broth media (MRS (rods) or ST (cocci)) were inoculated with 10% overnight, activated subcultures of LAB strains. The growth of cultures in broth media was monitored at 650 nm using spectrophotometer (Pharmacia LKB NOVASPEC II) for 6 h. At the early stationary growth phase, bacterial cells were harvested from the media by centrifugation of the culture (4000 g, 10 min, 4°C). The cell pellet was washed twice with 10 mL ringer solution and suspended in sterile reconstituted skim milk (12.5% W/V). This mixture was stored in ice at 4°C overnight. Sterile reconstituted skim milk (12.5% W/V) was inoculated with 2% of the cell pellet suspended in milk. The change in pH was determined using pH meter (Microcomputer pH-vision, model 05669-20) during six hours incubation at 42°C. The variation of pH (ΔpH) was calculated as the difference between the value immediately after inoculation and values at different time. The cultures were considered fast, when a ΔpH of 0.4 unit was achieved after 3 h.

Exopolysaccharides (EPS) production: The screening of EPS production was performed on the strains showing weak pellet formation after centrifugation. The screening of EPS consisted to reveal the presence of diffuse capsules surrounding bacterial cells as described by Prescott *et al*. (1996). For slime production, strains were streaked on the suitable media (MRS or ST) and incubated at 42°C for 24 h. Ropiness of colonies on agar surfaces was tested with a loop to observe the formation of slime.

Technological properties of fermented milk manufactured with single or mixed selected strains

Cultures and growth conditions: The strains of *Sc. thermophilus* (2FM, 20FM), *Lb. delbrueckii* ssp.

bulgaricus (285N) used in the preparation of fermented milk were selected based on their acidifying and exopolysaccharides (EPS) production abilities. The strains stored at -20°C were reactivated in ST broth (2FM, 20FM) and MRS broth (285N) and incubated overnight at 42°C. Inocula were prepared by incubating cultures in 12,5% reconstituted sterile skim milk for 16 h at 42°C and the freshly curdled cultures were used for the preparation of fermented milk.

In addition, one commercially available starter culture (MY900) was used as control in the manufacture of fermented milk. The MY900 culture consisted of *Lactobacillus delbrueckii* spp. *bulgaricus* and *Streptococcus thermophilus* was obtained from Rhodia Food (Saint Romain, France). It was then inoculated and propagated in sterile reconstituted skim milk.

Preparation of cow's fermented milk samples: Fresh cow's milk was obtained from the University Agricultural farm of Alexandria and pasteurized at 74°C for 15 sec. Dry skim milk powder (1% w/v) was added to pasteurized milk and stirred. The mixture was heated at 80°C and cooled to 45°C before inoculation. To prepare yoghurt from different cultures, milk was inoculated either with 0.3, 0.6 and 1.2% (v/v) each of *Streptococcus thermophilus* cultures (2FM or 20FM) or *Lactobacillus delbrueckii* ssp. *bulgaricus* (285N). Two mixed yoghurt cultures containing 1.2% (v/v) of *Sc. thermophilus* (2FM)/*Lb. delbrueckii.* bulgaricus (285N) and *Sc thermophilus* (20FM)/*Lb. delbrueckii.* bulgaricus (285N). The control was prepared using commercial starter MY900 at the levels of 0.3 and 0.5%.

The inoculated milk was divided aseptically into 100ml sterile plastic cups, sealed and incubated at 45°C for 5 to 7h until coagulation. After coagulation, the pots were stored at 4-5°C in the cold refrigerator. This fermentation procedure was repeated three times on separate days.

pH measurement: Changes in pH value were monitored continuously during the manufacture of fermented milk using the pH meter (Microcomputer pH-vision, model 05669-20).

Texture analysis: The texture properties of fermented milk samples were evaluated using texture analyzer (CNS/FARNELL LFRA, Borehamwoad, Hertfordshimre, England). Samples were removed from the refrigerator (4°C) and centrally positioned beneath the probe within the container in which they were packed. Tests were conducted at ambient temperature (approximately 20°C). The probe used was TA4, (ϕ = 38.1 mm) perpex cylinder. Data were collected on computer and the following texture parameters describing defined by Bourne (1978) were calculated from LFRA texture analyzer and computer interface: hardness (maximum force that is exerted on the sample), consistency (Total Positive Area (TPA)), adhesiveness (Total Negative Area (TNA)) and Adhesive Force)

Sensory evaluation of fermented milk: The sensory evaluation to evaluate the difference among the fermented milk of the various runs and the overall acceptability of each carried out by a panel of ten judges familiar with the quality attributes of fermented milk. The sensory evaluation was carried out after overnight storage on sample contained in 100 mL sterile plastic cups. The ratings of flavor, texture, appearance and color were based on hedonic scale ranging from 1 to 9. 9 representing like extremely and 1 dislike extremely. All measurements were carried out in triplicates samples. The results obtained were subjected to analysis of variance using super ANOVA V1.11 software to determine if or not any significant differences existed between the different types of fermented milk. Mean values were compared using student Newman Keuls test at $p<0.05$.

RESULTS

Identification of strains: As result of physiological and biochemical tests, four isolates (2FM, 8FM, 13FM, 20FM) were identified as *Streptococcus thermophilus.* The rod isolates (285N) was identified as *Lactobacillus delbrueckii.* bulgaricus (Table 1).

As shown in Fig. 1, SDS-PAGE results confirmed the phenotypic identification of all streptococci isolates. In this Fig. 1 lanes 3, 4, 5 and 6 were loaded with the protein extract of isolates 2, 8, 13 and 20FM, respectively. When compared to the profile pattern of reference strain (lanes 2 and 8) it was demonstrated that these reference strains of *Sc. thermophilus* were found to be the closest neighbors of our isolates. The reproducibility of the SDS-PAGE technique in this study was estimated by including duplicate runs of protein extract of *Psychrobacter immobilis* (LMG 1125) (lanes 1 and 7).

Assessment of technological performance and selection of strains: Results of acid-producing activity of the strains are shown in Fig. 2. No significant difference in acid-producing ability was found between strains, even within the same species. After 6 h incubation, decrease in pH among strains range from 1.2 to 1.8. All these strains were described as highly acidifying since the variation of pH of 0.4 units were achieved after within 4 h.

As shown in Fig. 3 strains 20FM and 285N produced exopolysacharide. Figure 3A and 3B presented strains

Table 1: Physiological and biochemical characteristics of streptococci and Lactobacilli strains

	Cocci strains				Lactobacilli strain
Characteristics	2FM	8FM	13FM	20FM	285N
Growth at					
10 °C	-	-	-	-	-
45 °C	+	+	+	+	+
Growth in					
6.5% NaCl	-	-	-	-	ND
SF Media	-	-	-	-	ND
CO_2 from glucose	-	-	-	-	-
Esculin hydrolysis	-	-	-	-	-
Production of acid from:					
D-Glucose	+	-	+	+	+
D-Fructose	-	-	-	+	+
Lactose	+	+	+	+	+
Sucrose	+	+	+	+	+
Identification	*Sc.thermophilus* (99,2 %)	*Sc. thermophilus* (92,8 %)	*Sc. thermophilus* (97 %)	*Sc. thermophilus* (98,2 %)	*Lb delbrueki* ssp. *bulgaricus* (99%)

All The 49 sugars of API 50CH gallery were tested for the identification of the bacilli and cocci strains. Only the sugars producing acid by at least one strain are reported in this table,+: Positive reaction;-: Negative reaction; ND: Not determined

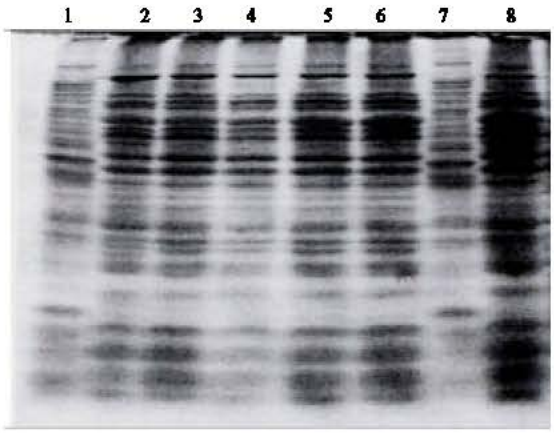

Fig. 1: Protein patterns after Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) of the whole cell free extracts of *Streptococcus thermophilus* strains. At the top of the gel, the numbers 1; 2, 3, 4, 5, 6, 7, 8 indicate the protein profile respectively for *Psychrobacter immobilis* (LMG1125), *Sc thermophilus* (5842), 2FM, 8FM, 13FM, 20FM and *Psychrobacter immobilis* (LMG1125) and *Sc. thermophilus* (3534). The reference strains used in this study was obtained in the database of the Laboratory of Microbial Biochemistry, University of Alexandria-Egypt

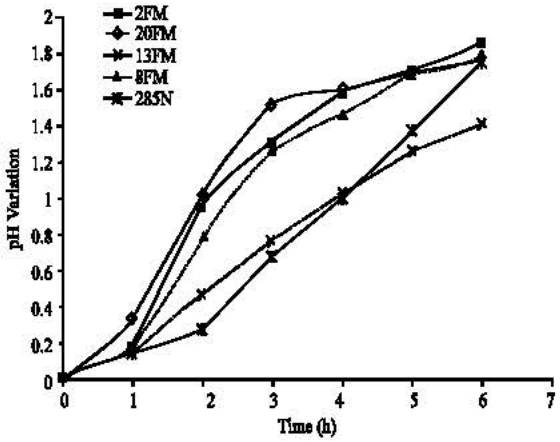

Fig. 2: Acidification profiles of *Lactobacillus delbrueckii*. bulgaricus (285N) and *Streptococcus thermophilus* (2FM, 8FM, 13FM, 20FM) strains during growth in milk at the constant temperature of 42°C

20FM, 285N, respectively with exopolysacharide in form of capsule surrounding the cells. These strains were poorly slime-forming lactic acid bacteria.

Based on these characteristics, *Sc. thermophilus* strains (2FM, 20FM) and *Lb. delbrueckii*. bulgaricus 285N were selected for further technological characterization in pure and mixed cultures.

Manufacture of fermented milks with pure and mixed cultures: The results concerning the effects of culture composition and inoculation rate on acidifying characteristics are summarized in Table 2. These data showed that kinetic behavior varied according to strain and as well as the inoculation rate. There was a high degree of heterogeneity in the time required to obtained pH = 4.8 due to lactic acid bacteria strain and the inoculation rate within the same strain. The time needed to bring the pH of standardized cow's milk from 6.6 to 4.8 was used as criterion to choose the best inoculation rate. For strains 2 and 20FM, the pH less than 4.8 was obtained within 6 hours and no significant variation between the inoculation rates was observed (Table 2). The slowest

Table 2: Effect of culture composition and inoculation rates on acidification characteristics of pures and mixed cultures grown on milk at 45°C until pH 4.7

Culture composition	Inoculation rate (%) (V/V)	pH during incubation time 0h	2h	4h	6h	7h	pH After 24h storage at 4°C	Time to achieve pH 4.8 (h)
2FM	0.3	6.47	6.26	5.20	4.80	-	4.67±0.05	6±0.5
	0.6	6.50	6.24	5.21	4.75	-	4.67±0.10	6±0.5
	1.2	6.44	6.18	4.88	4.65	-	4.63±0.06	5.5±0.5
20FM	0.3	6.46	6.27	5.20	4.80	-	4.67±0.06	6±0.5
	0.6	6.47	6.29	5.41	4.72	-	4.70±0.03	5.5±0.5
	1.2	6.40	6.26	5.27	4.70	-	4.64±0.08	5.5±0.5
285N	0.3	6.45	6.39	6.10	5.29	4.76	4.48±0.12	5.5±0.5
	0.6	6.45	6.37	6.05	5.15	4.78	4.70±0.05	6±0.5
	1.2	6.41	6.33	5.73	5.17	4.5	4.35±0.10	5±0.5
Mix1 2FM+285N(1:1)	1.2	6.36	6.12	5.02	4.71	-	4.68±0.05	5.5±0.5
Mix2 2FM+285N(1:1)	1.2	6.36	6.20	5.16	4.60	-	4.56±0.05	5±0.5
MY900	0.3	6.55	6.20	4.90	4.71	-	4.62±0.08	5±0.5
	0.5	6.50	6.17	4.88	4.68	-	4.60±0.09	5±0.5

2FM: Non-encapsulated strain of *Streptococcus thermophilus*, 20FM: Encapsulated strain of *Streptococcus thermophilus*, 285N: Encapsulated strain of *Lactobacillus delbrueckii* ssp. bulgaricus, MY900: commercial starter consisted of *Streptococcus thermophilus and Lactobacillus delbrueckii* ssp. bulgaricus

Table 3: Textural parameters of fermented milk made with single strain and different combination of streptococci and lactobacilli

Treatments	pH	Hardness of curd (g)	Consistency of curd (TPA) (g/s)	Rigidity of curd (modulus)	Adhesiveness of curd (AF) (g)	Resistance of curd to flow (TNA) (g/s)
Single strain fermented milk						
2FM (1.2%)	4.65	16.5±2.8^{a}	162.5±24.6^{a}	1.01±0.23^{a}	-0.50±0.28	-0.50±0.28^{a}
20FM (1.2%)	4.64	20.5±4.0^{a}	192.2±20.4^{a}	0.75±0.28^{a}	-0.75±0.28	-0.60±0.11^{a}
285N (1.2%)	4.35	18.0±2.3^{a}	182.4±39.7^{a}	0.85±0.17^{a}	-0.50±0.20	-0.25±0.14^{a}
My900 (0.3%)	4.65	29.0±9.2^{a}	195.0±27.7^{b}	1.55±0.40^{c}	-2.50±0.57	-1.25±0.28^{d}
Mixed strain fermented milk						
Mix1						
2FM+285N (1:1)	4.68	42.83±2.95	238.83±3.85^{ab}	2.43±0.28	-1.5±1	-2.17±1.73a
Mix2						
20FM+285N (1:1)	4.56	51.0±5.65	269.97±16.17^{a}	3.06±0.69	-3.16±117	-3.58±1.36
My900 (0.5%)	4.6	51.83±8.26	275.0±30^{b}	2.83±0,28	-2.83±0.23	-3.15±0.76

ab: For single fermented milk, means in each row bearing a common superscript differed significantly ($p<0.05$), 2FM: Non-encapsulated strain of *Streptococcus thermophilus*, 20FM: Encapsulated strain of *Streptococcus thermophilus*, 285N: Encapsulated strain of *Lactobacillus delbrueckii* ssp. bulgaricus, MY900: commercial starter consisted of *Streptococcus thermophilus and Lactobacillus delbrueckii* ssp. bulgaricus

A B

Fig. 3: Selected lactic acid bacteria stains negatively stained with India ink to show the diffuse capsule surrounding the cells. A) *Streptococcus thermophilus* 20FM negatively stained with India ink to show the diffuse capsule surrounding the cocci cells in chains. B- *Lactobacillus delbrueckii*. bulgaricus 285N) negatively stained with India ink to show the diffuse capsule surrounding the long bacilli cells

acidification rate was observed with strain 285N where the pH = 4.8 was achieved after 6.5±0.5 h incubation of milk for various inoculation rates.

In mixed cultured fermented milk, the inoculation rate was judiciously chose to obtain a pH = 4.8 for each sample within 6 h of incubation (Table 2).

Table 4: Sensory evaluation scores of fermented milk samples manufactured with mixed cultures of selected strains

Treatment	Flavor	Texture	Appearance and color	General comments on the overall acceptability
Mix1 2FM+285N (1:1)	8.61±0.03	8.50±0.31	8.80±0.42	Odor is pleasant; taste is slightly soft; curd is slightly acid. No texture defects are observed
Mix2 20FM+285N (1:1)	8.75±0.30	8.66±0.26	8.88±0.32	Pleasant taste and odor; very low level of syneresis No texture defects are observed observed
My900	8.52±0.26	8.45±0.37	8.92±0.64	Pleasant taste and odor; very low level of syneresis. No texture defects are observed

2FM: Non-encapsulated strain of *Streptococcus thermophilus*, 20FM: Encapsulated strain of *Streptococcus thermophilus*, 285N: Encapsulated strain of *Lactobacillus delbrueckii*. bulgaricus, MY900: commercial starter consisted of *Streptococcus thermophilus and Lactobacillus delbrueckii*. bulgaricus, 9 = Like very much; 8 = like much; 7 = Like moderately; 6 = Like little; 5 = Neither like nor dislike; 4 = Dislike little; 3 = Dislike moderately 2 = dislike much; 1 = Dislike very much

Table 3 presents the textural profile parameters of fermented milk calculated from LFRA texture analyzer. For single strain fermented milk manufactured by inoculating milk with 1.2% culture, a significant difference was observed for all textural parameters ($p<0.05$) except adhesiveness. The fermented milk manufactured with strain 20FM presented the greatest textural parameters. According to these parameters this sample is thicker with a more gel-like texture when compared to the order samples. The high initial force and the total area under both positive and negative regions of the profile curves justify these observations. In fact, the positive and negative areas beneath the curve measure the resistance encountered by the plunger withdrawal, which indicates the flowing characteristic of the fermented milk influenced by its consistency or viscosity. Thus, all the fermented milk samples manufactured with mixed culture were comparable to the one manufacture with starter MY900 (0.5%).

As observed in this Table 4, no specific defects were observed on different samples. However, compared to Mix2, the acidity of sample manufactured with Mix1 tended to remain higher, inducing an acid flavour, which was not liked much by the panellists. The best rating was fermented milk manufactured with Mix2, which is the association in equal ration of *Sc. thermophilus strain* (20FM) and *Lb. delbueckii*. bulgaricus strain (285N). A curd with pleasant flavour and taste, with no syneresis was obtained when Mix2 was used as culture for manufacture of fermented milk.

DISCUSSION

These results were in accordance to the physiological and biochemical characteristics described by Kandler and Weiss (1986). Lactose and sucrose were fermented by all isolates while the fermentation of glucose, galactose and fructose was strain dependent (Table 1). It has been proved that in *Streptococcus thermophilus* strain metabolism, lactose and sucrose are fermented more readily than their component monosacharides. Also, *Sc. thermophilus* and *Lb. delbrueckii*. bulgaricus are homofermentative and during their metabolism, lactose is taken up as free sugar and split with β-galactosidase (Thomas, 1976; Zourari, *et al*., 1992; Tinson *et al*., 1982; Thomas and Crow, 1983). Not all our *Streptococcus thermophilus* strains produced acid from galactose (Table 1). These strains follow an apparently obligatory homolactic fermentation, which may results from possession of lactate deshydrogenase activity (Zourari and Desmazeaud, 1991; Terence *et al*., 1984). Microbial identification based on biochemical and carbohydrate tests have been used with varying degrees of success (Bile *et al*., 1992). The API systems sometimes led to the misidentification of strain or no identification. In fact, for most of the strains, clear assignment to a particular species or subspecies is not possible because ambiguous results are obtained for sugar fermentation profile. These discrepancies prompted the utilization of SDS-PAGE analysis of whole cell proteins with proteins extracted from reference strains. A bacterial strain always produces the same set of proteins if grown under standardized conditions. The electrophoregrams produced under well-defined conditions can be considered as a sort of fingerprint of the bacterial strains from which they are obtained (Pot *et al*., 1994). Also, the rationale for the application of electrophoresis of cellular proteins in microbial systematics is that bacterial strains with 90 to 100% DNA relatedness display only minor differences in their protein fingerprints (Fig. 1). Strains with at least 70% DNA homology tend to have similarities in their protein electrophoregrams (Kersters, 1985).

Strain selection for industrial purposes must consider some specific technological activities such as acidification, flavoring or exopolysaccharide production. Streptopcci play a vital role in many commercial milk fermentations. Selected strains are deliberately added as starters and their primary role is to ferment lactose to lactic acid (Gisele *et al*., 2006). *Sc. thermophilus* is used in commercial milk fermentations in which the temperatures reached during product manufacture are relatively high (up to 55°C). This organism is normally used as part of mixed starter culture, the other component being a *Lb. delbrueckii*. bulgaricus for yoghurt. Data obtained from pure cultures do not consider phenomena related to interactions between microorganisms typical of mixed

cultures. Yoghurt is a simple ecosystem whose successful manufacture relies on interactions between two thermophilic lactic acid bacteria, *Sc. thermophilus* and *Lb. delbrueckii. bulgaricus*. This bacterial association had significant effect on acid production by strain of *Lb.* delbrueckii. bulgaricus, possibly because of a production of formic acid by the *S. thermophilus* strain (Zourari *et al*., 1992; Courtin and Rul, 2004). In previous study, Veringa *et al.* (1968) showed that all strains of *Sc. thermophilus* produced some formate, which is considered important in stimulating the growth of *Lb. delbrueckii. bulgaricus* in yoghurt cultures.

Some difference observed in sensory and rheological qualities of the fermented milk may have resulted from the ropy nature of culture used, which is linked to the production of exopolysaccharides (Figure 3A and 3B). The exopolysaccharides producing LAB strains are widely used in the dairy industry (Hassan *et al*., 1996; Zambou *et al*., 2004) in order to enhance the rheological quality and to prohibit syneresis in fermented milk. Hassan *et al.* (1996) proved that yoghurt made with encapsulated cultures presented more stable structure for shear, higher apparent viscosity and lower yield stress. Results from previous studies indicated that, the use of strains producing exopolysaccharides strengthened the protein network, resulting in a firmer fermented milk with better water binding properties (Hassan *et al*., 1996). Also it has been demonstrated that lactic acid bacteria producing exopolysaccharides are often used to increase viscosity of stirred fermented milks, such as yoghurt and reduce susceptibility to syneresis (Cerning, 1995; Hassan *et al*., 1996; De Vuyst and Degeest, 1999; Laws and Marshall, 2001; Patricia *et al*., 2002).

CONCLUSIONS

In this study, we were able to isolate, identify and select some interesting strains of LAB based on acidifying, flavouring potentials as well as the capability to produce exopolysaccharides. The selected strains were tested in the manufacture of fermented milk as single culture or mixed cultures. The associations of strain *Sc. thermophilus* (2FM and 20FM) with *Lb. delbueckii* bulgaricus (285N) in the rate of 1.2% (v/v) are interesting in the manufacture of fermented milk.

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