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Identification of lactic acid bacteria isolated from traditional Sudanese fermented camel's milk (*Gariss*)

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From 12 samples of *gariss*, a Sudanese traditionally fermented camel©s milk, 24 lactic acid bacteria were isolated and phenotypically characterized by biochemical tests and their ability to ferment different carbohydrates. Analysis of the observed data resulted in classifying the isolates into 11 groups, each group containing one or more isolates, 2 major groups were identified, the larger one composed of 7 isolates recognized as *Lactobacillus plantarum* (66.6% of the *gariss* isolates) and the second was composed of 6 isolates recognized as *Lactococcus raffinolactis* (33.3% of the *gariss* isolates). The remained minor groups were classified as *Lactobacillus animalis*, *Lactobacillus brevis*, *Lactobacillus divergens*, *Lactobacillus rhamnosus*, *Lactobacillus gasseri*, *Lactobacillus paracasei*, *Lactobacillus fermentum*, *Lactococcus alimentarium*, *Lactobacillus sp.* The genetic relationships of the isolates were determined by the random amplified polymorphic DNA (RAPD)-polymerase chain reaction (PCR), the results demonstrated a distinction comparative genetic clusters and their pattern was greatly related to the clustering obtained with the API 38 CHL group identification.

Key words: *Gariss*, camel's milk, lactic acid bacteria, RAPD, API CHL.

INTRODUCTION

Fermented foods and beverages constitute a major portion of people's diets in Africa (Oyewole, 1997). Lactic acid bacteria (LAB) are one of the microorganisms that dominate fermented food (Guasch-Jané et al., 2005; Robert, 2008). Today, LAB is of essential importance for their role in most industries of fermented foods as starter cultures. Various metabolic and enzymatic activities of LAB lead to production of volatile substances, which contribute to flavor, aroma and texture developments (Kleerebezemab et al., 2000). Certain LAB strain characterized by their ability to transform lactose and improves the digestibility of fermented dairy products (Weinberg et al., 2007) as well as their preservation (Abdelbasset and Djamila, 2008). They also employed for improvement of the taste, texture and viscosity in the manufacture of dairy products (Soukoulis et al., 2007). The ability of LAB to produce probiotics (Temmerman et al., 2002) and stimulation of the immune system (Kalliomäki et al., 2001) render this group of microorganisms essential importance dairy industry.

In Sudan, camel's milk and the fermented camel's milk (*gariss*) are widely consumed by the pastoralist communities living in the arid and semi-arid regions of the country. Camel's milk has more free amino acids and peptides than the bovine milk (Natasa et al., 2008). Moreover, Omer et al. (2007) stated that the nonprotein-bound amino acids in camel's milk are easily digested by microorganisms and therefore, camel's milk has a higher metabolic activity when used in a starter culture preparation. The beneficial microbiota of camel's milk represented by LAB could be a potential source of biological materials to be used in dairy technology. Therefore they should be more exploited to obtain new functional ingredients and natural food components and more efforts should be exerted towards their functional properties and genetic analysis.

This study was undertaken to isolate and identify the micro flora of Sudanese traditional fermented camel's milk (*gariss*) as a basis for possible development in technological knowledge related to LAB. This paper reports

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the appropriate identification of lactobacilli isolates using RAPD profiles and the discrepancy with API CHL system patterns.

MATERIALS AND METHODS

Samples collection

A total of 12 samples of traditionally prepared *gariss* were collected from different geographical regions of camel raising house hold in Sudan (Dongola, Kasala, El Gadarif, El Obied and Omdurman). Samples (250 ml) were collected in sterile screw-cap bottles and kept under low temperature using an ice-cooled box to be brought to the laboratory where they kept in a refrigerator (around 4°C) till the time of use.

Isolation of lactic acid bacteria

To favors better growth of bacteria, samples of *gariss* (1 ml) were homogenized with 9 ml sterile peptone physiological saline solution (5 g peptone, 8.5 g NaCl, 1000 ml distilled water, pH 7.0) . The homogenate was serial diluted and the appropriate dilutions were surface plated on M17 (Terzaghi and Sandine, 1975) and MRS agar (De Man Rogosa and Sharp, 1960). Plates were then incubated at 30°C for 3 days under anaerobic conditions using anaerobic jars.

Individual isolates from countable M17 and MRS agar plates were randomly- picked, representatives from all morphologically distinct colonies and were sub-cultured and purified 5 times on the appropriate agar medium. Pure strains, as judged by microscopic observations for homogeneity of cellular morphology and were maintained in MRS slants at -20°C. Bacterial isolates were further tested for gram reaction, catalase production oxidase activity and cell morphology according to the methods described by Kebede et al. (2007). 24 isolates of the gram-positive, catalase- negative, very short to very long rods, occurring singly, in pairs or in chains and grown on under anaerobic incubation, were randomly selected as presumptive LAB. The selected isolates were designated as SUD numbers.

API CHL assay

The carbohydrate fermentation pattern of the LAB isolates was characterized using the API 38 CHL system (KB009 Hi Carbohydrate Kit-Himedia) following the manufacturer's suggested protocol. All isolates were overnight cultured in MRS broth and 50 µl from each isolate were added individually to the substrate in the wells of the API 38 CHL strips. The inoculated strips were incubated at 30°C and then monitored for changes in the color of the medium after 1, 2 and 7 days. Change in color was represented by a positive sign (+), while a negative sign (-) represented no change. Discrimination between isolates was based on the principle of a pattern matching manual as described by the manufacturer.

Genomic DNA isolation from lactic acid bacteria

A single colony from each isolate was inoculated into 10 ml of the appropriate medium broth (kept in a 15 ml Falcon tubes) and incubated for overnight at 37°C. The cultivated culture was harvested by centrifugation at 5,000 rpm for 5 min and genomic DNA isolated by a modified genomic DNA isolation protocol (Sambrook and Russell, 2001). The collected pellet was re-suspended in 2.5 ml of TE buffer (1M Tris-HCl, 0.5 mM EDTA pH 8.0) containing 1% (w/v) SDS and washed twice with the same buffer. After washing the pellet was re-suspended into 500 µl of pre-warmed (65°C) lyses solution [(1.0 M Tris-HCl (pH 8), 0.5M EDTA, 5.0 M NaCl, 1.0% (v/v) -mercaptoethanol, 20 µl SDS (10% w/v) and 5 µl proteinase K (10 mg/ml)]. The cell suspension was incubated in a water bath at 65°C for 1 h with gentle shaking and then left to cool at room temperature for 10 min. The solution was emulsified gently by equal volume of TE buffer saturated phenol-chloroform (1:1), centrifuged at 5000 rpm for 5 min and the aqueous phase was transferred to a new tube. The aqueous solu-tion was then washed twice by an equal volume of chloroform: isoamylalcohol, 24:1 and centrifugation at 5000 rpm for 5 min. After centrifuging the aqueous phase removed to Eppendorf tube (400 µl/ tube) and RNA was removed by addition of 0.5 µl of RNAse and left at room temperature 30 min. Nucleic acids were precipitated by adding 200% volume of ice cooled absolute ethanol in presence of 10% of 5 M ammonium acetate solution and mixed gently to preci-pitate the DNA. The mixture was then centrifuged at 14000 rpm for 10 min, the supernatant was discarded and the formed pellet was washed twice by 70% ethanol. The remained ethanol was removed by leaving the pellet to dry at room temperature. The pellet was dissolved in TE buffer [1M Tris-HCl 0.5M EDTA (pH 8.0)]. The integrity and purity of the extracted DNA was observed according to Sambrook and Russell (2001).

RAPD analysis

The PCR procedure was carried out as described by Williams et al. (1990). A total of 20 random decamer oligonucleotide primers were screened for standardization of the amplification conditions only 8 primers were found to be suitable for amplification of the extracted total cellular DNAs. The 8 primers were selected due to the consistent reproducibility of their amplification products. Primers used in this study were P-1, P- 2, P-3, P - 5, P-6, P-7 and P-9 (obtained from 1st BASE Pte Ltd, Singapore) in addition to OPA -2 (obtained from operon technologies Inc., USA.

The PCR reaction mixtures were prepared in 25 µl volumes containing 2.5 µl of 10 X Taq buffer, 1.5 µl $MgCl_2$ (50 mM), 2.5 µl dNTPs (2 mM/µl), 2 µl random primer (10 pmol/ µl), 0.5 µl Taq. DNA polymerase (5 U/µl) and 1 µl of the extracted DNA (10 ng). The mixture was made up to 25 µl by addition of sterilized distilled water.

RAPD/PCR reactions were initiated using an Applied Biometra thermalcycler programmed to repeat the thermal profile. Setting of the PCR program was based on 3 steps. Step one, was an initial denaturation step at 94°C for 5 min. Step 2, was run for 40 cycles, each starting with denaturation at 94°C for 1 min, followed by annealing 36°C for 1 min and ended by extension at 72°C for 1 min. Step 3, was a final extension cycle that performed at 72°C for 7 min. The PCR machine was adjusted to hold the product at 4°C.

The amplification products were analyzed by electrophoresis. The DNA fragments and the standard marker (Hind III digested DNA) were then separated in 1.5% ethidium bromide-stained agarose gels. The separated fragments and their patterns were then visualized and photographed with gel documentation system.

Data analysis

All visible fragments by primers were scored under the heading of the total scored fragments. For each individual DNA fingerprints, only intense unambiguous RAPD bands were manually scored for their presence (1) or absence (0). Data were compiled for each

Table 1. Primers used, the sequences, number of produced bands, number of polymorphic bands and polymorphism.

Name of primer	Sequence of primer	No. of bands	No. of polymorphic bands	Polymorphis m (%)
P- 1	5-CAA ACG TCG G-3	5	5	100
P- 2	5-GGG TGT GGA G-3	5	3.75	75
P- 3	5-GGA TGC CAC T-3	8	8	100
P- 5	5-AGG GGT CTT G-3	7	7	100
P- 6	5-CAG CAC CCA C-3	3	3	100
P- 7	5-TGC GGC TTA C-3	5	3.75	75
P- 9	5-GTA GAC GAG C-3	6	3.6	60
OPA-25-GTT GCG ATC C -3		6	2.4	40
Total		45	36.5	650
Average		5.6	4.6	81.3

accession in a data matrix and were analyzed using the mathematical model for studying genetic variations (Nei and Li, 1979). Coefficient similarity trees were produced by clustering the similarity data with the un-weighted pair group method using statistical software package STATISTCA- SPSS (Stat Soft Inc). The similarity coefficient (Rohlf, 1993) was used to construct a dendrogram by the un-weighted pair group method with arithmetic averages (UPGMA).

RESULTS AND DISCUSSION

From the collected samples a total of 24 isolates randomly picked, after the original characterization. The selected isolate gave blue-purple color with gram staining, hence they are all gram positive bacteria. 16 of them were found to be bacilli with long and rounded ends mostly appeared as chains of 3 - 4 cells, pairs or single cells and these could tentatively determined as derivatives of the genus *Lactobacillus*. The rest of the selected isolates were cocci with spherical or ovoid morphology and appeared mostly as pairs or forming chains therefore they tentatively referred to *lactococcus*.

API CHL assay

In this study, a total of 24 LAB isolates were screened for their performance regarding growth characteristics in 35 carbon sources as supplied by the manufacturer. This characterization could be used as selection criteria for novel starter cultures in milk fermentations. The reproducibility of the fermentation tests was 100% and the results of carbohydrates fermentation by the tested 24 isolates of LAB are shown in Table 2. All isolates fermented lactose, fructose, galactose, trehalose, mellobiose, manose, xylitol and sorbose. Only 2 isolates could utilize esculin (SUD 13 and SUD 22). It was also observed that the isolates SUD1 and SUD 21 were not utilize citrate. Yet only the isolates SUD13, SUD 21 and SUD 23 showed positive result with malonate. None of the isolates could utilize ONGP. In exception of the mentioned results there was variation in the utilization of the carbohydrates sources of the API CHL system.

According to the analysis of the reading of the API CHL system the, isolates were resulted in 11 major groups containing one or more strains (Table 2). Group 1 contained one isolate (SUD 21) and it was closed to *Lactobacillus animalis* . Group 2 included 2 isolates (SUD 14 and SUD 22) and they were referred to *Lactobacillus brevis.* Group 3 formed from the isolate SUD23 which closed to *Lactobacillus divergens*. Group 4 is the largest group which composed of 7 isolates (SUD 5, SUD 7, SUD 11, SUD 12, SUD 15, SUD 19 and SUD 24) and all of them identified as *Lactobacillus plantarum*. Each of group 5 and 6 were composed of single isolate (SUD 8 and SUD 9) and they were closed to*, Lactobacillus rhamnosus* and *Lactobacillus gasseri* respectively. Group 7 was composed from 2 isolates (SUD 1 and SUD 3) which were closed to *Lactobacillus paracasei.* In group 8 stand the isolate SUD 16 and it was identified as *Lactobacillus fermentum*. Group 9 formed the second largest group that contained the isolates SUD 2, SUD 6, SUD 10, SUD 17, SUD 18, and SUD 20 and they were referred to the *Lactococcus raffinolactis.* Group 10 was formed from the isolate SUD 13 which was found to be closed to *Lactococcus alimentarium*. The last group (11) was not clearly identified but it could be referred to *Lactobacillus sp*. (Table 3 shows the suggested species of the LAB isolates and their percentage ratios).

Molecular characterization of the isolates

The results of strain typing of the LAB isolates by RAPD

Table 2. Carbohydrates fermentation by bacteria isolated lactic acid from traditional Sudanese fermented camel's milk (gariss) using API CHL identification systems.

Carbon sources	SUD isolates																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
Xylose	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+
Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Dextrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
Galactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Raffinose	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
Trehalose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Melibiose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	-	+
L-aeabinose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
Mannose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Inulin	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
Sodium gluconate	+	+	+	+	+	+	+	+			+	+	+	+	-	+	+	+	+	+	+	+	+	+
Glycerol	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
Salicin	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
Glucosamine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
Dulcitol	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+
Inositol	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
Sorbitol	+	+	+	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+
Mannitol	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
Adonitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-methyl-D-glucoside	+	+	+	+	+	+	+	+	+			+	+	+	+		+	+	+	+	+		+	+
Ribose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Rhamnose	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	-	+
Cellobiose	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+
Melezitose	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
-methyl-D-mannoside	+	+	+	+	+	+	+	+			-	+	+	+	-	+	+	+	+	+	-	+	+	+
Xylitol	+	+	+	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
ONPG	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Esculin	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-
D-Arabinose	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+
Citrate	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Malonate	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	+	-	+	-
Sorbose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Readings were done under anaerobic conditions after 24 h at 37°C. Key: + = Positive reaction, - = Negative reaction.

fingerprinting are shown in (Figures 1). Most of the RAPD patterns were distinct, with variations in the number of bands, fragment size and intensity. The patterns were highly reproducible, with variations only in relative band intensities. RAPD typing was capable of producing discriminating DNA fingerprints of the LAB isolates and indicated that there were genetic differences among them.

The obtained results indicated that the 8 primers showing at least 1 consistent polymorphic band. The selected primers generated distinctive RAPD fragments and

Table 3. Grouping of the lactic acid bacteria isolated from the Sudanese traditionally fermented camel's milk (*gariss*) based on API 38 CHL identification systems.

Group	Isolate	Suggested spp.	Ratio (%)
1	SUD 21	*L. animalis*	4.17
2	SUD 14 SUD 22	*L. brevis*	8.33
3	SUD 23	*L. divergens*	4.17
4	SUD 5 SUD 7 SUD 11 SUD 12 SUD 15 SUD 24 SUD 19	*L. plantarum*	29.17
5	SUD 8	*L. rhamnosus*	4.17
6	SUD 9	*L. gasseri*	4.17
7	SUD 1 SUD 3	*L. paracasei*	8.33
8	SUD 16	*L. fermentum*	4.17
9	SUD 2 SUD 6 SUD 10 SUD 17 SUD 18 SUD 20	*L. raffinolactis*	25.00
10	SUD 3	*L. alimentarium*	4.17
	SUD 4	*Lactobacillus spp.*	4.17

the fragment sizes were ranged between 250 and 3000 bp. The number of bands varied between 3 and 8. Some isolates exhibited significant similarity and common bands (Figure 1).

A total of 45 amplified fragments were distinguished across the selected primers and the statistical analysis showed 36.5 polymorphic bands among the 24 LAB isolates with an average of 4.6 polymorphic bands per primer. The maximum numbers of fragment bands were produced by the primer P-3 (8) with 100% polymorphism while the minimum numbers of fragments were produced by the primer P-6 (3) with 100% polymorphism. Pattern of RAPD fragments produced by the 8 mer-primers (P-1, P-2, P-3, P-5, P-6, P-7, P-9 and OPA-2) are shown in Table 1.

The pair- wise mean genetic distance value ranged from 2.0 to 44% (Table 1). The greater percentage of variation is (44%) was observed between isolates SUD 19, SUD 12 and SUD 24 (bacilli) when respectively compared with the isolates SUD 10, SUD 18 and the isolate SUD 20 (cocci). These were followed by 2 high values (39 and 38%) and both of them between isolates from 2 different LAB (bacillus and coccus). The dissimilarity within the same groups are small in general and most likely to be less in the bacillus rather than within the coccus such as the dissimilarity between SUD 8 and SUD 9 (2.0%).

According to the dendrogram (Figure 2) and cluster analysis, we found 4 major groups and within these group the isolates were clustered to form 2 main groups, one was the lactobacilli and the other was lactococci. Despite the location from where the sample was collected it was very obvious that there is a link between some individuals of groups. The apparent different in the clustering of the individuals confirmed the clustering according to the API CHL systems.

In this study, the isolates would not be positively identified solely by means of the phenotypic microscopic observation of cellular morphology but must be associated with other methods of phenotypic identification in particular API CHL systems. However; phenotypic characterization based on sugar fermentation pattern may not always provide sufficient basis for the reliable identify-cation of LAB, as reported by other researchers (Nigatu, 2000; De Angelis et al., 2001; Muyana et al., 2003) although it is a useful tool for presumptive classification. Commercially available systems based on carbohydrates fermentation should be combined with genotypic techniques which allowed more accurate identification. Therefore in the present investigations, the identification of lactic acid bacteria isolated from *gariss*, and the differrentiation between the isolates has been carried out using the RAPD-PCR fingerprinting technique and biochemical characteristics. When the selected arbitrarily primers used under well defined and optimized conditions they were capable of generating reproducible amplify-cation of random fragments of DNA from the isolated LAB and facilitated their genotyping differentiation.

The evaluation of the LAB isolated *gariss* revealed the predominance of *Lactobacillus* spp., which formed 66.7% while the remaining 33.3% were *Lactococuss* spp. Within the *Lactacillus* spp. wide diversity of strains were identified such as *Lactobacillus plantarum, L. animalis*, *L. brevis*, *Lactobacillus divergens*, *L. rhamnosus*, *L. gasseri*, *L. paracasei*, *L. fermentum*, *L. alimentarium* and *Lactobacillus sp.* These results confirm the predominance of LAB in traditionally fermented camel´s milk, as reported by other researchers (Sulieman et al., 2006; Omar et al., 2007) and comparable to those reported in previous studies on fermented milk products in Sudan and neighboring countries (Abdelgadir et al., 2001; Gonfa et al., 2001; Narvhus and Gadaga, 2003).

Development and research activities on domestic animals are mostly concentrated on species and breeds of animals available in the temperate zones of Europe and North America (Clemens, 2003). The camel (*Camelus dromedarius*) is certainly one of the most neglected species of the domestic animals. Although camel`s milk is an important part of the human diet in many parts of the

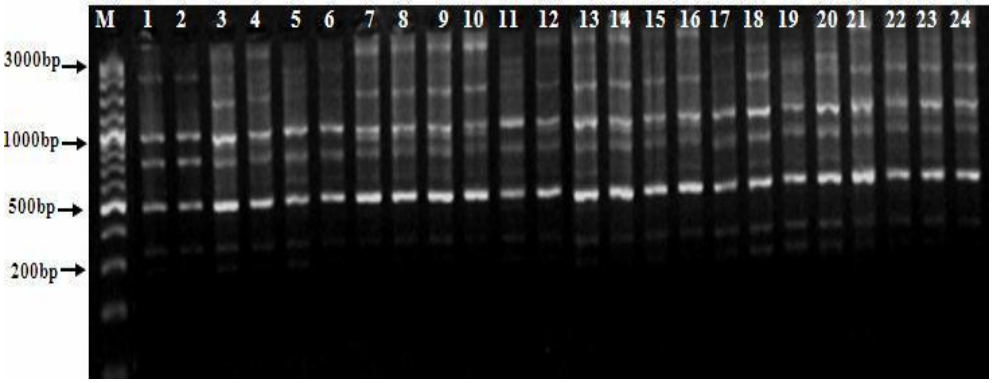

Figure 1. The RAPD-PCR products obtained from 24 LAB isolated from traditional Sudanese fermented camel's milk (*gariss*) using the primer P-2 (5-GGG TGT GGA G-3.

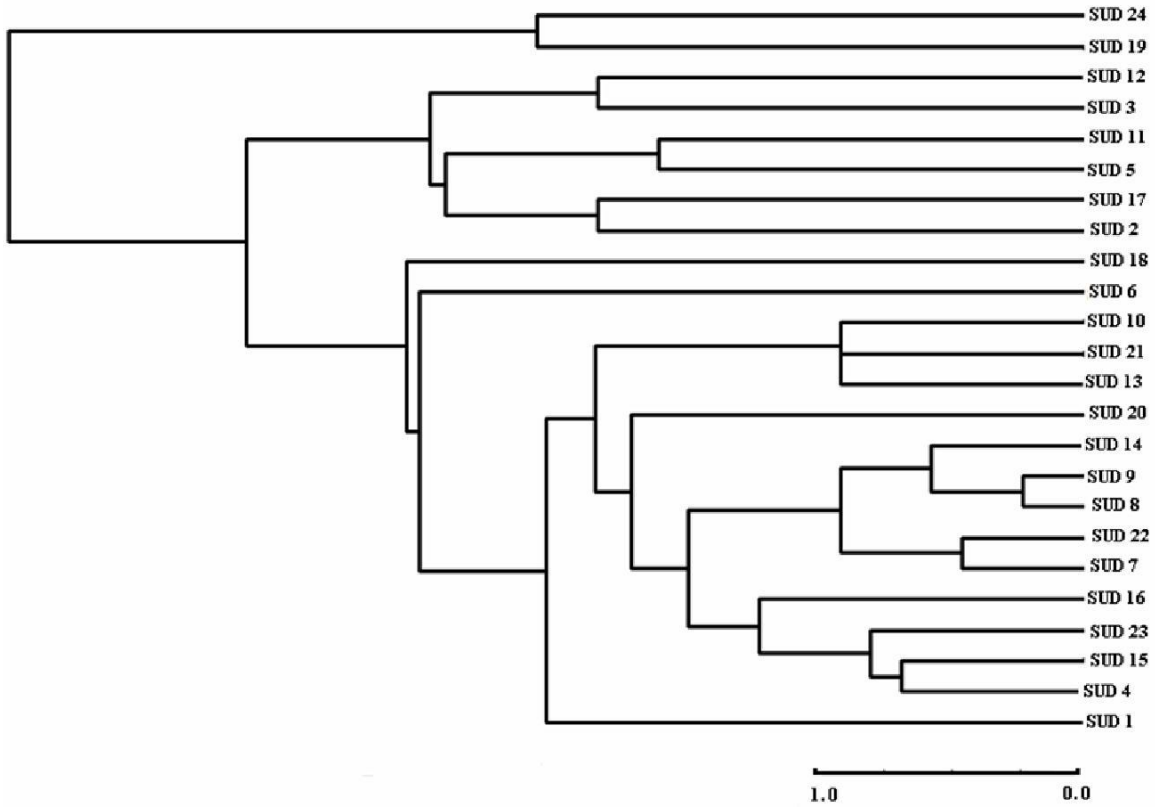

Figure 2. Dendrogram of LAB isolated from *gariss* based on RAPD-PCR. The dendrogram was constructed from a distance matrix using an UPGMA program.

world the majority of the studies conducted on camels concentrate mainly on its anatomical features and physiological adaptations to desert conditions (Lhoste, 2004).

In pastoral societies, milk is traditionally consumed predominantly in the form of fermented milk. Fermentation is the only means of preserving milk under warm condition (Farah and Fischer, 2004). In many arid areas, camels play a central role as milk suppliers where they are either home-consumed or sold (Lhoste, 2004). To prepare fermented camel milk, containers of calabash, clay pots, plant fiber vessels or hollowed wood vessels

are smoked by burning chips of *Olea Africana* or *Acacia busia*. The daily residual fresh milk is poured into the milk container. No starters are used and acidification develops after a few days, either from natural flora of milk when it is not boiled, or from the bacteria growing on the sides of the vessel. The milk is left in a quite place, often in a covered container sheltered from dust for usually 24 - 48 h until it becomes sour. The ambient temperature is normally between 25 and 35°C. Due to spontaneous nature of the fermentation, this traditional method results in a product with varying taste and flavor and often of poor hygienic quality.

To improve the spontaneous traditional fermentation, controlled fermentation using mesophilic lactic acid bacteria starter culture is a very important strategy for camel milk processing (Abu-Tarboush et al., 1998).

Since new starter cultures of lactic acid bacteria with an industrially important functionality are being developed, it would be very essential to search novel strains among traditional food stuff which can contribute to the microbial safety or offer one or more organoleptic, technological, nutritional, or health advantages. Lactic acid bacteria that can produce antimicrobial substances, sugar polymers, sweeteners, aromatic compounds, vita-mins, or useful enzymes, or that have probiotic properties could form an important step which could help in promotion of food industry.

Further research is needed to characterize the properties of isolated strains which have an influence on the sensorial characteristics of the industrial product, such as the determination of lactic acid production, proteolytic and lipolytic activity and the production of inhibitory substances.

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