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Research Article

# Isolation and Characterization of Lactic Acid Bacteria Strains from Raw Camel Milk for Potential Use in the Production of Yogurt

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## Abstract

The objective of this work is to formulate a starter lactic seen application camel milk to prepare fermented product yoghurt. Lactic acid bacteria isolated from camel milk has different characterization tests and selection: morphological study, catalase test, gram stain, use of citrate, acidifying power, lipolytic power and proteolytic power. These tests have to choose the strains: 1, 4, 5, 6, 7, 8, 9 and 10 which has ΔpH ≥ 0.3U after 6h as the most acidifying and EPS producing strains. All the strains showed a proteolytic activity with zone diameters and proteolysis was between 15 and 21mm. In addition, these lactic acid bacteria were considered low lipolytic but all having antimicrobial activity against 11 pathogenic strains. Then freeze-dried lactic acid bacteria were prepared from these strains starters (1, 4, 5, 6 and 9). These were used to inoculate three types of milk after pasteurization, using each time a combination of two strains. These strains were applied to goat, camel and cow's milk for the preparation of yoghurt. The monitoring of these fermented products shows the combinations of strain n°1 with strain n°6 in the goat milk and cow milk certainly give us the desired product (yogurt). Because of pH, titratable acidity and viscosity seem so similar to those of natural yoghurt.

**Keywords:** Camel milk; Fermentation; Lactic acid bacteria; Yoghurt

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## Introduction

In arid regions, camel milk is considered as one of the most important source of dairy products for human diet with potential therapeutic effects. Recent studies showed that camel milk is a natural source for probiotics [1]. The dominant and beneficial microflora in camel milk represented by LAB is a potential source of biological materials to be used in dairy technology [2]. Today, LAB are a focus of intensive international research for their essential role in most fermented food, for their ability to produce various antimicrobial compounds promoting probiotic properties [3], including antitumoral activity [4,5], reduction of serum cholesterol [6,7], alleviation of lactose intolerance [8], stimulation of the immune system [9] and stabilization of gut microflora [10]. LAB strains that produce Exopolysaccharide (ESP) are employed in the manufacture of fermented milk to improve its texture and viscosity [11,12]. Dairy products traditionally made are usually preserved due to spontaneous fermentation. However, modern large-scale production techniques generally make use of starter systems with defined strains so as to guarantee uniformity, safety and quality in the final product [13].

Camel milk has been used fresh or fermented in different regions of the world. Traditional fermented camel's milk is widely consumed in Africa and in Middle Eastern countries [14].

It is produced by spontaneous souring of camel's milk. In Tunisia, some practices of camel milk fermentation were known [15]. Development of new fermented functional camel milk in Tunisia can be promised. In fact, during the last decade, the interest of industries and consumers for functional foods has been exponentially increasing. The use of milk with particular nutritional properties such as camel milk, alone or in combination with bacterial strains having probiotic properties and/or producing physiologically active metabolites, represents one of the technology options for manufacturing dairy functional beverages [16]. The traditional method of milk fermentation results in a product with varying taste and flavor and often of poor hygienic quality.

Transformation of camel's milk into traditional Tunisian Yoghurt is achieved in addition of the yogurt starters *Streptococcus thermophiles* and *Lactobacillus bulgaricus.* Unfortunately, some of strains of *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* did not produce EPS or produce only low yields of EPS, which may affect the end products quality [17,18]. Therefore, screening LAB from natural sources has been one of the powerful means to obtain strains for the food industry. Thus, the objectives of this work are to isolate and characterize Lactic Acid Bacteria (LAB) from raw camel milk and to study their potential use in the production of fermented Tunisian dairy products like yoghurt.

## Materials and Methods

### Sampling

Milk samples were collected from camels (*Camelus dromadarius)* and goats (*Capra heircus*) belonging to the herd of the Arid lands

Institute (IRA Medenine). Cow milk was collected from a herd of cows in the same region of southern Tunisia. The samples were immediately cooled and brought to the laboratory in an isotherm containers and were analyzed upon arrival.

## Milk composition

**Physicochemical analysis:** pH is measured at 20ºC with a pH-meter type thermo-orion. and the titratable acidity (expressed as lactic acid %) was determined by titrating 10ml of homogenized fermented camel milk with 0.1N NaOH to the phenolphthalein end point.

The complex viscosity (in Pa s) was determined by applying a shear stress of 0.1Pa at an oscillation frequency of 1Hz for 1min with a Brookfield type viscometer (model DV-E, MA, USA).

Dry matter expressed in grams per litter milk is calculated after weighing the sample at 105ºC for 24h of its dry residue. The sample is 5g, Ash content, expressed in g/l of milk was determined after drying at 505ºC [19].

The fat content was measured by an acid-butyrometric method using a "Neusol solution" cited by Farah. This method is a direct reading on a butyrometer the amount of fat contained in 12ml of sample after centrifugation in the presence of amyl alcohol. The direct reading of graduations determines the amount of fat in g/l.

**Microbiological analysis:** The techniques used are conventional methods and reflect the recommendations of French law or official French method [20] which gives details of the technique followed. All samples studied have undergone a preliminary treatment to obtain the dilutions according to standard NF V08-0IO (March 96).

Milk samples (1ml) were diluted in buffered peptone saline ($10^{-1}$ to $10^{-3}$), mixed in stomacher bag. In order to quantify the various microbial groups, appropriate dilutions were surface plated:

Aerobic Total Plate Count (ATPC) (Sharlau Chemie S.A) was carried out on Plate Count Agar (PCA), incubated at 32ºC for 72h [21]. Yeast and moulds on Sabouraud Chloramphenicol (Pronadisa Micro & Molecular Biology) and incubated at 25ºC for 3 to 5 days. Total coliform were grown in Violet Red Bile Agar (VRBA) (AppliChem - Biochemica.Chemica Services) in double layer. After solidifying of the agar, the plates were incubated at 30ºC for 22h [22]. The lactic acid bacteria on MRS [23] are shown on the surface and then incubated 30ºC for 48h.

## Isolation and identification of strains

LAB were isolated on Man-Rogosa-Sharp (MRS) (Pronadisa) agar and incubated at 30ºC for 24 to 48h in order to apply the conventional tests for identification [24,25]. All isolates were initially examined for Gram staining and catalase production. Only Gram-positive and catalase-negative isolates were considered. Citrate utilization, in the presence of carbohydrates, was studied on Simmons citrate medium (Fluka Biochemica). The presence of a blue coloration (even locally only on the surface) indicated a positive reaction. These strains were tested from the growth in Na Cl (4, 6.5%), growth at different temperature (10-45ºC) and growth at different pH (4.2, 9.6).

## Strains conservation

The strains of LAB were stored without appreciable loss of properties in skimmed milk with 30% (v/v) glycerol at -20ºC [26,27]. Cultures were also kept on MRS agar or M17 agar slant at 4ºC and streaked every 4 weeks [26,28,29].

## Technological characterization

**Acidifying activity:** Acidifying activity of strains was measured according to the International Dairy Federation (IDF) standard 306, Kihal et al., and Alonso-Calleja et al. [28,30,31]. Acid production ability was assayed by inoculating 10% skim milk with 24h old cultures at 1% level and incubation at 30ºC. ΔpH was determined during 24h of incubation.

**Proteolytic activity:** To determine the proteolytic activity of LAB, MRS agar supplemented with 10% skim milk was poured, solidified and then dried. Sterile Whatman paper discs were deposited on the surface of the agar. Each disk received a volume of 20µl of a young culture. After incubation at 37ºC for 24h, proteolysis is indicated by clear zones around discs [32]. Proteolytic activity was determined from the diameter of lytic zone.

**Lipolytic activity:** To determine the lipolytic activity, the strains were inoculated on agar spot in Tween 80 (1, 3, 5%) [33]. Incubation was carried out at 25ºC for 72h. Strains with an opaque area due to the formation of esters with calcium liberated fatty acids were considered positive [34]. Lipolytic activity was determined from the diameter of lytic zone.

**Biomass production:** Strains were sub cultured on MRS broth; 100ml of the medium were inoculated with 10% of the active culture. Bacterial growth was monitored by measuring the Optical Density at 600nm (OD600) using a spectrophotometer (CECIL CE 2041/2000 Series) during 6h. The difference between the initial OD and the OD at which cells were collected (ΔOD) was taken as an indication for the growth amount. The maximum growth rate was determined from the slope of the linear part of curve representing Log OD versus time. At the early stationary phase, 30ml of culture were harvested by centrifugation (Sigma GmbH, Model 6K15, Gottingen, Germany) at 5000g for 30min at 4ºC. The dry weight was determined after drying the pellet at 105ºC for 24h. The remaining 70ml were used to study the separation of biomass by centrifugation and measurement of OD600 of supernatant [35].

**Exopolysaccharides production:** The cultures were streaked on modified MRS (m-MRS; glucose replaced with 100g/l sucrose) [36] and incubated at the optimum growth temperature for 24h, then tested for slime formation using the inoculated loop method [37]. Formed colonies were dragged up using a metal loop and the strains were considered positively slimy producer if the length of slime was above 1.5mm [35].

**Antibacterial effect:** For the antibacterial activity test, spot on lawn method was used. 18h cultures were spotted on MRS agar plates and incubated for 24h at 37ºC under anaerobic conditions. Overnight indicator strains (*Listeria inocua*, *Micrococcus luteus* and *Escherichia coli*) were overlaid in soft agar on MRS plates. Plates were incubated at 37ºC for 18h then, inhibition zone diameters were measured. Nisin (1mg/ml) was used as control.

**Lyophilization survival rate:** Cultures of LAB are prepared on liquid MRS medium. At the beginning of the stationary phase, the culture is stopped and centrifuged at 7000rpm, for 30min at 4ºC. 2% glycerol and 8% skimmed milk powder (proportional to the weight of the powder) are added to the pulp obtained. The mixture is mixed very well and placed in trays, the thickness of which must not exceed 5mm. They are frozen and then lyophilized with a lyophilizer

(CHRIST D-37520) for 48h at 4ºC. The survival rate after freeze-drying is given by the following equation:

$$\text{Survival rate (\%)} = \text{Ln N / Ln N0} * 100$$

With:

N: Number of viable cells after concentration,

N0: number of viable cells before concentration.

### Yogurt preparation

0.5g of the lyophilized ferment was weighed and dissolved in 5ml of the pasteurized milk. This pre-culture is incubated at 30ºC for 18h. Raw fresh camel milk consisted was preheated to 65ºC and homogenized. The homogenized milk was pasteurized at 65ºC for 30 minutes and cooled to 42ºC and portioned to three equal batches. The batches were inoculated with about 5ml starter cultures (SCC1-2+SCC1-15 and SCC1-13+SLch6) that were determined by the initial viable counts of yogurt. The initial viable counts of each batches were about $10^7$ CFU/mL measured by spread plate count method and incubated at 42ºC until the pH reached 4.6 (about 4 to 4.5h). Then yogurt samples were put into the refrigerating chamber (4ºC) for 12h to detect the pH, acidity, viscosity, aerobic total plate count and sensory of yogurts. Each test has three replications.

### Sensory evaluation

Twenty trained panelists (fourteen women and six men, aged 22-45) were asked to evaluate the sensory attributes of yogurt. The ratings were presented on a 9-point hedonic scale ranging from 9 (“like extremely”) to 1 (“dislike extremely”). Yogurt sensory parameters were evaluated by thickness, smoothness, fermented odor, finished flavor, and taste quality. To minimize bias, all groups were three digits coded. The yogurts were served to panelists after the cooling process. Result was given on averages of the three trials for each type of yogurt [38].

### Statistical analysis

Statistical analyses were performed using SPSS 14.0 software (SPSS Inc.; Chicago, IL, USA). Significant differences among treatments were tested by ANOVA followed by Tukey’s test with a level of significance at $\alpha = 0.05$. Data were expressed as Mean Values ± Standard Deviation (SD). All experiments were performed in duplicate and repeated three times.

## Result and Discussion

### Milk composition

The physicochemical characteristics of camel, cow’s and goat’s milk are given in table 1. There is a difference in the physicochemical characteristics of these three animal species. Camel milk (1,027) is less dense than goat milk (1,028) and cow’s milk (1,032), which could be explained by the difference in the stages of lactation and feeding of each animal species [39]. For pH and acidity, camel milk is more acidic than goat milk and cow’s milk. This is due to the presence of vitamin C (ascorbic acid) [40,41] which gives the milk a slightly acid taste [42]. This acidity could also be attributed to the richness of this milk in various organic acids (citric acid, orotic acid and butyric acid) [41].

The microbiological quality of the three type of milk was represented in table 2. The bacterial load is lower in camel milk than in goat milk and cow’s milk. According to El Hatmi et al., [39] this is due to its richness in soluble proteins which have an antimicrobial effect and to its richness in ascorbic acid which decreases the pH. In fact, the presence of factors limiting bacterial proliferation in raw milk has been demonstrated: high lysozyme content [43] and vitamin C [44]. TAPC count was reach to $2.44\times10^4$ UFC/ml in came milk. The main reason for these relatively high counts of bacteria should be ascribed to inadequate sanitary conditions during milking, collection and transport [41].

| Parameter | Cow Milk | Goat Milk | Camel Milk |
|---|---|---|---|
| pH | 6.77±0.00 | 6.63±0.06 | 6.736±0.005 |
| Acidity (ºD) | 17.1±0.9 | 16.2±0.9 | 13.8±0.5 |
| Density | 1.032±0.000 | 1.028±0.000 | 1.027±0.000 |
| Viscosity | 3.03±0.10 | 3.84±0.06 | 3.3±0.04 |
| Ash (g/l) | 8.43±0 .72 | 8.73±0.15 | 9.1±0.72 |
| Matter fat (g/l) | 20.33±4.61 | 60.33±7.50 | 23.66±11.01 |
| Dry matter (g/l) | 108.1±17 | 162.2±5.5 | 133.7±1.2 |

**Table 1:** Physicochemical characteristics of 3 types of milk (cow, goat and camel).

| | Aerobic Total Plate Count (UFC/ml) | Total Coliform (UFC/ml) | Yeast and Molds (UFC/ml) | Lactic Acid Bacteria (UFC/ml) |
|---|---|---|---|---|
| Camel milk | $4.7\times10^4$ | 0 | 0 | $8\times10^2$ |
| Cow milk | $2.09\times10^6$ | $2.15\times10^5$ | $8.3\times10^4$ | $6.5\times10^3$ |
| Goat milk | $2.39\times10^5$ | $2.67\times10^4$ | $3\times10^2$ | $1.15\times10^3$ |

**Table 2:** Microbiological characteristics of the 3 types of milk (cow, goat and camel).

### Isolation and identification of strains

Out of all strains obtained from Tunisian raw camels’ milk, 62 strains were Gram-positive, catalase negative and non-spore-forming. Only 29 of them were citrate positive. In microscopy, the cells had different shapes coccobacilli, cocci and bacilli, forming small chains of varying length, pairs or in clusters and were immobile. Ten strains were chosen according to the difference in cell morphology.

The ten strains encoded SCC1-2, SCC1-6, SCC1-7, SCC1-8, SCC1-13, SCC1-15, SCC1-24, SCC1-33, SLch6 and SLch14 are characterized by their ability to grow at different temperatures (10, 39 and 45ºC) at different salt concentrations (4, 6.5) while growth of these strains at 8% of salts concentration were not observed. All strains grow only at pH = 9.6 (Table 3).

### Technological properties of LAB isolates

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

**Acidifying activity:** In order to select a starter culture for lactic fermentation of camel milk, the strains were characterized on the basis of acid production ability. The acidity increased during the fermentation time and there was variability in acidification rate between the different strains used to inoculate milk (Figure 1). The strain is considered fast, medium and slow when ΔpH reached 0.4U for 3, 3 to 5 and >5h respectively [35]. This is applicable using cow’s milk as a substrate. In our case, only strains with $\Delta pH \geq 0.3U$ after 6h were kept for the next steps considering the antimicrobial activity of camel milk. Thus, the strains selected are: SCC1-33, SCC1-8, SCC1-7, SCC1-15,

SCC1-6, SCC1-24 and SLCch14. The fast acidifying strains are good candidate in the dairy fermentation process as primary starter organisms, whereas, the poor acidifiers strains can be used as adjunct cultures depending on their other important properties, e.g., proteolytic and autolytic activity.

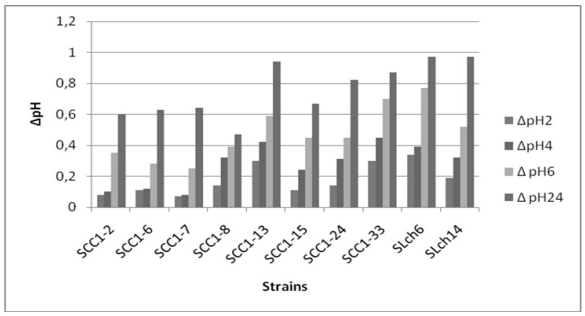


**Figure 1:** Evolution of ΔpH during the fermentation of camel milk after 2H (■), 4H (■), 6H and 24H (■) inoculated with different lactic strains and incubated at 30ºC.

The difference observed from one lactic acid bacteria species to another were explained by Badis et al. [26]. In fact, the acidifying activity of each strain is related to its specific capacity to break down the substances in the medium and render the capability of assimilation. On occasion, differences are also due to the presence or absence of nutrient transport systems [45].

**Proteolytic activity:** The results obtained during the implementation of this test are summarized in table 3. The table shows that all strains studied show growth with proteolytic activity resulted in the emergence of a clear halo around the discs. According to Vuillemard [32], the strain is called proteolytic if it has a zone of lysis of diameter between 15 and 21mm. Compared to these data, our strains revealed that proteolytic zone diameters were between 15 and 21mm.

The proteolytic activity of dairy lactic acid bacteria is essential for the bacterial growth in milk and involved in the development of organoleptic properties of different fermented milk products [46,47]. The production of high quality fermented dairy products is dependent on proteolytic systems of starter bacteria, since peptidase and amino acids formed have a direct impact on flavor or serve as flavor precursors in these products.

**Lipolytic activity:** The results of the lipolytic activity of lactic strains are shown in table 4. Lactic acid bacteria are considered weakly lipolytic [48] in comparison with other bacterial species such as *Pseudomonas*, *Acinetobacter* and *Flavobacterium* [49]. Yadav et al., [50] stated that the addition of autochthonous LAB on dairy products contributes to the production of free fatty acids and linoleic acid from milk fat lipolysis, providing a hipolipidemic effect in the host. These bacteria are found in large amounts on lactic foods due to their adaptation capacity in this substrate rich in proteins, lipids and fatty acids. Their wide distribution is a consequence of their lipolytic and proteolytic properties, their capacity to ferment/assimilate lactose and to use fatty acids.

**Biomass production and growth rate:** A starter is a microbial preparation of high cell density; therefore, it is necessary to select the starters to have significant biomass in the end of culture. A monitoring of OD was performed during strains culture on MRS broth. This allowed estimation of the maximum growth rate μmax.

The fermentation broth was centrifuged and the pellet was dried in order to determine biomass. The difference between the initial Optical Density (OD600) and the OD600 at which cells were collected (ΔOD600) as well as the dry weight of strains were used to reflect the growth amount (Table 5). Based on the biomass, cultures were divided into 3 groups: major yields when biomass ≥ 1.30mg/L, an average yield when the formed biomass ranged from 0.6 to 1.29mg/L, poor performance when the biomass was <0.6mg/L [35]. Strains SCC1-6, SCC1-15, SCC1-33 and SLCch14 were characterized by a high value of ΔOD600 and an important growth rate. The strains SCC1-24, SCC1-2, SCC1-13 and SCC1-13 presented a weak biomass and growth rate.

Indeed, the production of small quantities of biomass could be an inconvenient for the industrial use of these strains. However, this low yield could be explained by the loss of biomass during centrifugation and this was due to the production of exopolysaccharides that prevent the separation of bacterial cells and culture medium. This was visualized in the OD values of supernatant (Table 5). According to El-Soda et al., [35] a good separation of biomass was represented by an OD600 ranging between 0 and 0.1. The majority of strains had an OD600 <0.1 reflecting a good separation of biomass. Only two strains SCCl-8 and SCCl-13 had values greater than 0.1. As mentioned earlier, this was due to the production of EPS which prevent separation during centrifugation.

| Strains | Growth at Different Temperatures | | | Growth at Different pH | | Growth at Different [NaCl] | | |
|---|---|---|---|---|---|---|---|---|
| | 10ºC | 39ºC | 45ºC | 4.2 | 9.6 | 4% | 6.5% | 8% |
| SCC1-2 | + | + | + | - | + | + | + | - |
| SCC1-6 | + | + | + | - | + | + | + | - |
| SCC1-7 | + | + | + | - | + | + | + | - |
| SCC1-8 | + | + | + | - | + | + | + | - |
| SCC1-13 | + | + | + | - | + | + | + | + |
| SCC1-15 | + | + | + | - | + | + | + | - |
| SCC1-24 | + | + | + | - | + | + | + | - |
| SCC1-33 | + | + | + | - | + | + | + | - |
| SLch6 | + | + | + | - | + | + | + | - |
| SLch14 | + | + | + | - | + | + | + | - |

**Table 3:** Biochemical criteria of presumptive lactic species isolated from raw camel milk.

| Strains | Lipolytic Diameter Zone (mm) | | | Proteolytic Activity |
|---|---|---|---|---|
| | 1% tween 80 | 3% tween 80 | 5% tween 80 | Diameter Zone mm |
| SCC1-2 | 11,375 | 9 | 9 | 15±1.4 |
| SCC1-6 | 8,5 | 9 | 9,125 | 15±0.0 |
| SCC1-7 | 9,5 | 10 | 9,875 | 18±1.41 |
| SCC1-8 | 9,25 | 9 | 9,125 | 16±0.0 |
| SCC1-13 | 13,5 | 9,75 | 9,5 | 21±0.0 |
| SCC1-15 | 9,625 | 10,5 | 11,5 | 16.5±3.53 |
| SCC1-24 | 8,875 | 9,5 | 10,25 | 16.5±3.53 |
| SCC1-33 | 9,5 | 8,75 | 9,5 | 16.5±3.53 |
| SLch6 | 8,625 | 9,5 | 11 | 19±0.0 |
| SLch14 | 11,125 | 9 | 9,5 | 18±0.0 |

**Table 4:** Proteolytic and lipolytic activity of lactic acid bacteria.

| Strains | ΔOD600* | Biomass (g/l) | µmax (h-1) | EPS | OD600 Supernatant |
|---|---|---|---|---|---|
| SCC 1-2 | 0.44 | 0.53 | 0.05 | - | 0.003 |
| SCC1-6 | 1.13 | 0.81 | 0.13 | - | 0.008 |
| SCC1-7 | 0.67 | 0.69 | 0.07 | - | 0.01 |
| SCC1-8 | 0.86 | 0.20 | 0.08 | + | 0.10 |
| SCC1-13 | 0.73 | 0.06 | 0.07 | + | 0.11 |
| SCC1-15 | 1.32 | 0.79 | 0.12 | + | 0.02 |
| SCC1-24 | 0.73 | 0,06 | 0,07 | + | 0.04 |
| SCC1-33 | 1 .32 | 0.79 | 0,12 | + | 0.05 |
| SLch6 | 0.68 | 0.887 | 0.14 | + | 0.06 |
| SLCch14 | 1.93 | 0.98 | 0.13 | + | 0.02 |

**Table 5:** Characteristics of growth strains.

* ΔOD 600, difference between the initial optical density and optical density after 6h of culture; +, EPS producing strains; -, non EPS producing strains.

**Exopolysaccharide production:** Lactic acid bacteria have the ability to synthesize and excrete during their growth, extracellular sugar polymers called polysaccharides or Exopolysaccharide (EPS), which can improve the texture and viscosity of the final product [51]. In general, the presence of polysaccharides in fermented products such as yogurt can increase the homogeneity of the product and make its presentation more enjoyable [12]. The texture of fermented milk depends also on the interactions between bacteria and the different proteins (spatial conformation, interaction, pH, ionic strength) [52]. Our results showed that seven strains (SCC1-8, SCC1-13, SCC1-15, SCC1-24, SCC1-33, SLch6 and SLch14) were able to produce EPS (Table 5).

**Antagonism Effect:** The ten strains used in this study were tested for their antagonism effect. 10µl cultures were used and halos of inhibition were ranged between 10 to 26mm against *Listeria inocua, Micrococcus luteus* and *Escherichia coli*. (Figure 2). The results revealed that the antibacterial activity of the selected LAB could inhibit all tested pathogenic bacteria however at different inhibition levels as shown in figure 2. All isolates showed the most antibacterial potency to *Escherichia coli*. The strain SCC1-24 presents no inhibition against *Micrococcus luteus*. The difference in inhibition potential among the selected strains was considered to be due to the different intrinsic factors induced by different food origins [53]. The inhibitory action of LAB bacteria is mainly due to the accumulation of main primary metabolites such as lactic and acetic acids, ethanol and carbon dioxide.

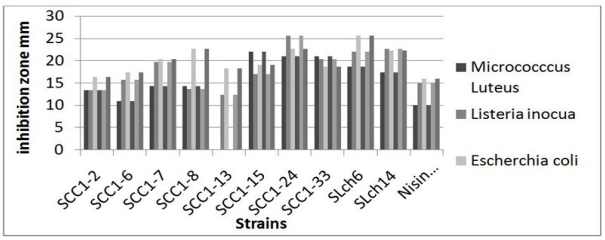


**Figure 2:** Antagonism effect of strains against Micrococcus luteus (■), Listeria inocua (□) and Escherichia coli (■).

**Stability of lyophilized cultures:** Lyophilization is considered as a standard practice for type culture collections, that technique was frequently reported in preserving and distributing lactic starter cultures [54]. The survival of lactic acid bacteria differ from one strain to another depending on the concentration of bacteria before freeze-drying and cryprotector used (Table 6). The strain SLch14 presents the higher survival rate (128.5%). The effect of lyophilization on the viability and activity of LAB is reported by several workers [55,56].

| Strains | Survival Rate Before Lyophilization | | Survival Rate After Lyophilization | |
|---|---|---|---|---|
| | CFU/ml | % | CFU/ml | % |
| SCC1-2 | 8 $10^8$ | 100 | 2.3 $10^8$ | 28.75 |
| SCC1-6 | 7.6 $10^8$ | 100 | 5.6 $10^8$ | 73.68 |
| SCC1-7 | 6.5 $10^8$ | 100 | 5.3 $10^8$ | 81.53 |
| SCC1-8 | 8.9 $10^7$ | 100 | 6.7 $10^6$ | 75.28 |
| SCC1-13 | 5.7 $10^8$ | 100 | 5.9 $10^8$ | 103.5 |
| SCC1-15 | 9.6 $10^7$ | 100 | 5.3 $10^8$ | 55.21 |
| SCC1-24 | 8 .1 $10^8$ | 100 | 5.1 $10^6$ | 62.96 |
| SCC1-33 | 9.2 $10^7$ | 100 | 5.2 $10^6$ | 56.52 |
| SLch6 | 7.2 $10^8$ | 100 | 6.3 $10^8$ | 87.50 |
| SLch14 | 5.6 $10^8$ | 100 | 7.2 $10^6$ | 128.5 |

**Table 6:** Survival rate of strains before and after lyophilization.

## Application of strains in the preparation of yoghurt from goat, cow and camel milk

In this study four performance isolates were used showing a high acidifying rate, high proteolytic capacity and high EPS production in preliminary experiments.

Camel, cow and goat milk were inoculated with these selected strains to prepare the pre-culture. After preparing dairy product, pH, acidity, and microbial count were measured. The results were shown in figures 3, 4 and table 7.

The fermentation profiles obtained for the three types of milk (camel, cow and goat) are similar. The acidity increases rapidly over time in different dairy products; this is explained by the importance the inoculum ($210^6$) and its adaptation to the fermentation because the pre-culture is carried out on the same type of milk. Indeed, the fermentation of lactose into lactic acid lowers the pH and promotes the proteolysis of proteins [57]. The increase in acidity is accompanied by a decrease in pH to a final value 4.45, 4.83 and 5.04 respectively for camel, goat and cow milk in day 1 and this decrease continue after 24h (Figures 3 and 4).

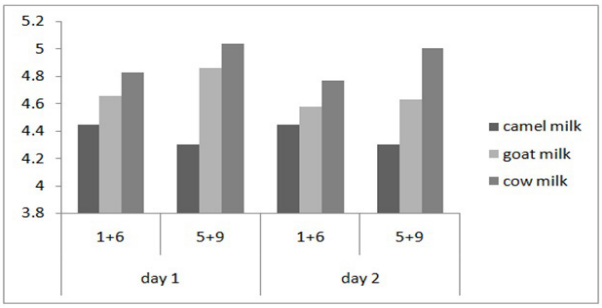


**Figure 3:** Strains effect on the pH in yoghurt from camel, cow and goat milk. Strains 1+6: SCC1-2+SCC1-15 and Strains 5+9: SCC1-13+SLch6.

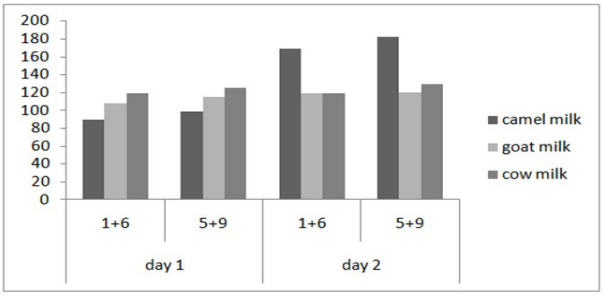


**Figure 4:** Strains effect on the acidity in yaourt from camel, cow and goat milk. Strains 1+6: SCC1-2+SCC1-15 and Strains 5+9: SCC1-13+SLch6.

| | Day 1 | | Day 2 | |
|---|---|---|---|---|
| | (1+6) | (5+9) | (1+6) | (5+9) |
| Camel milk | $172\times10^{7}$ | $8\times10^{10}$ | $4\times10^{7}$ | $4.1\times10^{10}$ |
| Cow milk | $3.16\times10^{9}$ | $5.36\times10^{9}$ | $>300\times10^{9}$ | $>300\times10^{9}$ |
| Goat milk | $>300\times10^{7}$ | $>300\times10^{7}$ | $>300\times10^{7}$ | $>300\times10^{7}$ |

**Table 7:** Strains effect on the total microbial count in yoghurt from camel, cow and goat Strains 1+6: SCC1-2+SCC1-15 and Strains 5+9: SCC1-13+SLch6.

Thus, it is observed that the pH of the fermented milks for the combination of strain 1 with strain 6 appear closest to being included in the pH range of plain yogurt (4.2 to 4.3) cited by Maurice M [58].

In some countries the yoghurt-equivalent product may contain others organisms beside *Lactobacillus bulgarius* and *S. thermophilus*. For example, in India, other bacteria are used (*Lactobacillus plantarum* and *Lactococcus lactis subsp lactis*; Marshall) [59], while Chander et al., [60] have used only mixed cultures comprising *Lac. lactis biovar diacetylactis* and *cremoris*. *Lactobacillus plantarum* is a heterofermentative lactobacillus producing acetate in addition to lactate, and the lactococci produce only diacetyl and no acetataldehyde.

### Sensory evaluation

The scores for sensory characteristics of the yogurt samples were presented in figure 5. For texture, the goat yoghurt was better than camel and cow yoghurt. For the taste quality, the goat yoghurt has the preferable taste. For odor, camel yoghurt was the best. The results of sensory evaluation indicated that the camel yoghurt with the selected strains had the potential to replace the imported commercial starter.

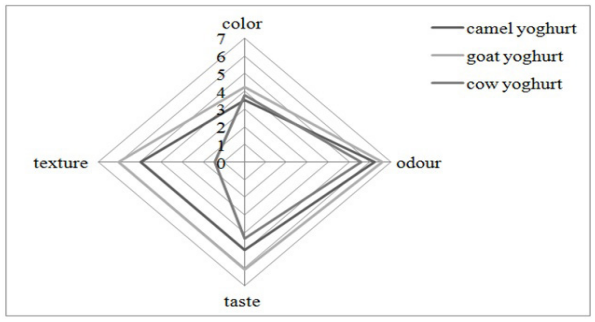


**Figure 5:** Sensory value for different starter fermented milk.

## Conclusion

The present study described the technological potential of combination of 2 selected strains of Lactic Acid bacteria isolated from camel milk and their use as starters for yoghurt preparation.

Based on the overall evaluation of the obtained results, the strains selected have high acidifying activity, high proteolytic activity and sensitive reaction to antibiotics. Among 10 strains, only 2 which have high acidification rate and high yield of biomass at the end of fermentation were applied to prepare dairy product and these dairy product present lowest pH values, highest acidity and lowest microbial cell count. This work may have important implication to put in the market yoghurt based of camel, cow and goat milk using a combination of lactic acid bacteria other than *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. Bulgaricus*. After sensory evaluation, these implied that our own authority starter could produce the yogurt with similar or better quality compared with the commercial starters.

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