# Characterization of Lactic Acid Bacteria from Camel Milk and their Technological Properties to Use as a Starter Culture

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**Abstract:** Proper selection and balance for starter culture is critical for the manufacture of fermented products of desirable texture and flavor. The objective of this study was to characterize lactic acid bacteria (LAB) from camel milk and elucidate their properties to use as a starter culture. Twenty-one lactic acid bacteria species were isolated from 30 samples of camel milk collected from Babile Woreda, eastern Ethiopia. Isolates were characterized phenotypically and their technological properties such as acidification, exopolysaccharide production (EPS), proteolytic and antimicrobial activities were studied following standard procedures. The results revealed that the isolated LAB strains belonged to *Lactobacillus, Lactococcus, Streptococcus* and *Enterococcus* genera. All lactic acid bacteria strains showed proteolytic activity with different degrees of clear zones. The lactic acid bacteria strains exhibited either high to low acidification activities. About 85% of the lactic acid bacterial strains had significant exopolysaccharide production (EPS). Three LAB strains showed maximum antagonistic properties against indicator organisms (*Staphylococcus aureus, Escherichia coli, Salmonella typhi,* and *Pseudomonas aeruginosa*). It could be concluded that *Lactobacillus plantarum* HUM19, *Lactobacillus acidophilus* HUM20, and *Streptococcus cremoris* HUM8 had high acidifying, antimicrobial and proteolytic activities, and EPS production among all other lactic acid bacteria isolates.

**Keywords**: Acidifying activity; Antimicrobial activity; Exopolysaccharide production; Fermentation; Proteolytic activity; Technological properties

## 1. Introduction

Fermented foods and beverages constitute a major portion of people's diets in Africa (Oyewole, 1997). Microorganisms are important in dairy products. One of the most important groups of acid producing bacteria in the food industry is the lactic acid bacteria (LAB) which are used to prepare starter culture for different dairy products. The proper selection and balance for starter culture is critical for the manufacture of fermented products of desirable texture and flavor (Temmerman *et al.*, 2002).

Currently, LAB are a focus of intensive international research for their essential role in fermented foods as a starter culture and for their ability to produce various antimicrobial compounds promoting probiotic properties (Temmerman *et al.,* 2002), various production of metabolic and enzymatic substances, which contribute to flavor, aroma and texture developments (Kleerebezemab *et al.*, 2000), for their ability to transform lactose and improve the digestibility of fermented dairy products (Soukoulis *et al.,* 2007; Weinberg *et al.,* 2007) as well as for their preservative quality (Abdelbasset and Djamila, 2008).

The microbiological quality of milk and milk products is influenced by the initial flora of raw milk (Ritcher and Vadamuthu, 2001). When camel milk is left to stand, its acidity rapidly increases due to the presence of LAB (Ohris and Joshi, 1961). It has also been recognized that LAB are capable of producing inhibitory substances other than organic acids (lactate and acetate) that are antagonistic toward other microorganisms (Daeschel, 1989).

Ethiopia is a country where vast arid regions exist. In these regions, long spells of dry period without any rain are common. Under such conditions, the only livestock, which can successfully survive and produce substantial quantities of good quality milk is the camel. But accessing market for camel milk is low for producers due to remoteness from towns and roads, and cannot be used in a fresh state and goes wasted. During peak production season, it can be saved and effectively utilized through converting it into fermented camel milk product by using starter culture of lactic acid bacteria (Seifu *et al.*, 2012). Therefore, the present study was aimed at characterizing lactic acid bacteria obtained from camel milk and elucidates their technological properties to use as a starter culture for prolonging the shelf life of the milk by preserving its taste and flavour.

## 2. Materials and Methods

### 2.1. Description of Study Area

The study was carried out in Eastern Ethiopia, Babille Woreda (district), which is predominantly an agro-pastoral Woreda (Tofik, 2014). The town is situated at the latitude of 09°13'N 42°20'E and longitude of 07°16'N 32°13'E and elevation of 1648 meters above sea level. The town is located at the distance of 30 km east of the town of Harar and 535 km from Addis Ababa, eastward. The study sites of the pastoral areas are located at the distance of about 15 km from the

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town of Babile. The rainfall pattern is bimodal but unpredictable and erratic in distribution. The mean annual temperature is between 34°C and 38°C (www.weather-forecast.com).

**2.2. Sample Collection**

A total of 30 samples of camel milk were collected aseptically from the pastoral households of Babille Woreda in Ethiopia. Camel milk samples were collected using sterile bottles and transported to Haramaya University, Microbiology Laboratory in an icebox for analysis. Aseptic sampling was followed as described by the Health Protection Agency HPA (2004) and the Food and Drug Administration FDA (2003). In the laboratory, the camel milk samples were kept at the temperatures below 4°C and analyzed within 48 h of collection (HPA, 2004).

**2.3. Isolation of Lactic Acid Bacteria (LAB)**

Lactic acid bacteria (LAB) were isolated from the camel milk samples using de Man Ragosa and Sharpe (MRS) and M17 agar according to the method described by Harrigan and McCance (1976) by pour plate method in triplicates. Ten (10) ml of unpasteurized camel milk samples were homogenized with a 90 ml sterile saline solution (0.85%, w/v NaCl) to make an initial dilution ($10^{-1}$). The suspension was used for making suitable serial dilutions up to $10^{-8}$ by incorporating 1 ml into 9 ml of sterile NaCl solution (0.85%, w/v) in sterile tubes. From appropriate dilutions of $10^{-2}$ to $10^{-4}$, 1 ml of the samples were pour plated into MRS medium, incubated anaerobically for 72 hrs at 37°C, for isolation of *lactobacilli* and M17 medium, incubated anaerobically for 48 hrs at 30°C, for isolation of *Lactococci*. After incubation, colonies were enumerated and recorded as colony forming units (cfu) per ml of milk. Then desired glistening colonies were picked up from the MRS and M17 agar plates by a sterile platinum loop and sub culturing was continued until the pure culture was obtained.

The presumptive lactic acid bacteria (LAB) isolates were inoculated in MRS/M17 broth, incubated at 30°C and checked for purity by streaking on their respective isolation media until only a single type of colony was present. The preliminary isolation of lactic acid bacteria was made on the basis of Gram staining and catalase reaction followed by microscopic examination to observe cell arrangements and morphological characteristics as described by Harrigan and McCance (1976). Only Gram positive, catalase negative, non-motile, cocci and bacilli shaped isolates were considered as presumptive lactic acid bacteria according to Savadogo *et al.* (2004). The cultures were stored and maintained at -20 on MRS and M17 agar slants supplemented with 10% (v/v) glycerol for further studies.

**2.4. Characterization of LAB Isolates**

Presumptive isolates that showed the general characteristics of lactic acid bacteria were selected randomly and subjected to different tests that included growth at different temperatures (10, 15 and 45°C), at different NaCl concentration (2%, 4%, and 6.5%), gas production from glucose and arginine hydrolysis was carried out according to the method described by Harrigan and McCance (1976). Identification to species level was conducted by subjecting isolates to various carbohydrates fermentation (Starch, Amygdalin, Arabinose, Cellobiose, Fructose, Galactose, Glucose, Lactose, Maltose, Mannitol, Mannose, Melizitose, Melibiose, Raffinose, Rhamnose, Ribose, Sucrose, Salicin, Sorbitol, Trehalose and Xylose) in MRS/M17 broth containing 1% solution of carbohydrate and added to 0.025% bromocresol purple as indicator according to Schillinger and Lucke (1987). Results were recorded after 48 h of incubation at 37°C. Based on the results, the isolates were then identified to species level using the species identification procedure of Bergey's manual of Determinative Bacteriology (Holt *et al.,* 1994), and by comparing the result with previously published scientific research work of Bettache *et al.* (2012).

**2.5. Technological Properties**

**2.5.1. Acidifying Activity**

The acid production by the isolated lactic acid bacteria species was determined after inoculating the isolates into sterile reconstituted skim milk powder (10% w/v) at a rate of 1-2% inoculums /100 ml milk in sterile bottles of 200 ml capacity according to the method described by Attia *et al.* (2001). The inoculated skim milk medium was incubated at 30°C for mesophilic and at 38°C for thermophilic lactic acid bacteria (Farah *et al.,* 1990; De Vuyst and Degeest, 1999; Attia *et al.,* 2001). Change in pH was monitored at different intervals by taking samples at 0 h (initial), 12 h, 24 h, 48 h and 72 h until the pH of the medium reached 4.6 (iso-electric point) (Patrignani *et al.*, 2007). The isolated lactic acid bacteria species were characterized as fast acid producers (less than12 h to reach pH 4.6), medium acid producers (12-48 h to reach pH 4.6) or slow acid producers (more than 48 h to reach pH 4.6) based on their acid production potential according to Seifu *et al.* (2012).

**2.5.2. Proteolytic Activity**

To determine the proteolytic activity of lactic acid bacteria isolates, MRS/M17 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 disc received a volume of 20 µl of a young culture. After incubation at 37°C for 24 h, proteolysis was indicated by clear zones around discs (Vuillemard *et al.*, 1986), which were recorded as positive activity. All strains with positive reaction in

MRS/M17 with 1% skimmed milk were considered as strains with a slight proteolytic activity (Lasagno *et al.,* 2002).

**2.5.3. Antimicrobial Activities of LAB Isolates**

**2.5.3.1. Indicator Strains**

The indicator strains including *Staphylococcus aureus, Escherichia coli, Salmonella typhi,* and *Pseudomonas aeruginosa* were used for the antimicrobial test of the lactic acid bacteria isolates. All indicator strains were obtained from the Pasture Institute in Addis Ababa.

**2.5.3.2. Preparation of Cell-Free Supernatant (CFS)**

Each LAB isolate was inoculated in 10 ml of MRS broth and incubated at 30°C for 48 hrs. After incubation, a cell-free supernatant was obtained by centrifuging the bacterial culture at 6000 rpm for 15 minutes, followed by the filtration of the supernatant through 0.20 µm pore size filters.

**2.5.3.3. Screening for Antimicrobial Activities**

The agar-well diffusion method was employed in the screening of LAB for antimicrobial activities. Indicator microorganisms were prepared by inoculating 20 ml of molten agar media seeded with $1x10^7$ cfu/ml of an overnight culture of each indicator organism and allowing them to solidify in a Petri dish. 50 µl of the filtered cell-free supernatant of test strains was separately placed into the wells. The plates, prepared in triplicate, were kept at 4°C for 24 h according to Bonade *et al.* (2001) to allow pre-diffusion of the CFS into the agar and then incubated at 37°C for 24 h. They were then observed for possible clearing of zones (inhibition zones). The antimicrobial activity was determined by measuring the diameter of the inhibition zones around the well using a caliper in mm.

**2.5.4. Exopolysaccharide (EPS) Production**

The screening of the isolates for EPSs production was carried out according to the method described by Guiraud (1998). The isolates cultured on MRS agar were streaked onto LTV agar (0.5% (w/v) tryptone, 1% (w/v) meat extract, 0.65% (w/v) NaCl, 0.8% (w/v) potassium nitrate, 0.8% (w/v) sucrose, 0.1% (v/v) Tween 80, and 1.7% (w/v) agar, pH 7.1± 0.2) Sawadogo *et al.* (2007) and incubated at 30°C for 48 h. The sticky aspect of the colonies was determined by testing them for slime formation using the inoculated loop method as described by Knoshaug *et al.* (2000). The isolates were considered positively slimy producer if the length of slime diameter was above 1.5 mm.

The positive isolates were confirmed by growing them on MRS sucrose broth and incubating them at 30°C for 24 hrs. A volume of 1.5 ml of the 24 h culture was centrifuged at 5000 rpm for 10 minute at 4°C and 1 ml of the supernatant was put in a glass tube and an equal volume of ethanol 95% was added. An opaque link formed at the interface of the tube indicated the presence of EPSs.

**2.6. Data Analysis**

Obtained experimental data of the study were analyzed using descriptive statistical methods.

## 3. Results and Discussion

**3.1. Isolation and Identification of LAB**

In the present study, a total of 95 lactic acid bacteria colonies were isolated, of which 51 and 44 colonies were obtained from MRS and M17 agar media, respectively. A total of 9 isolates selected from MRS agar plates were found to belong to the genus *Lactobacillus*. According to the biochemical test, *Lactobacillus brevis* HUM14, *Lactobacillus paracasei subsp. tolerans* HUM15, *Lactobacillus casei subsp. casei* HUM18 and *Lactobacillus plantarum* HUM19 produced gas from glucose. This is in agreement with the results of Seifu *et al.* (2012) that all *Lactobacillus* strains isolated from *ititu* were able to produce gas from glucose. All *Lactobacillus* isolates did not produce ammonia from arginine, catalase negative, and are non-motile. Regarding growth at different temperature, *Lactobacillus paracasei subsp. tolerans* HUM15, *Lactobacillus delbrueckii subsp. bulgaricus* HUM16, *Lactobacillus amylophilus HUM17, Lactobacillus casei subsp. casei* HUM18, and *Lactobacillus plantarum* HUM19 grew at 15°C. The results also showed that *Lactobacillus delbrueckii subsp. lactis* HUM13, and *Lactobacillus acidophilus* HUM17 *grew* at 45°C whereas *Lactobacillus delbrueckii subsp. bulgaricus* HUM20 and *Lactobacillus helveticus* HUM21 were able to grow at 15 and 45°C. According to pH resistant test, all *lactobacillus* isolates were able to grow at pH 5.0, while *Lactobacillus delbrueckii subsp. bulgaricus* HUM20 and *Lactobacillus delbrueckii subsp. lactis* HUM13 were able to grow at pH 4.0. Regarding growth of isolates at different NaCl concentration, *Lactobacillus delbrueckii subsp. lactis* HUM13, *Lactobacillus brevis HUM14,* and *Lactobacillus paracasei subsp. tolerans* HUM15, grew at 2% NaCl. The growth of isolates in a medium containing 2% NaCl observed in the present study is similar to the findings of Hutkins *et al.* (1987), where all *lactobacilli* isolated from camel milk were able to grow at 2% NaCl. The results also showed that *Lactobacillus amylophilus* HUM17, *Lactobacillus casei subsp. casei* HUM 18, *Lactobacillus plantarum* HUM19 and *Lactobacillus helveticus* HUM21, grew at 2 and 4% NaCl, whereas *Lactobacillus delbrueckii subsp. bulgaricus* HUM20 grew at 4 and 6.5% NaCl. Different *lactobacilli* strains isolated from camel milk are shown in Table 3, while the results of different physiological and biochemical tests are given in Table 2.

The other isolates were referred to the genus *Lactococcus*. They were identified by their morphological, cultural, physiological and biochemical characteristics. Ten isolates picked from M17 agar plates were found

to belong to the genus *Lactococcus*. All *Lactococcus* were unable to produce gas from glucose, show arginine hydrolysis by some strains, catalase-negative and non-motile which is indicator of *Lactococcus* strains. Arginine hydrolysis indicated that *Lactococcus lactis subsp. lactis* HUM1, *Pediococcus acidilactici* HUM4, *Pediococcus pentosaceus* HUM5, and *Lactococcus garviae* HUM6 were able to produce ammonia from arginine. Regarding growth at different temperature, *Lactococcus lactis subsp. cremoris* HUM2 was able to grow at 15°C, whereas *Lactoccoccus raffinolactis* HUM3, *Pediococcus acidilactici* HUM4, *Lactococcus garviae* HUM5, *Pediococcus pentosaceus* HUM6, *Streptococcus cremoris* HUM8, *Streptococcus lactis* HUM9, *Pediococcus damnosus* HUM10, and *Streptococcus salivarius subsp. thermophilus* HUM12 grew at 45°C. According to growth at different pH range; *Lactoccoccus raffinolactis* HUM3 was able to grow at pH 2.0 and 4.0. On the other hand, *Lactococcus garviae* HUM5, *Streptococcus cremoris* HUM8, *Streptococcus lactis* HUM9, and *Streptococcus salivarius subsp. thermophilus* HUM12 were able to grow at pH 4.0 and 5.0, whereas *Lactococcus lactis subsp. lactis* HUM1, *Lactococcus lactis subsp. cremoris* HUM2, *Pediococcus acidilactici* HUM4, *Pediococcus pentosaceus* HUM6, and *Pediococcus damnosus* HUM11 were able to grow at pH 5.0. Regarding growth at different NaCl concentration, *Lactococcus lactis subsp. lactis* HUM1, *Lactococcus lactis subsp. cremoris* HUM2, *Lactoccoccus raffinolactis* HUM3, and *Lactococcus garviae* HUM5 grew at 2 and 4% NaCl whereas *Pediococcus acidilactici* HUM4 and *Pediococcus pentosaceus* HUM6 were able to grow at 4.0 and 6.5% NaCl. Similar observations were reported by Togo et *al.* (2002) who indicated that Lactococcus isolates were able to grow at higher NaCl (4% and 6.5%). The different *Lactococci* strains isolated from camel milk are shown in Table 3 while the results of different physiological and biochemical tests are given in Table 1.

Two isolates selected from M17 agar plates were identified as *Enterococcus* strains, which included *Enterococcus faecalis* HUM7. This isolate was able to grow at 15 and 45°C, pH 4.0, and in a medium containing 6.5% NaCl, whereas *Enterococcus casseliflavus* HUM2 was able to grow at 15°C, pH 5.0, and in a medium containing 2% and 4% NaCl. *Enterococcus* was observed to be the only genera that showed growth at a high NaCl concentration (6.5%) which is similar with an earlier finding by El-Hadi *et al.* (2006), Gram-positive and catalase negative bacteria that are capable of growing at 15 and 45°C, and in a medium containing 6.5% NaCl were considered to be *Enterococci* (Table1).

In the current study, more growth of *Lactobacillus* species in camel milk as compared to others isolates were observed on their selective culture media and comprised 72.60% of the total lactic acid bacteria (Table 5). These findings are in accordance with Khedid *et al.* (2006), who reported that *Lactobacillus* species isolated from camel milk was the dominant genus with 37.5% of the total lactic acid bacteria isolates. Bettache *et al.* (2012) indicated that members of the genus *Lactobacillus* isolates dominated in all Dhan samples as well as in the traditional butter. Consistent with the results of this study, Abu-Tarboush (1994) reported that camel milk provided support to the growth of *L. acidophilus*.

Table 1. Physiological and biochemical characteristics of *cocci* strains.

| Characteristics | *Lactococci* isolates | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | HUM 1 | HUM 2 | HUM 3 | HUM 4 | HUM 5 | HUM 6 | HUM 7 | HUM 8 | HUM 9 | HUM 10 | HUM 11 | HUM 12 |
| Gas from glucose | - | - | - | - | - | - | - | - | - | - | - | - |
| Cell shape | cocci | cocci | cocci | cocci | cocci | cocci | cocci | cocci | cocci | cocci | cocci | cocci |
| Ammonium from arginine | + | - | v | + | + | + | v | - | - | - | v | - |
| Motility | - | - | - | - | - | - | - | - | - | - | - | - |
| Catalase test | - | - | - | - | - | - | - | - | - | - | - | - |
| Aerobicity | f.a | f.a | f.a | f.a | f.a | f.a | f.a | f.a | f.a | f.a | f.a | f.a |
| Growth at different temperature | | | | | | | | | | | | |
| 10°C | - | - | - | - | - | - | - | - | - | - | - | - |
| 15°C | - | + | - | - | v | - | + | - | - | - | + | - |
| 45°C | + | - | + | + | + | + | + | + | + | + | - | + |
| Growth at different pH | | | | | | | | | | | | |
| 2.0 | - | - | + | v | - | - | - | - | - | - | - | - |
| 4.0 | - | - | + | v | + | - | + | + | + | - | - | + |
| 5.0 | + | + | - | + | + | + | - | + | + | - | + | + |
| Growth in the presence of NaCl | | | | | | | | | | | | |
| 2% | + | + | + | - | + | - | - | - | - | - | - | - |
| 4% | + | + | + | + | + | + | - | - | - | - | - | - |
| 6.5% | - | - | - | + | v | + | + | v | - | - | v | v |

***Note:*** + = positive reaction; - = negative reaction; v = variable reaction; f.a = facultative anaerobic; HUM1 = *Lactococcus lactis subsp. lactis*; HUM2 = *Lactococcus lactis. subsp. cremoris*; HUM3 =*Lactococcus. raffinolactis*; HUM4 = *Pediococcus acidilactici*; HUM5 =*Lactococcus.garviae*; HUM6 = *Streptococcus lactis*; HUM7 = *Enterococcus faecalis*; HUM8 = *Streptococcus cremoris*; HUM9 = *Streptococcus salivarius subsp. Thermophiles*; HUM10 = *Pediococcus damnosus*; HUM11 = *Enterococcus casseliflavus*; HUM12 = *Pediococcus pentosaceus; n = 2*

Table 2. Physiological and biochemical characteristics of *Lactobacillus* strains.

| Characteristics | *Lactobacilli* isolates | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | HUM 13 | HUM 14 | HUM 15 | HUM 16 | HUM 17 | HUM 18 | HUM 19 | HUM 20 | HUM 21 |
| Gas from glucose | - | + | + | - | - | + | + | - | - |
| Cell shape | bacilli | bacilli | bacilli | bacilli | bacilli | bacilli | bacilli | bacilli | bacilli |
| Ammonia from arginine | - | - | - | - | - | - | - | - | - |
| Motility | - | - | - | - | - | - | - | - | - |
| Catalase test | - | - | - | - | - | - | - | - | - |
| Aerobicity | f.a | f.a | f.a | f.a | f.a | f.a | f.a | f.a | f.a |
| Growth at different temperature | | | | | | | | | |
| 10°C | - | - | - | - | - | - | - | - | - |
| 15°C | - | - | + | - | + | + | + | - | + |
| 45°C | + | - | - | + | - | - | v | + | + |
| Growth at different pH | | | | | | | | | |
| 2.0 | - | - | - | - | - | - | - | - | - |
| 4.0 | + | - | - | - | - | - | - | + | - |
| 5.0 | + | + | + | + | + | + | + | + | + |
| Growth in the presence of NaCl | | | | | | | | | |
| 2% | + | + | + | - | + | + | + | - | + |
| 4% | - | - | - | - | + | + | + | + | + |
| 6.5% | - | - | - | - | - | - | - | + | v |

***Note:*** + = Positive reaction; - = Negative reaction; v = Variable reaction; f.a = Facultative anaerobic; HUM13 = *Lactobacillus delbrueckii subsp. Lactis*; HUM14 = *Lactobacillus brevis*; HUM15 = *Lactobacillus paracasei subsp. Tolerans*; HUM16 = *Lactobacillus delbrueckii subsp. Bulgaricus*; HUM17 = *Lactobacillus amylophilus*; HUM18 = *Lactobacillus casei subsp. Casei*; HUM19 = *Lactobacillus helveticus*; HUM20 = *Lactobacillus acidophilus*; HUM21 = *Lactobacillus plantarum; n =2*

Table 3. Carbohydrates fermentation profile of lactic acid bacteria (LAB) species.

| Isolates number | Carbohydrates | | | | | | | | | | | | | | | | | | | | Species identification |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Lac | Mal | Glu | Gal | Mos | Man | Mlz | Sal | Mel | Cel | Rha | Suc | Rib | Xyl | Str | Amy | Ara | Fru | Sor | Tre | Raf | |
| HUM 1 | + | + | + | + | + | - | - | + | - | + | - | - | + | v | + | - | - | + | - | v | - | *Lactococcus lactis subsp.lactis* |
| HUM 2 | + | - | + | + | + | - | - | - | - | + | - | - | - | v | + | - | - | + | - | - | - | *Lactococcus lactis subsp.cremoris* |
| HUM 3 | + | + | + | + | + | + | + | + | + | - | - | + | - | + | + | - | - | + | - | + | + | *Lactococcus raffinolactis* |
| HUM 4 | v | - | + | + | + | - | - | - | - | + | + | + | + | + | - | - | v | + | - | + | - | *Pediococcus acidilactici* |
| HUM 5 | + | - | + | + | + | - | - | + | - | + | - | - | + | - | - | + | - | + | - | + | - | *Lactococcus garviae* |
| HUM 6 | v | + | + | + | + | - | - | + | - | + | + | + | + | v | - | + | + | + | - | + | - | *Pediococcus pentosaceus* |
| HUM 7 | + | + | + | v | + | + | + | + | + | - | - | + | + | - | + | - | - | - | + | + | - | *Enterococcus faecalis* |
| HUM 8 | + | + | + | - | - | - | + | + | + | + | + | - | - | - | - | + | + | + | + | + | v | *Streptococcus cremoris* |
| HUM 9 | + | - | + | + | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | v | *Streptococcus salivarius subsp.thermophilus* |
| HUM 10 | - | v | + | - | + | - | v | | v | + | - | v | - | - | - | - | - | + | + | - | - | *Pediococcus damnosus* |
| HUM 11 | + | + | + | - | + | + | - | + | + | - | + | + | + | + | - | - | - | - | + | + | + | *Enterococcus casseliflavus* |
| HUM 12 | + | - | - | + | + | + | - | v | v | - | - | + | + | + | + | - | - | - | + | v | - | *Streptococcus lactis* |
| HUM13 | + | + | + | v | - | + | - | + | - | - | - | - | - | - | - | + | - | + | - | - | - | *Lactobacillus delbrueckii subsp.lactis* |
| HUM 14 | v | | + | + | - | - | - | v | v | v | - | - | - | - | - | - | + | + | - | - | - | *Lactobacillus brevis* |
| HUM 15 | + | - | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | *Lactobacillus paracasei subsp.tolerans* |
| HUM 16 | + | - | + | - | - | - | - | - | - | - | - | - | + | - | - | - | - | + | - | - | - | *Lactobacillus delbrueckii subsp.bulgaricus* |
| HUM 17 | - | - | + | + | - | v | - | - | - | - | - | v | - | - | + | - | - | - | - | - | - | *Lactobacillus amylophilus* |
| HUM 18 | - | v | + | + | + | + | v | + | + | + | - | - | - | - | - | + | - | - | + | + | - | *Lactobacillus casei subsp.casei* |
| HUM 19 | + | + | + | + | + | + | + | | - | + | | - | - | + | - | + | - | + | + | + | - | *Lactobacillus plantarum* |
| HUM 20 | + | + | + | + | + | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | *Lactobacillus acidophilus* |
| HUM 21 | + | - | + | + | v | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | *Lactobacillus helveticus* |

***Note:*** Tre = Trealose; Suc = sucrose; Amy = Amygladin; Str = Starch; Mlz = melizitose; Sor = Sorbitol; Ara = Arabinose; Gal = Galactose; Rib = Ribose; Rha = Rhamnose; Xyl = Xylose; Mal = Maltose; Cel = cellubiose; Mel = melibiose; Sal = Salicin; Man = Mannitol; Mos = Mannose; Fru = Fructose; Glu = Glucose; Lac = Lactose; Raf = Raffinose.

### 3.2. Acidifying Activity

To select a potential candidate starter culture, the lactic acid bacteria strains isolated from camel milk were characterized on the basis of acid production capability. The acidity was increased during the fermentation, and there was variability in acidification rate between the different strains used to inoculate the milk.

According to acidifying activity, *Lactobacillus acidophillus* HUM20*,* and *Lactobacillus plantarum* HUM19, were considered to be fast acid producer among *Lactobacilli* strains as they reached to a final pH value of 4.33 and 4.50 from an initial value of 6.75 and 6.77, respectively within 8 h of incubation. This is in agreement with the results of Fguiri *et al.* (2016) that *Lactobacillus plantarum* was selected as fast acid producer *Lactobacillus* isolate from camel milk. On the other hand, *Lactobacillus brevis* HUM14, *Lactobacillus helveticus* HUM16, *Lactobacillus delbrueckii subsp. bulgaricus* HUM2*,* and *Lactobacillus casei subsp. casei HUM18* were found to be medium acid producers from an initial value of (6.77, 6.73, 6.67, and 6.79 to a final pH value of 4.44, 4.43 4.34 and 4.40, respectively) within 48 h of incubation. This is in accord with the findings of Seifu *et al.* (2012) who reported that *Lactobacillus delbrueckii subsp. bulgaricus* showed medium acidification activity and reduced the pH of the skim milk powder from an initial values of 6.77 to a final pH values of 4.57 within 48 h of incubation. On the other hand, *Lactobacillus paracasei subsp. tolerans* HUM15, *Lactobacillus delbrueckii subsp. lactis* HUM13 and *Lactobacillus amylophilus* HUM17 reduced the pH of the skim milk from an initial value of 6.75 to a final pH value of 4.42, 4.46, and 4.6, respectively, within 72 h of incubation.

Among *Lactococcus* strains, all *Lactococcus* isolates were slow acid producers except, *Pediococcus pentosaceus* HUM6, and *streptococcus cremoris* HUM8 reduced the pH of the skim milk fast from an initial value of 6.78 and attained a final pH value of 4.56, and 4.38 respectively, within 10 h of incubation. This is in agreement with the results of Fguiri *et al.* (2016) who reported that *pediococcus pentosaceus* was selected as fast acid producer among *Lactococcus* isolate from camel milk.

The difference observed in acidifying activities between each strain of lactic acid bacteria species may be associated with specific capacity to break down the carbon and nitrogen substrates in the medium and the capability to assimilate the nutrients essential for growth (Badis *et al.*, 2004). On occasions, differences are also due to the presence or absence of nutrient transport systems (Albenzio *et al.,* 2001).

### 3.3. Proteolytic Activity

According to proteolytic test all investigated, lactic acid bacteria isolates showed different diameter of a clear zone around the discs. According to Vuillemard *et al.* (1986), a strain is called proteolytic if it has a zone of lysis of diameter between 15 and 21 mm. Compared to these data, our strains revealed that proteolytic zone diameters were between 15 and 21 mm. The results obtained during the investigation of Proteolytic test are shown in Table 4.

Table 4. Proteolytic activities of lactic acid bacteria species.

| Isolates | Diameter of inhibition zone (mm) |
|---|---|
| *Lactococcus lactis subsp. lactis* HUM1 | 16±0.03 |
| *Lactococcus lactis subsp. cremoris* HUM2 | 16±0.00 |
| *Lactococcus raffinolactis* HUM3 | 15±0.10 |
| *Pediococcus acidilactici* HUM4 | 15±0.31 |
| *Lactococcus garviae* HUM5 | 17±1.40 |
| *Pediococcus pentosaceus* HUM6 | 17±3.00 |
| *Enterococcus faecalis* HUM7 | 15±0.00 |
| *Streptococcus cremoris* HUM8 | 21±0.01 |
| *Streptococcus salivarius subsp. thermophilus* HUM9 | 15±0.00 |
| *Pediococcus damnosus* HUM10 | 16±0.00 |
| *Enterococcus casseliflavus* HUM11 | 15±0.00 |
| *Streptococcus lactis* HUM12 | 15±0.01 |
| *Lactobacillus delbrueckii subsp. lactis* HUM13 | 15±0.40 |
| *Lactobacillus brevis* HUM14 | 18±0.03 |
| *Lactobacillus paracasei subsp. tolerans* HUM15 | 15±0.00 |
| *Lactobacillus delbrueckii subsp. bulgaricus* HUM16 | 18±0.00 |
| *Lactobacillus amylophilus* HUM17 | 15±1.30 |
| *Lactobacillus casei subsp. casei* HUM18 | 16±0.02 |
| *Lactobacillus plantarum* HUM19 | 19±0.10 |
| *Lactobacillus acidophilus* HUM20 | 20±0.00 |
| *Lactobacillus helveticus* HUM21 | 17±2.30 |

***Note:*** The value indicated is means ± SD; n = 4

Among all lactic acid bacteria isolates, *Lactobacillus acidophilus* HUM20, *Lactobacillus plantarum* HUM19, and *Streptococcus cremoris* HUM8 had a high proteolytic activity with different diameters of clear zones. Proteolytic activity is essential for the development of organoleptic properties of different fermented milk products (Axelsson 1998; Christensen *et al.,* 1999). The production of high-quality fermented dairy products is dependent on the proteolytic systems of starter bacteria, as the peptides and amino acids formed have a direct impact on flavour or serve as flavour precursors in these products (Axelsson, 1998; Christensen *et al.,* 1999). Several peptidases with different specificities have been identified in lactic acid bacteria. All peptidases have been found to be intracellular and liberated in fermented milk products after cell lysis (Law and Haandrikman, 1997; Axelsson, 1998).

### 3.4. Antimicrobial Activities

The antimicrobial properties of lactic acid bacteria isolates from camel milk are shown in Table 5. The LAB strains were able to inhibit the selected indicator organisms to varying degrees of the zones of inhibition. Similar to our findings, Kivanc (1990) and Tadesse *et al.*

(2005) observed varying degrees of inhibition of various food borne pathogens by cell-free filtrates of LAB. Afolabi *et al.* (2008) showed that antimicrobial producing microorganisms had the ability to inhibit the growth of other bacteria which included both Gram-negative and Gram positive bacteria. Such antimicrobial activities were also demonstrated in the works of other researchers such as Adesokan *et al.* (2008) where LAB species were tested against *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, Escherichia coli,* and *Proteus vulgaris*. It has been also demonstrated that, the antimicrobial compounds produced by LAB can inhibit the growth of pathogenic bacteria of possible contaminants in fermented products (Raccah *et al.,* 1979; Smith and Palumbo, 1983; Cintas *et al.,* 1998).

The ability to inhibit other organisms is due to the fact that LAB produces substances which are injurious to the indicator organisms depending on the concentration or quantity produced (Axelsson, 1998; Christensen *et al.,* 1999). These substances serve as competitive advantage to LAB when in mixed culture especially during fermentation and hence the dominance of LAB during fermentation of milk, cereals and vegetables (Afolabi *et al.*, 2008). Wakil and Osamwonyi (2012) indicated that LAB isolates showing antimicrobial activity were discovered to produce antimicrobial substances like lactic acid, hydrogen peroxide, and diacetyl, showing that the ability to inhibit other organisms was directly related to the ability of these organisms to produce these substances. Daeschel (1993) reported the ability of LAB to produce lactic acid, thereby reducing the pH of the fermenting medium. The lactic acid produced serves to reduce the pH of the medium, thereby making it acidic which is not conducive for the survival of spoilage bacteria which may have found their way into the fermenting substrate during spontaneous fermentation. Lactic acid is a natural preservative that inhibits putrefying bacteria and is responsible for the improved microbiological stability and safety of the food (Raccah *et al.,* 1979).

Table 5. Antimicrobial activities of lactic acid bacteria species against pathogenic microorganisms.

| LAB isolates | Indicator strains | | | |
|---|---|---|---|---|
| | *S. aureus* | *S. typhi* | *E. coli* | *P. aeruginosa* |
| *Lactococcus lactis subsp. lactis* HUM1 | + | + | + | + |
| *Lactococcus lactis subsp. cremoris* HUM2 | + | + | + | ++ |
| *Lactococcus raffinolactis* HUM3 | ++ | + | + | + |
| *Pediococcus acidilactici* HUM4 | + | ++ | + | + |
| *Lactococcus garviae* HUM5 | ++ | + | + | ++ |
| *Pediococcus pentosaceus* HUM6 | + | ++ | + | + |
| *Enterococcus faecalis* HUM7 | + | + | + | + |
| *Streptococcus cremoris* HUM8 | +++ | + + | +++ | ++ |
| *Streptococcus salivarius subsp. thermophilus* HUM9 | + | + | + | ++ |
| *Pediococcus damnosus* HUM10 | ++ | ++ | + | ++ |
| *Enterococcus casseliflavus* HUM11 | + | + | + | + |
| *Streptococcus lactis* HUM12 | ++ | + | + | ++ |
| *Lactobacillus delbrueckii subsp. lactis* HUM13 | + | ++ | + | + |
| *Lactobacillus brevis* HUM14 | ++ | + | + | ++ |
| *Lactobacillus paracasei subsp. tolerans* HUM15 | ++ | ++ | + | + |
| *Lactobacillus delbrueckii subsp. bulgaricus* HUM16 | ++ | + | + | + |
| *Lactobacillus amylophilus* HUM17 | + | + | + | + |
| *Lactobacillus casei subsp. casei* HUM18 | ++ | + | + + | ++ |
| *Lactobacillus plantarum* HUM19 | ++ | + + | +++ | + ++ |
| *Lactobacillus acidophilus* HUM20 | +++ | + + | +++ | ++ |
| *Lactobacillus helveticus* HUM21 | + | + | + | + |

*Note:* + = [1–4 mm]; ++ = (4–8 mm]; +++ = (8–12 mm]
Source: Akabanda *et al.*, 2014

**3.5. Exopolysaccharide Production (EPS)**

The results showed that all the groups of LAB strains tested produced exopolysaccharide (EPS) with slime length diameter above 1.5 mm. This finding is in agreement with the findings of Patel and Prajapati (2013), who reported most of the LAB producing EPS belonged to the genera *Streptococcus, Lactobacillus, Lactococcus, Leuconostoc,* and *Pediococcus.* According to Bridget and Lordsday (2011) a total of 77% of LAB strains produced exopolysaccharides under the experimental condition from Nigerian yoghurt.

Exopolysaccharide production is a desirable feature of bacteria applied in dairy products because EPSs act as natural biothickener leading to higher consistency and

viscosity of the product and reduced syneresis Ruas-Madiedo *et al.* (2006). However, most of them are chemically or enzymatically modified in order to improve rheological properties (e. g. cellulose, starch, pectin and alginate) (Ruas-Madiedo *et al.* (2006), and therefore, their use is strongly restricted for food applications. The EPSs of microbial origin have unique rheological properties because of their capability of forming very viscous solutions at low concentration and their pseudoplastic nature (Becker *et al.,* 1998). Some strains of LAB have been reported to produce EPS and gain increasing attention over the last few years because of their contribution to the rheology and texture of fermented milk and food products (Cerning and Marshall, 1999). EPS-producing LAB has a greater ability to withstand technological stresses and survive the passage through the gastrointestinal tract compared to their nonproducing bacteria (Stack, 2010). Hence, the choice of EPS-producing starter culture seems to give several advantages over nonproducing ones.

Table 6. Frequency distribution of lactic acid bacteria of different genera based on their carbohydrate fermentation profile.

| Genus | Species | Number of isolates | % of total isolates |
|---|---|---|---|
| *Lactobacillus* | *Lactobacillus delbrueckii subsp. lactis* | 12 | 8.22 |
| | *Lactobacillus brevis* | 2 | 1.37 |
| | *Lactobacillus paracasei subsp. tolerans* | 5 | 3.42 |
| | *Lactobacillus delbrueckii subsp. bulgaricus* | 8 | 5.48 |
| | *Lactobacillus helveticus* | 2 | 1.37 |
| | *Lactobacillus casei subsp. casei* | 9 | 6.16 |
| | *Lactobacillus plantarum* | 18 | 12.33 |
| | *Lactobacillus acidophillus* | 35 | 23.97 |
| | *Lactobacillus amylophilus* | 15 | 10.27 |
| *Lactococcus* | *Lactococcus lactis subsp.lactis* | 5 | 3.42 |
| | *Lactococcus garviae* | 3 | 2.05 |
| | *Lactococcus raffinolactis* | 4 | 2.74 |
| | *Lactococcus lactis subsp. cremoris* | 2 | 1.37 |
| *Streptococcus* | *Streptococcus cremoris* | 4 | 2.74 |
| | *Streptococcus lactis* | 3 | 2.05 |
| | *Streptococcus salivarius subsp.thermophilus* | 5 | 3.47 |
| *Enterococcus* | *Enterococcus faecalis* | 2 | 1.37 |
| | *Enterococcus casseliflavus* | 2 | 1.37 |
| *Pediococcus* | *Pediococcus pentosaceus* | 2 | 1.37 |
| | *Pediococcus damnosus* | 5 | 3.42 |
| | *Pediococcus acidilactici* | 3 | 2.05 |
| Total | | 146 | 100 |

## 4. Conclusion

This study has demonstrated that 21 species of lactic acid bacteria were isolated from camel milk. The most dominant lactic acid bacterial species was *Lactobacillus* that comprised 72.6% of the total lactic acid bacteria isolates. Based on the overall technological properties, *Lactobacillus acidophilus* HUM20, *Lactobacillus plantarum* HUM19, and *Streptococcus cremoris* HUM8 were high in acidifying and proteolytic activities, exopolysaccharide production (EPS), and antimicrobial activities, implying that these bacteria could be used as starter cultures for the industrial processing of camel milk under controlled environments in the future. However, further research should be conducted to elucidate performance in mixed cultures, EPS quantification and lipolytic activities, aroma production and other desirable characteristics of the isolates as well as their molecular attributes to determine their suitability for commercial production of fermented camel milk products.

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