

Carbohydrate Fermentation Profile and Physiological Studies of Lactic Acid Bacteria from Native Raw Cow Milk

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Authors' contributions

This work was carried out in collaboration between both authors. Author ABA designed the study, wrote the protocol and wrote the first draft of the manuscript. Author ABA also managed the analyses of the study. Author SA gave scientific suggestions, managed the literature searches, results and discussion. Both authors read and approved the final manuscript.

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ABSTRACT

The aim of this study was to identify and evaluate the physiological studies of lactic acid bacteria from native raw cow milk. Cow milk samples were collected purposively from four different locations in Ibadan. The proximate analysis, pH and acidity of the milk samples were examined using standard procedures. Lactic acid bacteria (LAB) were isolated, characterized, and identified using both morphological, biochemical test, and Analytical profile index (API) system. The identified LAB were subjected to various physiological conditions such as growth at different temperature (15, 30, 45°C), pH (4, 6, 8) and NaCl concentrations (4, 6, and 8%). The heterotrophic counts ranged between 3.1×10^7 to 4.2×10^7 CFU/mL and lactic acid bacteria counts ranged from 2.2×10^7 to 3.8×10^7 CFU/mL. Thirty-five LAB isolates were randomly picked and identified as *Lactobacillus* (57.15%), *Streptococcus* (14.29%), *Leuconostoc* (8.57%), *Pediococcus* (8.57%), *Lactococcus* (5.71%) and *Enterococcus* (5.71%). The LAB isolates were further identified as *Lactobacillus plantarum*, *Leuconostoc mesenteroides*, *Lactococcus lactis*, *Lactobacillus acidophilus* and

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Lactobacillus bulgaricus based on API 50 test kit, and were able to grow at different physiological parameters. This study shows that *Lactobacillus* strains isolated from raw cow milk had better physiological attributes. These LAB could be recommended for further assessment such as evaluation of probiotic potential properties and genomic analysis.

Keywords: Isolation; identification; lactic acid bacteria; physiological; raw cow milk.

1. INTRODUCTION

Raw cow milk is a pale liquid produced by the mammary glands of female cattle. It contains protein, water, ash, fat, lactose and minerals. Moreover, milk itself is known as one of the natural habitats of lactic acid bacteria (LAB), hence composition of milk vary considerably between cows of different, and same breeds [1,2]. Milk, and other dairy products are nutritious food items containing numerous essential nutrients, and characterized by a rich biodiversity of microorganisms. Raw milk is the most used product for obtaining useful cultures in food industry [3]. LAB plays important role in the development of organoleptic characteristics (flavors and nutritional qualities) of fermented dairy products like yoghurt, kefir, and cheese.

Moreover, the selection of lactic acid bacteria during manufacture of various fermented dairy products is based on the production of lactic acid, diacetyl, peptides and aromatic compounds. These bacterial flora (lactic acid bacteria) has been the subject of several research studies and are still in existence. [4]. These LAB could produce antimicrobial compounds that promote probiotic properties as a result of inhibition of spoilage and food borne pathogens in both dairy and non-dairy products [5].

LAB are gram-positive bacteria, non-spore forming, cocci or rods, which produce lactic acid as the major end product during the fermentation of carbohydrates. The LAB group comprises the genera *Lactobacillus*, *Streptococcus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Aerococcus*, *Alloicoccus*, *Dolosigranulum*, *Enterococcus*, *Globicatella*, *Lactospaera*, *Oenococcus*, *Carnobacterium*, *Tetragenococcus*, *Vagococcus* and *Weissella*. Historically, the genera *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus* are the core LAB group [6,7]. The most frequently isolated LAB genera from raw milk and dairy products are *Enterococcus*, *Lactococcus*, *Lactobacillus*, *Leuconostoc*, and *Streptococcus*. The raw milk microflora is also important for the ripening of cheese [8].

However, lactic acid bacteria possessed nutritional and therapeutic benefits, and information concerning their physiological studies and identification procedures using API system are scarce. Therefore, there is need to identify lactic acid bacteria from samples of raw cow milk using rapid kit, and evaluate their physiological studies.

2. MATERIALS AND METHODS

2.1 Collection of Samples

A total number of 8 samples of raw cow milk were collected from 8 different lactating cows at 4 different milking point in Ibadan, Oyo state, Nigeria. These locations are Research and Dairy Farm at University of Ibadan, *Kara* at Bodija market, Akinyele market and Sabo cattle market. The raw cow milk samples of 100 mL each were collected aseptically in sterile sample bottles, kept in an iced packed container at 4°C, and transported to the Central Laboratory, University of Ibadan, Oyo State for onward microbiological assessment.

2.2 Culture Medium and Sterilization Procedures

For isolation of Lactic acid bacteria, the culture media used was de Man Rogosa and Sharpe Agar (MRS agar) while Nutrient agar (NA) was used for total heterotrophic counts. The culture medium was weighed using weighing machine and then poured into one litre Erlenmeyer flask, and 1000 mL of distilled water was added. The solution in the flask was homogenized on hot plates for 10 minutes to dissolve the component. The culture media was sterilized by autoclaving for 15 minutes at 121°C, and allowed to cooled to 40-45°C before pouring into plates. All glasswares were autoclaved at 121°C for two hours.

2.3 Proximate Analysis of Raw Cow Milk Samples

The determination of protein, carbohydrate, fat, moisture, and mineral content were carried out

on raw cow milk samples using standard procedures according to method of [9].

2.4 Determination of Titratable Acidity and pH of Cow Milk Samples

The titratable acidity was determined by titrating 20 mL of cow milk samples with 0.1M of NaOH and 3 drops of phenolphthalein indicator (0.5% in 50% alcohol). This was done at 0, 24 and 48 hours. Each milliliter of N NaOH is equivalent to 90.08 mg of lactic acid. The titratable acidity acid was calculated according to [9].

$$\text{Titratable acidity} = \frac{\text{M1 NaOH} \times \text{N NaOH} \times \text{M.E.} \times 100}{\text{Volume of sample}}$$

Where MI NaOH= Volume of NaOH used,
N = NaOH= Normality of NaOH solution
M.E.=Equivalence Factor

The pH of the cow milk samples was also determined during incubation time of 0, 24 and 48 hours with a pH meter (pHep ® H198128 by HANNA)

2.5 Isolation and Characterization of Lactic Acid Bacteria from Raw Cow Milk

The raw milk sample was fermented for a period of 24 hours. Aseptically, 1 mL of each milk sample were added into 9 mL of sterile water and mixed thoroughly and a serial dilution of 10^{-6} was made. One mL of the appropriate dilution (10^{-5}) was then plated on MRS (de Mann Rogosa and Sharpe) agar using pour plate method, and it was further incubated anaerobically at 37°C for 48 hours. After incubation, colonies having different morphology, shape and size were sub-cultured until pure culture were confirmed. All pure cultures were maintained as stocks in MRS broth at -4°C with 15% glycerol. However, cultural morphology such as size, shape colour and cellular characterization (Gram's staining) were assessed using macroscopic and microscopic techniques, respectively according to the methods described by [10]. Biochemical tests such as catalase, indole, oxidase, motility, endospores, arginine production from ammonia, starch hydrolysis, fermentation of sugars (fructose, glucose, sucrose, mannitol, sorbitol), methyl red, and voges-proskauer were conducted on the LAB isolates.

2.6 Identification of Isolates

The isolates obtained were preliminarily identified by reference to Bergey's Manual of Systematic Bacteriology and an approach to the classification of Lactobacilli. A rapid kit known as API 50CHL was used as confirmatory test of identification. Moreover, identification of lactobacillus was accomplished by using API 50 CHL micro-identification systems (Bio Mérieux, France) and incubated at 37°C for 48 hours. However, Lactococcus was identified using API 20 STREP (Bio Mérieux, France) according to the manufacturer's instructions. In this study, API 20 STREP strips (acetoin production; hypurate, esculin and arginine hydrolysis; pyrrolidonyl- aralamydase, α-galactosidase, βgalactosidase, β-glucuronidase activity and utilization of ribose, arabinose, mannitol, sorbitol, lactose, trehalose, inulin, raffinose, starch, glycogen and glycerol) were incubated at 37°C and examined after 24 hours of incubations The interpretation of the fermentation profiles was facilitated by the use of the computer-aided database "APIWEB" (Bio Mérieux).

2.7 Physiological Studies of Identified LAB Isolates

The identified LAB isolates were assessed for growth at 10°C, 15°C and 45°C, growth at 4%, 6% and 8% NaCl concentrations, growth at pH 4, 6 and 8, including haemolysis (α) and haemolysis (β) test. It was carried out using the method of [9] and [11].

3. RESULTS

Table 1 shows the proximate analysis of cow milk samples obtained from four (4) different locations. Samples F and G had the least protein content of 3.20% while E had the highest protein content 3.63%. The highest carbohydrate and fat content was observed in sample A with the value of 6.40% and 3.90%, respectively but with the least mineral content of 0.60%. Sample A had the least moisture content of 85.60% but there was considerable little variation in other samples collected.

Fig. 1 shows the pH and titratable acidity (TA) of raw cow milk samples. Sample A had the highest pH(6.90,6.32 and 5.82) at 0, 24 and 48 hours respectively, while samples F and G had the lowest pH value (6.10) at 0 hour. In all the samples, it was observed that the pH values decreased at 0 to 48 hours of incubation. The

titratable acidity ranged from 0.28 to 0.10 as shown in Fig. 1. The lowest TA (0.10) was observed in samples C and D at 0 hour while the highest TA (0.28) was observed in sample H at 48 hours which increases over time. The highest TA was observed in every samples at 48 hours.

The total heterotrophic and LAB counts in raw cow milk samples are shown in Table 2. Sample A had the highest total heterotrophic counts and LAB counts of 4.2×10^7 CFU/mL and 3.8×10^7 CFU/mL, respectively and the least heterotrophic and LAB counts was observed in Sample F with value of 3.1×10^7 CFU/mL and 2.2×10^7 CFU/mL respectively.

The morphological, biochemical characterization and identification of lactic acid bacteria are shown in Table 3. Thirty-five presumptive LAB were isolated from raw cow milk samples. All isolates were non-motile, microaerophilic, gram-positive rod to cocci, and non-spore formers. They were also negative to Voges- proskauer (V.P), Methyl red (M.R), Indole, oxidase and catalase test.

A total of nineteen LAB isolates were found to be rod-shaped strains with long and rounded ends, mostly appeared as chains, pairs or single cells, fermented all the six sugars used and they belong to the genus *Lactobacillus*. However, two of the isolates were cocci, fermented all sugars except sucrose and sorbitol and were characterised as *Lactococcus* sp. while two isolates characterized as *Pediococcus*, were able to ferment all the six sugars except sorbitol.

Futhermore, five of the isolates were cocci (*Streptococcus*), fermented glucose and lactose, variability in fructose, maltose and sucrose and unable to ferment sorbitol, while three of the isolates characterized as *Enterococcus* with cocci to ovoid shape were able to ferment glucose, fructose and lactose, and unable to ferment sorbitol. Also, three of the isolates identified as *Leuconostoc* sp. with cocci to ovoid cell shape were able to ferment glucose, lactose, maltose, sucrose but unable to ferment sorbitol and fructose.

Fig. 2 shows the percentage occurrence of lactic acid bacteria isolated from raw cow milk samples. They are members of the genus *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Streptococcus*, *Enterococcus* and *Pediococcus*. *Lactobacillus* sp. has the highest occurrence (57.15%), followed by *Streptococcus* sp

(14.29%). *Pediococcus* sp. and *Leuconostoc* sp. had percentage occurrence of 8.57% each while the least (5.71%) was observed by *Lactococcus* sp and *Enterococcus* sp.

In addition to the preliminary phenotypic tests. the fermentation profile of carbohydrate for the LAB isolates is summarized in Table 4. Table 4 shows the identification of lactic acid bacteria isolated from raw cow milk using API 50CHL. The LAB were identified as *L. plantarum*, *L. mesenteriodes*, *Lactococcus. lactis*, *L. acidiphilus* and *L. bulgaricus*. Among the isolates, only *L. mesenteriodes* was able to produce carbon dioxide from glucose fermentation.

The physiological studies of lactic acid bacteria is shown Table 5. The selected LAB showed good growth towards different temperature. At 10°C, 60% of the LAB isolates had least growth while 40% did not grow. At 15°C, 60% had abundant growth, 20% showed moderate growth while 20% had least growth. During growth at 45°C, 60% had no growth, 20% grew moderately and 20% had abundant growth. *Lactobacillus plantarum* and *L. bulgaricus* were the only LAB that grew at 45°C. All the isolates grew moderately in 6% salt concentration but had least growth in 8% NaCl except *L. lactis* with moderated growth. All isolates did not grow at 4% NaCl except *L. plantarum* that showed least growth. However, none of the strains produced haemolysin (α -hemolysis and β -hemolysis) from sheep's blood agar. All LAB were able to grow at pH 4, 6 and 8 except *L. mesenteriodes* which did not grow at pH 4.

4. DISCUSSION

The nutritional analysis of the raw cow milk samples shows that they are of appreciable nutritional status. There was slight variation in the protein, carbohydrate, fat, minerals and moisture content between samples from different cows. The average protein content of raw milk obtained in this study is in agreement with the reported value (3.48%) from Sudan [12]. The lowest fat content observed from Sample G could be attributed to inability of the cow to consume more feeds. The variation may be considerable low due to the kind of feeds utilized by the cows before and during the stage of lactation. Quadghiri et al., [19] have reported that the composition of raw milk is mainly influenced by the stage of lactation, time of year, and kind of feeds. The least carbohydrate content of 4.41%

was from Sample C which could also be due to difference in breed, feeding and management practices which have important effects on milk composition and quality [13]. Protein content from all samples were in accordance with the USDA standard which state that the standards for protein content of unprocessed whole cow milk should not be less than 2.97% [14].

Table 1. Proximate analysis of raw cow milk (%)

Samples	Proximate contents (%)				
	Protein	Carbohydrate	Fat	Mineral	Moisture
A	3.50±0.1*	6.40±0.55	3.90±0.14	0.60±0.01	85.60±0.20
B	3.50±0.01	4.50±0.30	3.70±0.01	0.80±0.01	87.50±0.22
C	3.61±0.20	4.41±0.12	3.75±0.02	0.83±0.30	87.40±0.01
D	3.52±0.01	4.63±0.31	3.70±0.15	0.65±0.11	87.50±0.25
E	3.63±0.52	4.80±0.02	3.87±0.02	0.80±0.02	86.90±0.45
F	3.20±0.20	4.90±0.14	3.70±0.10	0.70±0.03	87.50±0.50
G	3.20±0.11	5.00±0.20	3.60±0.05	0.70±0.01	87.50±0.16
H	3.25±0.03	5.00±0.01	3.70±0.01	0.85±0.20	87.20±0.20

Key: *Values are means of duplicates ± Standard deviation

pH and Titratable acidity of raw cowmilk

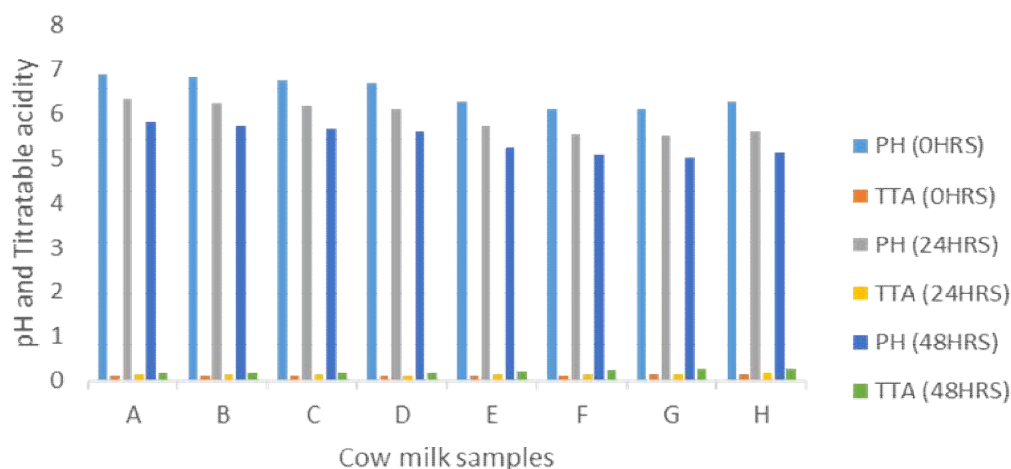


Fig. 1. pH and titratable acidity (TA) of raw cow milk

Table 2. Total heterotrophic and LAB counts in raw cow milk samples ($\times 10^7$ CFU/ml)

Samples	Total heterotrophic counts	Lactic acid bacteria counts
A	4.2	3.8
B	4.1	3.6
C	4.1	3.4
D	3.6	3.3
E	3.5	2.8
F	3.1	2.2
G	3.5	2.7
H	3.2	2.50

Table 3. Morphological and preliminary biochemical characterisation of Lactic acid bacteria from raw cow milk samples

Isolates	Cell morp.	Gr	Mo	Sp	Mr/Vp	In	Ox	Ca	So	Ma	Fr	La	Su	Gl	Organism
A ₁	Rod	+	-	-	-	-	-	-	+	+	+	+	+	+	<i>Lactobacillus sp.</i>
A ₂	Rod	+	-	-	-	-	-	-	+	+	+	+	+	+	<i>Lactobacillus sp.</i>
B ₁	cocci chain	+	-	-	-	-	-	-	-	±	±	+	±	+	<i>Streptococcus sp.</i>
B ₂	cocci/ovoid	+	-	-	-	-	-	-	-	+	-	+	+	+	<i>Leuconostoc sp.</i>
C ₁	Cocci	+	-	-	-	-	-	-	-	+	+	+	+	+	<i>Pediococcus sp.</i>
C ₂	Cocci	+	-	-	-	-	-	-	-	+	+	+	-	+	<i>Lactococcus sp.</i>
C ₃	Cocci	+	-	-	-	-	-	-	-	+	+	+	+	+	<i>Pediococcus sp.</i>
C ₄	cocci/ovoid	+	-	-	-	-	-	-	-	+	-	+	+	+	<i>Leuconostoc sp.</i>
D ₁	Rod	+	-	-	-	-	-	-	+	+	+	+	+	+	<i>Lactobacillus sp.</i>
D ₂	cocci chain	+	-	-	-	-	-	-	-	±	±	+	±	+	<i>Streptococcus sp.</i>
D ₃	cocci/rod	+	-	-	-	-	-	-	-	+	+	+	-	+	<i>Lactococcus sp.</i>
D ₄	Rod	+	-	-	-	-	-	-	+	+	+	+	+	+	<i>Lactobacillus sp.</i>
D ₅	Rod	+	-	-	-	-	-	-	+	+	+	+	+	+	<i>Lactobacillus sp.</i>
E ₁	Cocci	+	-	-	-	-	-	-	-	+	+	+	+	+	<i>Pediococcus sp.</i>
E ₂	Rod	+	-	-	-	-	-	-	+	+	+	+	+	+	<i>Lactobacillus sp.</i>
E ₃	Rod	+	-	-	-	-	-	-	+	+	+	+	+	+	<i>Lactobacillus sp.</i>
E ₄	cocci/ovoid	+	-	-	-	-	-	-	-	+	-	+	+	+	<i>Leuconostoc sp.</i>
F ₁	cocci/chain	+	-	-	-	-	-	-	-	±	±	+	±	+	<i>Streptococcus sp.</i>
F ₂	Rod	+	-	-	-	-	-	-	+	+	+	+	+	+	<i>Lactobacillus sp.</i>
F ₃	Rod	+	-	-	-	-	-	-	+	+	+	+	+	+	<i>Lactobacillus sp.</i>
G ₁	cocci chain	+	-	-	-	-	-	-	-	±	±	+	±	+	<i>Streptococcus sp.</i>
G ₂	Rod	+	-	-	-	-	-	-	+	+	+	+	+	+	<i>Lactobacillus sp.</i>
H ₁	Rod	+	-	-	-	-	-	-	+	+	+	+	+	+	<i>Lactobacillus sp.</i>
H ₂	Rod	+	-	-	-	-	-	-	+	+	+	+	+	+	<i>Lactobacillus sp.</i>
H ₃	Rod	+	-	-	-	-	-	-	+	+	+	+	+	+	<i>Lactobacillus sp.</i>
H ₄	Rod	+	-	-	-	-	-	-	+	+	+	+	+	+	<i>Lactobacillus sp.</i>
H ₅	Rod	+	-	-	-	-	-	-	+	+	+	+	+	+	<i>Lactobacillus sp.</i>
H ₆	cocci/ovoid	+	-	-	-	-	-	-	-	±	+	+	±	+	<i>Enterococcus sp.</i>

Isolates	Cell morp.	Gr	Mo	Sp	Mr/Vp	In	Ox	Ca	So	Ma	Fr	La	Su	Gl	Organism
H ₇	Rod	+	-	-	-	-	-	-	+	+	+	+	+	+	<i>Lactobacillus sp.</i>
H ₈	cocci/ovoid	+	-	-	-	-	-	-	-	±	+	+	±	+	<i>Enterococcus sp.</i>
H ₉	Rod	+	-	-	-	-	-	-	+	+	+	+	+	+	<i>Lactobacillus sp.</i>
H ₁₀	cocci/ovoid	+	-	-	-	-	-	-	-	±	+	+	±	+	<i>Enterococcus sp.</i>
H ₁₁	Rod	+	-	-	-	-	-	-	+	+	+	+	+	+	<i>Lactobacillus sp.</i>
H ₁₂	cocci/chain	+	-	-	-	-	-	-	-	±	±	+	±	+	<i>Streptococcus sp.</i>
H ₁₃	Rod	+	-	-	-	-	-	-	+	+	+	+	+	+	<i>Lactobacillus sp.</i>

Key: + = positive, - = negative, ± = variables, Cell mor = cell morphology, Gr = gram's reaction, Mo = motility, Sp = spore formation, In=indole, Ox = oxidase, Ca= catalase, So=sorbitol, Ma = maltose, Fr = fructose, La = Lactose, Su = sucrose, Gl = glucose

Table 4. Carbohydrate fermentation profile of Lactic acid bacteria using API 50CHL system

Sugar fermentation	LAB isolates				
	A1	C4	D3	G2	H9
Glycerol	-	-	-	-	-
Erytritol	-	-	-	-	-
D-arabinose	-	-	-	-	-
L-arabinose	+	+	+	-	-
Ribose	+	+	+	-	-
D-xylose	-	+	-	-	-
L-xylose	-	-	-	-	-
B-methi-xyloside	-	-	-	-	-
Galactose	+	+	+	-	-
D-glucose	+	+	+	+	+
D-fructose	+	+	-	+	+
D-mannose	+	+	+	+	+
L-sorbose	-	-	-	-	-
Rhaminose	-	-	-	-	-
Inositol	-	-	-	-	-
Mannitol	+	-	-	-	-
Sorbitol	+	-	-	-	-
α-methyl-mannoside	+	-	-	-	-
α-methyl-Dglucoside	-	+	-	-	-
Nacetylglucosamine	+	Wg	-	-	-
Esculine	+	-	-	-	-
Cellobiose	+	+	-	+	-
Maltose	+	+	-	Wg	-
Lactose	+	Wg	-	+	+
Melibiose	+	+	-	-	-
Saccharose	+	+	Wg	+	-
Trehalose	+	+	+	+	-
D-raffinose	Wg	+	-	-	-
Starch	-	-	-	-	-
D-fucose	-	-	-	-	-
L-fucose	-	-	-	-	-
D-arabitol	Wg	-	-	-	-
L-arabitol	-	-	-	-	-
Percentage of identity	99%	95%	99%	95%	99%
API identification	<i>Lactobacillus plantarum</i>	<i>Leuconostoc mesenteroides</i>	<i>Lactococcus lactis</i>	<i>Lactobacillus acidophilus</i>	<i>Lactobacillus bulgaricus</i>

Key: +=positive, -=negative, A1, C4,D3, G2 and H9 = LAB isolates codes, wg=weak reaction

The pH of the studied cow milk samples had an appreciable values of less than 6.0 at incubation time of 48 hours. This conforms with the work done by [15] who reported a significant value at 48 hours. This could be attributed to the metabolism rate and strain of the LAB that metabolized the milk. The pH of raw cow milk can increase to 7.2 due to clinical mastitis, hence lower pH is considered inhibitory to growth of pathogenic microorganisms [16].

However, titratable acidity (TA) is normally used to estimate the freshness of milk, and to monitor the production of lactic acid during fermentation. Our results agreed with the findings of [15] which revealed the TA ranged between 0.55 and 1.2% with a mean value of $0.76 \pm 0.018\%$ at 0 hour, hence the higher the pH the lower the titratable acidity. The higher acidity of milk obtained may be due to the high bacterial growth, metabolism and multiplication during transportation of the milk to the laboratory. The total heterotrophic

counts and lactic acid bacteria counts were within the range of 10⁷ CFU/mL. In most areas of the world, the LAB counts are usually between the range of 10⁷ to 10⁹ CFU/mL in raw cow milk. It was suggested that LAB counts are higher in raw milk than powdered or dried milk.

In this study, LAB were isolated and characterized. The cultural, cellular and biochemical characteristics of the isolated LAB were similar with the findings of [17]. These

microorganisms are usually found in raw cow milk due to their ability to utilise the substrate for growth and metabolism. The dominance of *Lactobacillus plantarum* observed in this study could be as a result of decreased pH. In another study, lactococcal strains were isolated from raw milk in Camembert cheese area and identified by using both phenotypic criteria (physiological and biochemical tests). The classification of lactic acid bacteria into different genera is largely based on morphology, mode of glucose

Table 5. Physiological studies of identified lactic acid bacteria from raw cow milk

Test	LAB isolates				
	<i>L. plantarum</i>	<i>L. mesenteriodes</i>	<i>Lac. Lactis</i>	<i>L. acidophilus</i>	<i>L. bulgaricus</i>
Growth at different temp. (°C)					
10	+	+	+	-	-
15	+++	+	++	+++	+++
45	+	-	-	++	+++
Growth at different NaCl Concentrations (%)					
4	+	-	-	-	-
6	++	++	++	++	++
8	+	+	++	+	+
Growth at different pH					
4	++	-	+++	+	++
6	++	++	++	++	++
8	++	+++	+	+	++
Hemolysis (β)	-	-	-	-	-
Hemolysis(α)	-	-	-	-	-

Key: +++ = Abundant growth, ++ = Moderate growth, + = Least growth, - = No growth
 L= *Lactobacillus*, Lac= *Lactococcus*

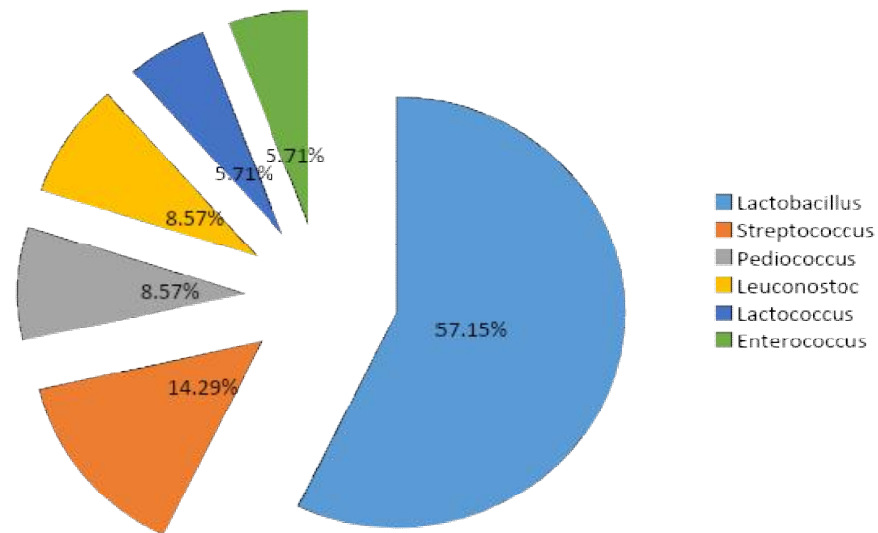


Fig. 2. Percentage occurrence of Lactic acid bacteria (LAB) isolated from raw cow milk

fermentation, growth at different temperature, configuration of the lactic acid produced, ability to grow at high salt concentrations, and acid or alkaline tolerance. These organisms can be divided into two groups based on the end-products formed during the fermentation of glucose, and such organism could be homofermentative (*Streptococcus*, *Lactococcus* and some *lactobacilli*) or heterofermentative lactic acid bacteria (*Weissella*, *Leuconostoc*. and some *lactobacilli*) that produce equimolar amounts of lactate, CO₂ and ethanol from glucose via the hexose. The highest percentage occurrence of *Lactobacillus* is in agreement with the findings of [18] who reported *Lactobacillus* sp. as the relatively dominating species of cow milk. *Leuconostoc* sp. had low percentage which agreed with the studies of [18] who reported lowest occurrence probably due to their inability to compete with other LAB in mixed cultures environment.

The studied LAB were able to tolerate 6-8% NaCl concentrations which revealed their effectiveness and activities when grown in this particular salt. [19] reported the tolerance of some *Lactobacillus* species from raw cow milk to 4- 10% NaCl concentrations in Jordan. However, reports achieved from this experimental studies are similar to the work done by [11], on the tolerance of LAB to 4- 8% NaCl.

Moreover, the studied LAB were able to grow at temperature 10°C, 15°C, and 45°C which is similar to the temperature of some psychrophiles and thermophiles. Moreover, since they can grow in this temperature range, this indicates they can resist cold or high conditions which is essential parameter for them to be more effective. The results of this experimental temperature test were similar to the work of [11] which reported LAB could grows at 10°C and 24°C.

Furthermore, the identified LAB were subjected to both acidic pH, and alkaline conditions. The reasons for adapting to low pH(acidic pH) was suggested to be controlled by several genes synthesized by most LAB which can be called pre challenge adaptation and transient adaptation, but occurs during low pH. This study reveals that most of the studied LAB were able to survive the hostile environment similar to stomach, indicating production of organic acids, and ability to live within the medium they grow in.

In addition, this work reveals that LAB isolated from raw cow milk were able to survived pH 4

similar to the stomach, and alkaline conditions (pH 8) similar to the small intestine. The ability for them to survive could also be intrinsic or inherent. This is in agreement with work of [11], that revealed growth of *Lactobacillus* in pH 2.5 to pH 8.5. The reason for choosing this pH range was to determine whether the isolated strains could grow in both acidic and alkaline conditions. Similar research presented that both acidic and alkaline conditions are usually used to evaluate physiological studies of lactic acid bacteria. The studied LAB were also non- α -hemolysis and β -hemolysis strains in blood agar media, indicating them as safe organisms.

5. CONCLUSION

Lactic acid bacteria such as *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Streptococcus*, *Enterococcus* and *Pediococcus* were isolated, characterized and identified from raw cow milk samples. This study demonstrated that *Lactobacillus plantarum* grew well at different pH, salt concentrations and temperature, indicating better physiological attributes which could serve as beneficial candidates in future researches. Further studies will be required on molecular characterization and assessment of probiotic potential.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Abebe BZ, Ajebu NY. Hygienic and microbial quality of raw whole cowmilk produced in Ezha district of the Gurage zone, Sourthern Ethiopia wudpecker Journal of Agric. 2012;1(11):459-465.
2. Delavenne E, Mounier J, Déniel F, Barbier G, Le Blay G. Biodiversity of antifungal lactic acid bacteria isolated from raw milk samples from cow, ewe and goat over one-year period. Int. J. Food Microbiol. 2012; 155(3):185–190.
3. Asfour HA, Gamal IM, Darwish SF. Phenotypic characterization and molecular identification of some lactic acid producing bacteria in raw milk of different animal species. Assiut Veterinary Medical Journal. 2016;62:47-59.
4. Mchiouer K, Bennani S, El-Gendy NS, Meziane M. Identification and antibiotic resistance of *Lactobacilli* isolated from raw

- cow's milk of Oujda City (Morocco). Energy Sources, Part A: Recovery, Utilization, and Environmental Effects. 2016;38:3572-3577.
5. Sumathi V, Reetha D. Screening of Lactic acid bacteria for their antimicrobial activities against pathogenic bacteria. International journal of pharmaceutical and biological Archives. 2012;3(4): 802-808.
 6. Hayek SA, Ibrahim SA. Current Limitations and Challenges with Lactic Acid Bacteria: A Review. Food and Nutrition Sciences. 2013;4:73-87.
 7. Luana MP, Luis AN. Antagonistic Lactic Acid Bacteria isolated from goat milk and identification of a novel nisin variant *Lactococcus lactis*. BMC Microbiology. 2014;14:36-39.
 8. Corroler D, Mangin I, Desmasures N, Gueguen M. An ecological study of lactococci isolated from raw milk in the Camembert Cheese Registered Designation of Origin area. Appl. Environ. Microbiol. 1998;64:4729-4735.
 9. AOAC. Official Methods of Analysis. 15th ed. Washington DC: Association of Official Analytical Chemist; 2013. ISBN 2-93558442-0
 10. Dykes GA, Britz TJ, Von Holy A. Numerical taxonomy and identification of Lactic acid bacteria from spoiled, vacuum packed Vienna sausages. Journal of Applied Bacteriology. 1994;76:246-252.
 11. Hoque F, Akter KM, Hossain MSM, Rahman MM, Billah, Islam KMD. Isolation, identification and analysis of probiotic properties of *Lactobacillus spp.* From Selective Regional Yoghurts. World Journal of Dairy & Food Sciences. 2010;5(1):39-46.
 12. Elrahman Abd, Ahmad SM, El Owni AM, Ahmed MK. Microbiological and physicochemical properties of raw milk used for processing pasteurized milk in blue Nile dairy company (Sudan)". Australian Journal of Basic and Applied Sciences. 2009;3(4): 3433-3437.
 13. Mchiouer K, Bennani S, El-Gendy NS, Meziane M. Identification and antibiotic resistance of *Lactobacilli* isolated from raw cow's milk of Oujda City (Morocco). Energy Sources, Part A: Recovery, Utilization, and Environmental Effects. 2016;38:3572-3577.
 14. Teshome G. Review on lactic acid bacteria function in milk fermentation and preservation. Afr. J. Food Sci. 2015;9(4): 170-175.
 15. El-kholy SK, El-shinawy SH, Meshref AM, Korney AM. Screening of antagonistic activities of probiotic bacteria against some food-borne pathogens. Journals of Applied and Environmental Microbiology. 2014;2(2): 53-60.
 16. Barat ZY, Morteza KA, Alireza SM, Seid MJ. Hygienic quality of camel milk and fermented camel milk (chai) in Golestan province, Iran. Journal of Microbiology Research. 2014;4(2):98-103.
 17. Quadghiri MV, Naser SP, Evers DG, Lefebvre KF, Amar MA. Identification of Lactic acid bacteria in Moroccan raw milk and traditionally fermented skimmed milk "Iben". Journal of Applied Microbiology; 2008.
 18. Steele J, Broadbent J, Kok J. Perspective on the contribution of lactic acid bacteria to cheese flavor development. Current Opinion in Biotechnology. 2013;24(2):135-141.
 19. Al-Mahadin A, Abushelaibi SK, El-Tarabily N, Shah P, Ayyash M. Characterization potential probiotic lactic acid bacteria isolated from camel milk. LWT-Food Science and Technology. 2017;79:316-325.

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