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Isolation, Characterization and Identification of Industrially Beneficial Probiotic Lactic Acid Bacteria from Goat Milk

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ABSTRACT

Purpose: *Goat's milk is enriched with many nutrients and diverse lactic acid bacteria (LAB). This research was aimed at isolating and characterizing industrially beneficial probiotic lactic acid bacteria present in goat milk and to investigate their ability to produce lactic acid.*

Research Method: *A total of 100 bacterial isolates were obtained from 20 fresh goat's milk samples from Western and North Western provinces in Sri Lanka. These isolates were characterized based on their colony and cell morphologies, physiological and biochemical characteristics, tolerance to in-vitro gastro intestinal tract (GIT) conditions and industrial properties. Species level identification of the isolates was performed based on the obtained phenotypic properties according to the Bergey's manual of systematic bacteriology.*

Findings: *The selected eight probiotic LAB isolates did not produce hemolytic enzymes, therefore, could primarily consider as safe for live consumption. According to the phenotypic properties, the isolates were of Lactobacillus pentosus, Lactobacillus plantarum, Streptococcus thermophilus, Streptococcus bovis, Lactococcus lactis and Enterococcus faecium species. They were able to coagulate skimmed milk which was indicated by the decrease of final pH (4.8-5.9), tolerate to high (45°C) and low (15°C) temperatures, grow in different NaCl concentrations (2%, 4%, and 6.5%) and different pH levels (1.5-3.9). HPLC analysis revealed that, Lactobacillus pentosus (33 mg/L) and Streptococcus thermophiles (14mg/L) possessed the highest lactic acid production abilities.*

Research Limitations: *The species level identification of the isolates should be continued employing genotypic methods. The safety of the isolates for live consumption should confirm with in-vivo methods.*

Originality/Value: *The raw goat milk microbiota is considered as a good source of novel lactic acid bacteria (LAB) strains that can be exploited for use as industrial starters and probiotics. Lactic acid produced by the microorganisms; specifically, lactic acid bacteria is an important commodity chemical having many numbers of applications. However, very less research work has been done on the characterization of beneficial lactic acid bacteria present in goat milk produced in Sri Lanka.*

Keywords: *Biochemical properties, Goat's milk, Lactic acid production, Lactobacillus, Morphological properties*

INTRODUCTION

Goat's milk is one of the closest alternatives to bovine milk for human consumption. It is highly nutritious, easily digestible and possesses many beneficial health effects (Zhang *et al.,* 2017). Lactic acid bacteria (LAB) are a group of Gram-positive, non-spore forming, cocci or rod shaped, catalase-negative organisms that produce large amounts of lactic acid from the fermentation of carbohydrates (Maslanka *et al.,* 2015). They are considered as 'Generally Recognized as Safe

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(GRAS) organisms and naturally present in milk (Chowdhury *et al.,* 2012; Misganaw and Teketay, 2016). *Lactobacillus*, *Micrococcus*, *Pediococcus, Streptococcus* and *Leuconostoc* are the major members of the lactic acid bacterial group. Most research targets to isolate new *Lactobacilli* strains as they are the main fermentative agents used in the dairy industry (Patil *et al.,* 2010).

However, only few studies have isolated the LABs in goat's milk, though it's a rich source for obtaining beneficial LAB species with a potential for promoting health, including probiotic properties (Vanniyasingam *et al.,* 2019). Further, scientific investigations on physicochemical and microbiological characteristics of goat milk and utilizing it for value added products are limited throughout the globe. Scientific evidence on using LABs isolated from goat milk as a fermentative agent for dairy products are extremely rare (Park, 2010; Sharma *et al.,* 2013). Moreover, for the best of our knowledge no comprehensive studies are being carried out to investigate the beneficial LAB species in goat milk in Sri Lanka.

The unique physical and chemical properties of goat's milk have made differences in the microbial compositions in raw goat milk. In particular, goat's milk contains lower level of casein compared to cow milk, with very little to no alfa-s1- casein, which leads to lower coagulation ability (Hodgkinson *et al.,* 2018; Ranadheera *et al.,* 2019). Thus, goat's milk shows lower coagulating properties in yoghurt and cheese making. Due to these unique characteristics of goat milk, the LAB typically used for the cow's milk fermentation, which probably isolated from cow's milk may not be suitable for the fermentation of goat's milk (Zhang *et al.,* 2017).

Furthermore, LAB species are famous for producing lactic acid as the major fermentation product which is an interesting compound because it can be utilized in the food, pharmaceutical and chemical industries. In addition, lactic acid has recently gained more interest due to the possibility of manufacturing poly lactic acid, which is a green polymer with numerous industrial and pharmaceutical applications (Bernardoa *et al.,*2016).

Therefore, the aim of this study was to isolate, characterize and identify the industrially beneficial probiotic lactic acid bacteria present in goat milk and to investigate their lactic acid production abilities for future industrial applications.

MATERIALS AND METHODS

Sample collection

Twenty *(n = 20)* fresh goat milk samples were collected during August to October of the year 2017 following the methods explained in the Sri Lanka Standard Institute Specification for milk and milk product sampling (SLS 1404:2010) from goat farms located in Western and North Western provinces of Sri Lanka. Samples were collected by hand expression after cleaning the udder area into sterilized polypropylene tubes (50 mL, disposable). Approximately 50 mL milk samples collected from each goat were cooled immediately and transported on ice to the microbiology laboratory of Food Technology Section, Industrial technology Institute, Sri Lanka to be stored at 4 °C for analysis, for no longer than 3-4 hours.

Isolation of LAB

Milk samples were aseptically homogenized, serially diluted and then plated in triplicate by pour and spread plate techniques respectively on de Man, Rogosa and Sharp (MRS) and M17 agar (Oxoid, UK) for isolating LABs (Colombo et al, 2018). The MRS agar was mainly used for the isolation of bacilli and the M17 agar was used for the isolation of cocci (Guessas and Kihal, 2004). Cycloheximide 0.01% (v/v) was added to MRS and M17 media in order to prevent the growth of yeast and fungi. The plates were incubated under anaerobic conditions in anaerobic jars (Oxoid, UK) containing gaspack (AnaeroGen, Oxoid, UK) at 37 °C for 2-3 days. Colonies with distinct colony morphologies were selected and purified by repeated streaking on respective agar pates.

Identification

***Morphological characterization of isolates*:** Each colony formation was observed and distinct morphological differences based on color, form, texture, size, elevation, colony forming units (CFU), margin and surface smoothness were recorded as explained in the Bergey's Manual of Systematic Bacteriology. The selected individual colonies with distinct morphologies were streaked onto fresh agar plates of the respective culture medium and incubated for further 48 hours at 37 °C under anaerobic conditions.

***Biochemical characterization*:** The isolated bacteria were identified as LAB species by battery of biochemical tests including Gram staining, motility test, endospore test, catalase test, indole test, urease test, oxidase test, Voges-proskauers, H_2S production test, arginine hydrolysis test, methyl red, acid/gas production from glucose, citrate test and SIM mortality test as described by Bennani *et al.,* 2017.

***Carbohydrate fermentation pattern of the isolates*:** All the isolates were also characterized according to their fermentation profiles of different carbohydrates (Glucose, D-trehalose, D-sucrose, D-mannitol, D-melezitose, D-mannose, L-arabinose, D-maltose, D-fructose, L-rhamnose, D-cellobiose, D-galactose, D-salicin, D-raffinose, D-sorbitol, D-melibiose and D-ribose) by adding the specific carbohydrate to the basal broth media (MRS and M17) prepared without adding sugars. Acid production was identified by a change in color and gas production was detected by observation of gas collection in the inverted Durham tubes following the method of Tserovska *et al.,* 2002 as explained by Thakur *et al.,* 2017.

In-vitro safety assessment of the isolates: Screening of hemolytic organisms (hemolytic test) was performed as described below. Sterile blood agar plates (Columbia blood agar plates, Oxoid, UK) were used for the screening of hemolytic organisms. Blood agar plates were inoculated with 20 µL of broth cultures and incubated at 37 °C for 2-3 days under anaerobic conditions and observed for the signs of hemolysis (α, β or ϒ hemolysis) (Silva *et al.,* 2019).

Determination of probiotic and technological potentials of isolates

***Temperature tolerance*:** Tolerance to temperature was determined according to the method of Khalid, (2011) with some modifications. Fresh LAB (24 hours old) cultures were prepared in sterilized MRS and M17 broth media and adjusted to 0.6 OD (optical density) at 600 nm (UV Visible spectrophotometer, Spectra max plus 384) to determine the temperature tolerance. Cultures were transferred to 96 -well micro titer micro plate and incubated aerobically at 15 °C, 37 °C and 45 °C over 5 hours. After every hour of incubation, samples were periodically drawn out to determine the cell concentration by measuring OD at 600 nm. All the experiments were replicated twice. Sample incubated at 37 °C was taken as the control experiment. Growth of the isolates was determined by comparing the hourly growth by measuring absorbance using the equation given below (Aswathy *et al.,* 2008).

1.0 OD at 600nm = 1 x 10^8 CFU/mL

***pH tolerance*:** Tolerance to different pH levels was determined following the method of Khalid, 2011 with some modifications. MRS broth was prepared and adjusted to different pH levels (1.5, 3 and 9) by using 1M HCl. Isolates were inoculated into pH adjusted media and incubated aerobically at 37 °C. The tolerance was measured following the method described under the temperature tolerance A control experiment was maintained without adjusting the medium pH (pH 6.5±0.2) and all the experiments were performed with replicates.

***NaCl tolerance*:** Tolerance to NaCl was determined according to the method of Khalid, 2011 with some modifications. MRS broth was prepared and adjusted to different NaCl levels by adding 2%, 4% and 6.5% NaCl. Isolates were inoculated to pH adjusted media and incubated aerobically at 37 °C. The tolerance was measured as described under the temperature tolerance. A control experiment was maintained without NaCl and all the experiments were performed with replicates.

Determination of Lactic acid production

Quantification of Organic Acid (HPLC method): The MRS and M17 broths were prepared and autoclaved at 121 °C and 15 psi pressure for 15 minutes. Around 1 mL of prepared media was added to each 2 mL eppendorf tube and inoculated with 300 µL of culture broth. Inoculums were incubated at 37 °C for 18-24 hours. Samples were centrifuged at 10000 rpm for 10 minutes and supernatant was filtered through 0.45 µm filter membranes prior to HPLC analysis. Agilent 1260 Infinity HPLC (Agilent, USA) equipped with a quaternary gradient pump, PDA detector (λ=210 nm) and Thermo stated column compartment was used for the analysis. Chromatographic separation was achieved with a RezexROA Organic Acid H+ 8% (7.8 mm × 300 mm, 5 µm, Phenomenex) analytical column and a Guard column (4.6 mm × 12.5 mm, 5 µm, Phenomenex). Mobile phase was consisted of 0.005 NH_2SO_4 with a flow rate of 0.7 mL/min and a total run time of 30 minutes. Injection volume was 10.0 µL. Column compartment was thermo stated at 30 °C. Lactic acid production was measured by peaks of the chromatograms resulted in HPLC (Sharma *et al.,* 2013).

***Detection of fermentation abilities of the isolates*:** Sterilized skim milk 10% (w/v) was inoculated with 3% from each isolate and fermented at 37 °C until the medium gets coagulated (Fguiri *et al.,* 2017). The pH of the samples was monitored at 0 hour, 2 hours, 4 hours, 6 hours and after 24 hours (during the fermentation period) using a digital pH meter (Thermo Scientific/Orion 3 STAR pH Bench top) calibrated with buffers at pH 4.0 and 7.0. The measurement of titratable acidity (TA) in terms of percentage lactic acid, was done by titrating 10 mL sample with 0.1N NaOH using phenolphthalein as the indicator as per AOAC (2016). Titratable acidity (TA) in terms of percentage lactic acid was calculated following the equation given below.

TA% = Vol.0.1N NaOH x 0.9/10 mL sample

Each 1mL of NaOH= 0.009 g of lactic acid

Statistical analysis

Each experiment was independently replicated three times and data were summarized as mean values and standard deviations. Statistical analysis of the obtained data was done by using the IBM SPSS Statistical Software Package version 20.0 (Armonk, NY: IBM). The results from *in-vitro* transit tolerance tests and lactic acid production were analyzed using one-way analysis of variance (ANOVA) with Tukey's test as a post-hoc test.

RESULTS AND DISCUSSION

Characterization of isolates

Around 100 LAB isolates were obtained from the twenty goat milk samples analyzed. The morphological characterization of isolates were followed by the presumptive identification tests (gram staining test, catalase test, endospore staining test and motility test) as explained by Schaeffer and Fulton, 1933; Murray *et al.,* 2003. Most of the isolated colonies were circular in shape with wet surface, creamy and raised with entire margins. The presence of circular, wet, creamy, raised and white color, colonies were common to *Lactobacillus* species (Tserovska *et al.,* 2002; Hussain *et al.,* 2013). Twenty isolates (n=20) were Gram positive (Figure 01.), rod shaped, non-motile, catalase negative and without endospores.

Out of those 20 isolates, 60% were hemolysis positive (35% were β-Hemolytic and 25% were α-Hemolytic) and only 40% isolates were hemolysis negative (ϒ-Hemolytic) or could consider as safe organisms for live consumption, though further *in-vivo* tests and animal trials are necessary for the confirmation (Araya *et al.,* 2002). Hemolytic bacteria have the ability to produce exoenzymes that lyse red blood cells and degrade the hemoglobin. Such isolates are not suitable for food products, therefore, removed them from further analysis (Anas *et al.,* 2008). Results of presumptive identification tests and biochemical characteristics of the selected non-haemolytic eight isolates (L03, L11, L20, L17, L74, L77, L87 and L97) are presented in Table 01, and Table 02.

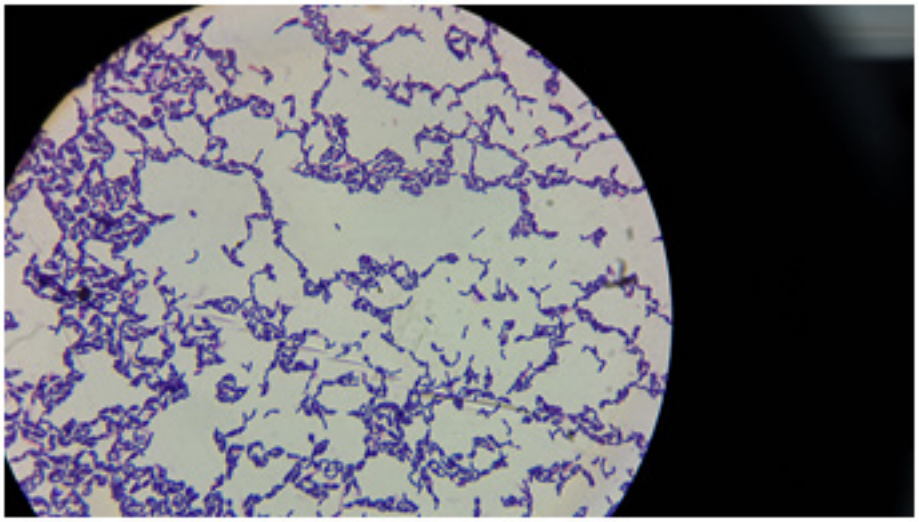

Figure 01: **Gram-positive *Lactobacillus* spp. under the light microscope**

Table 01: **Results of presumptive identification tests of selected eight LAB isolates**

Isolates	Cell Morphology	Gram's test	Catalase test	Endospore test	Motility test
L003	Small cocci	+	-	-	-
L011	Small diplococci	+	-	-	-
L017	Small cocci	+	-	-	-
L020	Small bacilli	+	-	-	-
L074	Small cocci	+	-	-	-
L077	Small cocci	+	-	-	-
L087	Medium bacilli	+	-	-	-
L097	Large cocci	+	-	-	-

+ positive reaction; - negative reaction

Table 02: **Biochemical characteristics of selected eight LAB isolates**

Isolates	L03	L11	L17	L20	L74	L77	L87	L97
Indole test	-	-	-	-	-	-	-	-
Methyl red test	+	+	+	+	+	+	+	+
Voges-Proskauer test	-	-	-	-	-	-	-	-
Urease test	-	-	-	-	-	-	-	-
Oxidase test	-	-	-	-	-	-	-	-
H_2S Production	-	-	-	-	-	-	-	-
Arginine hydrolysis test	-	+	+	+	-	-	-	-
Acid/Gas production from glucose	+	+	+	+	+	+	+	+
Citrate test	-	-	-	-	-	-	-	-
SIM Mortality	-	-	-	-	-	-	-	-

- negative reaction; + positive reaction

According to the results, those eight isolates were negative for indole, Voges-proskauer, urease and Oxidase tests, H_2S production and SIM Mortality test. The negative Indole test might be a result of incapability to degrade amino acid tryptophan to indole, pyruvic acid and ammonia (Murray *et al.,* 2003). Negative Voges-proskauer test results exhibit the inability of LAB isolates to produce acetyl methyl carbinol (Murray *et al.,* 2003). The selected isolates failed to hydrolyze urea and also to produce hydrogen sulfide (H_2S) and convert citrate into oxoloacetate (Rhaiem *et al.,* 2016). Isolates L03, L74, L77, L87 and L97 were unable to hydrolyze arginine but, the rest (L11, L17, L20) were able to form ammonia from arginine (Rhaiem *et al.,* 2016). All isolates were positive for acid/gas production test and methyl red test indicating their ability to form acid from some carbohydrates and fermenting dextrose (Murray *et al.,* 2003).

Isolates were also categorized based on their physiological properties. Usually, psychrophilic microorganisms grow at 15-20 °C, mesophilic at 15-45 °C and thermophilic grow above 45 °C (Yelnetty *et al.,* 2014). The results indicated that all LABs were mesophilic as they grew well at 15 °C as well as 45 °C as shown in Table 03.

Determination of probiotic and technological attributes of selected LAB isolates

Growth of isolates under simulated GIT conditions was estimated comparing initial and final cell densities.

***pH tolerance*:** To reach the intestine, probiotics must first pass through the stomach, which secretes hydrochloric acid and several digestive enzymes. The alkalinity of stomach can also rise as high as pH 6 or more after food ingestion (Huang and Adams, 2004). Therefore, probiotic microrganisms should have the ability to survive in different pH levels to survive in different parts of the digestive tract. As a result, resistance to pH 1.5, 3 and 9 were often used in *in-vitro* assays to determine the tolerance of isolates during digestive process for 0 hour to 5 hours (Evans *et al.,* 1988).

According to the results, all eight isolates were able to survive at pH 1.5. However, growth of L74 was decreased after 4 hours incubation at pH 1.5 whereas, the highest growth was observed in L77. Isolates L87, L20, L74, L17 and L97 were able to survive but, growth was comparatively lower than the rest. Isolates L11 and L3 survived at pH 1.5, but there was no significant growth (p-values>0.05) compared to initial inoculum level during the incubation period.

At pH 3, the highest growth was shown by L87 and L17 isolates. Isolates L20, L74, L77 and L97 were also continued to grow throughout the incubation period. The highest growth at pH 9 was shown by the L87 and the other seven isolates were also able to tolerate pH 9. According to the results, isolate L87 well tolerated the simulated pH conditions of the digestive tract. The other isolates were also survived under those conditions except, the isolate L3 and L11.

Table 03: Growth of the selected LAB isolates at different temperature, pH and NaCl conditions

Isolate	Growth/Tolerance to different temperatures (°C)			Growth/Tolerance at different pH levels			Growth/Tolerance at different NaCl concentrations (%)		
	15°C	37°C	45°C	1.5	3	9	2%	4%	6.5%
L03	+	+	+	+	-	+	+	+	+
L11	+	+	+	+	-	+	+	+	+
L17	+	+	+	+	+	+	+	+	+
L20	+	+	+	+	+	+	-	-	-
L74	-	+	+	+	+	+	+	-	+
L77	-	+	+	+	+	+	-	+	-
L87	-	+	+	+	+	+	+	+	+
L97	-	+	+	+	+	+	+	-	-

+ positive reaction - negative reaction

***Temperature tolerance*:** In the temperature tolerance test, four isolates were able to survive at 15 °C. The highest growth was observed in the isolates L11, L17, L20 and L3. However, isolates L74, L77, L87 and L97 were unable to grow at 15 °C. Their cell densities rapidly decreased during the test period. Most probiotic products are stored under lower temperature conditions therefore, tolerance to lower temperatures could consider as an additional technological property of a probiotic for the application in product developments (Fenster *et al.,* 2019). At 37 °C, all eight isolates were able to survive, however, the best survivor was L11 which also exhibited highest growth at 45 °C. Other seven isolates also survived at these temperatures throughout the test period indicating their ability to survive under the gastrointestinal temperatures. According to the results, L11 isolate had the best survivability under 15 °C, 37 °C and 45 °C temperatures.

***Tolerance to different NaCl concentrations*:** The basis of NaCl tolerance test is to check the ability of the LAB isolates to survive in digestive tract of humans containing sodium chloride (NaCl) and also to confirm their ability to grow in the presence of various NaCl concentrations used in food processing (Albarracin *et al.,* 2011; Divya *et al.,* 2012). Some fermented foods have low (~2%) or moderate (~4%) or high NaCl concentrations (~6.5%) for preservation purposes (Halder *et al.,* 2017). Among the eight isolates, five isolates were able to survive in 2% NaCl concentration. The highest growth was shown by L3 and L87; isolates and the isolates L11, L74 and L97 were also survived during the study period. However, isolate L77 and L20 were unable to survive under 2% NaCl concentration.

At the 4% NaCl concentration, the highest growth was showed by L11 and the isolates L17, L3, L74 and L87 also survived under that condition. However, isolates L20 and L97 were unable to exhibit any growth at 4% NaCl. At 6.5% NaCl concentration, the highest growth was presented by L11 isolate. The L17, L3, L74 and L87 L20, L77 and L97 isolates were unable to survive under that osmotic pressure condition. The L11 was the best survivor under all three osmotic pressure conditions. The rest were also survived under the provided conditions except L20 and L97 isolates.

***Milk coagulation*:** Milk coagulation ability indicates the organic acid production potential during incubation and change in pH with time (Yelnetty *et al.,* 2014). The pH of milk is mostly between 6.6 - 6.7. Before the fermentation, an initial apparent acidity of 0.16% in nonfat milk containing 9% solids increased 0.02% acidity for each additional 1% increase in solids reconstituted, accompanied by a slight decrease in pH from 6.6 to 6.4 (Wilkowske, 1954). The tested isolates were able to ferment skimmed milk at 37 °C within 24 hours (Table 04).

Table 04: The pH reduction of milk during fermentation at 37 °C as affected by the LAB isolates

Isolate	Mean difference of pH of milk inoculated with the test isolates during fermentation (mean ±SD)				
	00hr	02hr	04hr	06hr	24hr
L03	$6.27^{b} \pm 0.04$	$6.20^{abc} \pm 0.03$	$6.05^{a} \pm 0.07$	$5.63^{a} \pm 0.04$	$5.35^{c} \pm 0.02$
L11	$6.13^{b} \pm 0.04$	$6.11^{ab} \pm 0.03$	$6.08^{a} \pm 0.04$	$5.55^{a} \pm 0.07$	$5.37^{c} \pm 0.02$
L17	$6.36^{b} \pm 0.05$	$6.24^{bc} \pm 0.04$	$6.04^{a} \pm 0.02$	$5.65^{a} \pm 0.01$	$5.33^{a} \pm 0.01$
L20	$6.31^{b} \pm 0.01$	$6.13^{abc} \pm 0.01$	$6.06^{a} \pm 0.02$	$5.52^{a} \pm 0.07$	$4.92^{c} \pm 0.03$
L74	$6.27^{b} \pm 0.03$	$6.12^{ab} \pm 0.03$	$6.07^{a} \pm 0.03$	$5.71^{a} \pm 0.02$	$4.95^{a} \pm 0.01$
L77	$6.34^{b} \pm 0.01$	$6.26^{c} \pm 0.01$	$6.14^{a} \pm 0.03$	$5.62^{a} \pm 0.01$	$5.11^{a} \pm 0.01$
L87	$6.35^{b} \pm 0.02$	$6.01^{a} \pm 0.02$	$6.06^{a} \pm 0.03$	$5.64^{a} \pm 0.06$	$5.05^{ab} \pm 0.01$
L97	$6.31^{b} \pm 0.01$	$6.15^{abc} \pm 0.07$	$6.09^{a} \pm 0.04$	$5.67^{a} \pm 0.03$	$4.95^{a} \pm 0.07$

**Values in the same column with different superscripts are statistically significant at 95 % confidence interval.*

As presented in Table 04, all isolates performed equally better in reducing pH of milk during fermentation. However, L20 was the fastest acid producer which dropped the pH level to 4.92 after 24 hours while others could reduce only up to pH 4.9-5.3 level. Ability to ferment milk, indicated by the reduction of pH is important in starter culture performance for manufacturing of cheese as optimum pH of cheese production is in the range of 5.0-5.2. Good quality cultured butter milk is obtained from final pH near to 4.5 to 5 (Wilkowske, 1954). Therefore, the selected LAB isolates of the present study are suitable for making cheese or cultured butter milk.

Titratable acidity measures the total acid concentration contained within a food (AOAC, 2010). The L17 isolate produced the highest titratable acidity and also had the highest lactic acid production potential. However, it was not significantly different from L11 and L77 isolates ($p>0.05$), hence, those two isolates were also equally better performers in lactic acid production. The other isolates were also able to produce lactic acid at a range of 0.35-0.50 as presented in Figure 02. All isolates had good lactic acid production abilities and therefore, are suitable candidates to use as starter cultures in fermented products.

Determination of lactic acid production abilities by HPLC analysis

Isolates with an ability to ferment the sugars in to lactic acid, and lactic acid productivity was determined by using HPLC method and the yield was calculated by the equation given by the calibration curve (Figure 03.) as performed by Sharma *et al.* (2013). The amount of lactic acid produced by the isolates are presented in Table 05, and the highest lactic acid concentration was produced by the isolate L87. Isolates L3, L11, and L17 producde less lactic acid content compared to the rest of the isolates (L87, L20, L74 and L77). Therefore, isolates L87, L20, L74 and L77 can be used as starter cultures for the dairy industry, due to their high lactic acid production abilities and can also use as industrial lactic acid producers for numerous industries.

Categorization of isolates based on their morphological, biochemical and physiochemical properties

Lactococci species could grow in 15 °C, pH 3 and tolerant to 4% and 6.5% of NaCl concentrations (Kacem *et al.,* 2003). This species could also ferment arabinose, ribose, trehalose and unable to ferment raffinose and rhamnose and unable to hydrolyze arginine as well (Kacem *et al.,* 2003). Therefore, by characterizing the isolates based on the properties presented in Table 06, the L03 isolate could assume as belonging to *Lactococcus lactis* species based on characteristics reported by Sharma *et al.* (2003).

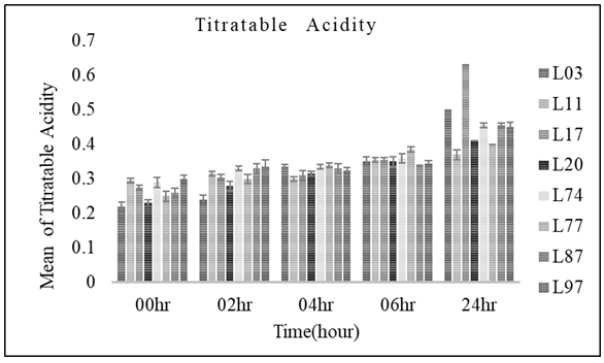

Figure 02: **Acid production indicated as titratable acidity (TA%) by the selected LAB isolates during fermentation**

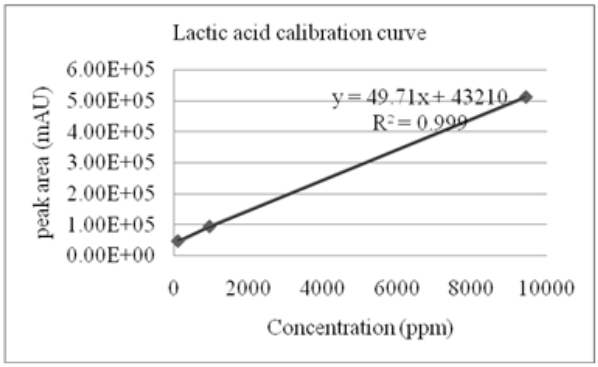

Figure 03: **Lactic acid calibration curve obtained through HPLC chromatograms**

Table 05: Different quantities of lactic acid produced by the isolates

Isolate	Lactic acid production (mg/L)
L03	1.4
L11	3.2
L17	3.7
L20	28
L74	14
L77	13.1
L87	33
L97	8.2

Table 06: Summary of physiochemical properties and carbohydrate fermentation profiles of the selected LAB isolates

Test profile	LAB isolates							
	L03	L11	L17	L20	L74	L77	L87	L97
Growth at 15 ^{0}C	+	+	+	+	-	-	-	-
Growth at 45 ^{0}C	+	+	+	+	+	+	+	+
Growth at 1.5% [NaCl]	+	+	+	-	+	-	+	+
Growth at 3% [NaCl]	+	+	+	-	-	+	+	-
Growth at 6.5% [NaCl]	+	+	+	-	+	-	+	-
Arginine hydrolysis	-	+	+	+	-	-	-	-
Growth at pH 4	-	-	+	+	+	+	+	+
pH Range for fermentation	ND	ND	ND	0.98	ND	ND	1.33	ND
Lactic acid concentration (mg/L)	1.4	3.2	3.7	28.0	14.0	13.1	33.0	8.2
Sugar fermentation								
D-Raffinose	-	+	+	+	+	+	+	+
L-Arabinose	+	+	+	+	+	+	+	+
D-Ribose	+	+	+	+	-	-	-	+
D-Trehalose	+	+	+	+	+	+	+	+
D-Glucose	+	+	+	+	+	+	+	+
D-Mannitol	+	+	+	+	+	+	+	+
D-Melizitose	+	+	+	-	+	+	+	+
D-Sucrose	+	+	+	+	+	+	+	+
D-Mannose	+	+	+	+	+	+	+	+
D-Fructose	-	+	+	+	+	+	+	+
L-Rhamnose	-	-	-	-	-	-	-	-
D-Galactose	+	+	+	+	+	+	+	+

ND not determined, + tolerant / positive, - intolerant /negative

Table 07: Presumptive identification of selected LAB isolates based on their phenotypic properties

Isolate	Species
L03	*Lactococcus lactis*
L11	*Enterococcus faecium*
L17	*Enterococcus faecium*
L20	*Lactobacillus plantarum*
L74	*Streptococcus thermophilus*
L77	*Streptococcus bovis*
L87	*Lactobacillus pentosus*
L97	*Streptococcus thermophilus*

The isolates L11 and L17 could assume as belonging to *Enterococcus faecium* species (Table 07) as they were able to grow in 15 °C, 45 °C temperatures and in 6.5% NaCl concentration (Table 06). Most enterococcus species posess those features and could also hydrolyze arginine and ferment arabinose, ribose and trehalose (Kacem *et al.,* 2003) as exhibited by L11 and L17 isolates.

Isolates L74 and L97 from goat milk could assume as members of *Streptococcus thermophiles* species (Table 07) as they were able to grow at 15 °C, 45 °C temperatures, pH 3.0, tolerate 2 % and 4% of NaCl concentrations and considering their carbohydrate fermentation profile as presented in Table 06. Tolerance to above temperatures, pH and NaCl concentrations are features usually exhibited by *Streptococcus thermophiles* species (Sharma *et al.,* 2003; Sharma *et al.,* 2013). Their carbohydrate fermentation profile (Table 06) also tallies with the members of *Streptococcus thermophilus* species (Rhaiem *et al.,* 2016; Domingos-Lopes *et al.,* 2017).

Isolate L77 exhibited characteristics of *Streptococcus bovis* (Table 07) as they did not grow at 6.5% NaCl concentration and was unable to hydrolyze arginine. They had the ability to grow in 45 °C and in 3% NaCl concentration (Table 06) thus, tallies with the specifications given by Kacem *et al.* (2003).

Isolates L87 and L20 were the only bacilli members isolated from analyzed goat milk samples. Their morphological, bio chemical and physiological characteristics (Table 06) were similar to *Lactobacillus* genera as described by Vos *et al.,* 2011. The pH reduction abilities during fermentation was the only awailable characteristic in the present study for the differentiation of those isolates to *Lactobacillus plantarum* and *Lactobacillus pentosus* species using the characterization data of Yelnetty *et al.,* 2014. The pH reduction during fermentation of the isolate L87 (*Lactobacillus pentosus*) showed the largest drop from 6.34 to 5.01 while the isolate L20 (*Lactobacillus plantarum*) showed the smallest drop from 6.32 to 5.34 in milk fermentation (Table 04). The L87 also produced the highest lactic acid concentration (33 mg/L) after 24-hour fermentation process compared to L20 (28 mg/L) (Table 05).

CONCLUSIONS

The LABs isolated from goat milk could possibly belong to *Lactococcus lactis* (L003)*, Enterococcus faecium* (L011, L017), *Lactobacillus plantarum* (L020), *Streptococcus thermophilus* (L074, L097), *Streptococcus bovis* (L077)*, Lactobacillus pentosus* (L087*)* species based on their phenotypic properties. However, it is necessary to perform genotypic tests for species confirmation in the future. The isolates were able to ferment milk effectively and survived during exposure to several stressful conditions exsisting in the GIT and applied in food fermentation processes. Furthermore, the findings of this study revealed that L087, L020,

L074 and L097 as the best lactic acid producers as indicated by lactic acid production. Therefore, goat milk is a good sorce for the isolation of probitotic LAB species with desirable probiotic and technological properties for functional food applications. The isolates also possessed lactic acid production potential for commercial use, though further optimization and scaleup trials are necessaty to perform.

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