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Probiotic, Antimicrobial and Bioactive Properties of Lactic Acid Bacteria Isolated from Goat Milk

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Abstract

This study aimed at isolating and identifying lactic acid bacteria (LAB) from goat milk and the determination of their probiotic, antimicrobial and antioxidant potential. Raw milk samples were collected into sterile corked bottles from Sokoto Red and the West African dwarf goats and transported in ice packs to the laboratory. LAB were isolated and identified following standard techniques and were subjected to different probiotic assays including bile salt and acid tolerance. Their lipolytic and proteolytic activities, their antimicrobial activity against selected pathogens, and their capability to produce lactic acid, diacetyl and hydrogen peroxide were also determined. Safety assessment tests to determine if they can produce haemolysin, gelatinase, DNase and lecithinase were also performed. Their bioactive potential was assessed. The isolates were finally subjected to a number of antibiotics. A total of 34 LAB strains were isolated and were identified to belong to 10 species namely; Lactobacillus plantarum (26.5%), Lactobacillus casei (14.7%), Pediococcus spp. (8.8%), Streptococcus thermophilus (8.8%), Lactobacillus acidophilus (8.8%), Lactobacillus helviticus (14.7%), Lactobacillus brevis (5.9%), Lactobacillus delbrueckii (5.9%), Lactobacillus bulgaricus (2.9%) and Lactobacillus fermentum (2.9%). Of all the 34 LAB isolates, only two exhibited desirable probiotic, antimicrobial and antioxidant properties. These two LAB isolates are useful candidates as starter cultures in food fermentation.

Keywords	Goat Milk; Lactic Acid Bacteria (LAB); Probiotic; Antimicrobial; Bioactive Properties
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Background

Milk is a nutrient-rich secretion from the mammary glands of mammals and serves as the primary food for young animals (Pandya and Ghodke, 2007). Cow and goat milk, widely consumed globally, are processed into staple dairy products, but their susceptibility to microbial spoilage, particularly by lactic acid bacteria (LAB), leads to souring as lactic acid production lowers pH and causes curdling (Pandya and Ghodke, 2007). Fermentation became a traditional method to extend milk's shelf life, giving rise to diverse dairy products worldwide (Mechai and Kirane, 2008). Products such as yoghurt, cheese, *wara*, *nunu*, and *ergo* have evolved from traditional knowledge, and the demand for fermented products has sharply increased in recent decades due to innovations involving probiotic cultures, bioactive fortification, and improved sensory attributes (Sfakianakis and Tzia, 2014).

LAB are central to dairy fermentation because they convert glucose and galactose into lactic acid, creating the distinctive aroma, flavor, and texture of fermented foods. They often dominate natural microbial communities in raw milk, reflecting their ubiquity and importance in spontaneous fermentation (Guetouache et al., 2015). However, modern socio-economic changes threaten traditional fermentation methods and the indigenous microorganisms associated with them, making it essential to investigate and preserve these microbial resources (Akabanda et al., 2017). Although pasteurization and hygienic packaging reduce microbial diversity in modern dairy products, fermented foods remain important to human nutrition (Okonkwo, 2011).

Current research in food biotechnology focuses heavily on probiotic strains, especially those from *Lactobacillus* and *Bifidobacterium* (Arthure et al., 2002). Microorganisms isolated from naturally fermented foods are being evaluated for health-promoting properties and used as starter cultures in commercial products (Birollo et al., 2000). LAB contribute to product preservation through lactic acid production and enhance flavor, texture, and nutritional value. Their documented benefits include immune stimulation, anti-carcinogenic activity, and antagonism against pathogens (Chassard et al., 2010). Despite their potential, probiotic bacteria must meet strict criteria such as surviving gastrointestinal acidity, bile salts, and processing conditions before being deemed suitable for food applications (Birollo et al., 2000).

As Gram-positive, Generally Regarded as Safe (GRAS) organisms, LAB are essential in industrial fermentation. Their metabolites including lactic acid, acetic acid, exopolysaccharides, and bacteriocins exhibit antimicrobial activity and significantly improve the shelf life, sensory quality, and safety of fermented products (Bennama et al., 2012; Saranraj et al., 2013; Shah and Prajapati, 2013; O'Sullivan et al., 2002).

Materials and Methods

Sample Collection

Goat milk samples were collected from two different breeds of goat; the West African Dwarf and Sokoto Red from Bodija (Ibadan North Local Government) and Kara Akinyele (Akinyele Local Government) in sterile corked bottles and transported immediately in ice packs to the Food Microbiology laboratory of the Department of Microbiology, University of Ibadan, Nigeria for further processing.

Isolation of Lactic Acid Bacteria

Isolation was made from samples using pour plate techniques according to the method described in Compendium of Methods for the Microbiological Examination of food (Harrigan and McCance, 1966). Ten-fold serial dilutions were done for the two milk samples separately and using sterile pipettes, 1mL of dilutions 10^{-3}, 10^{-5} and 10^{-7} were plated out by mixing with 20mL of molten de Man Rogosa and Sharpe (MRS) medium contained in McCartney bottle and poured aseptically into sterile Petri dishes. The plates were gently swirled to allow even distribution of the inocula and thereafter incubated at 37°C for 48 hours in microaerophilic condition (de Carvalho Lima *et al*., 2009). Afterwards, distinct resultant colonies were subcultured via subsequent streaking to obtain pure cultures. Thereafter, pure cultures were inoculated in cryovials containing 12% (w/v) glycerol-nutrient broth medium and incubated until visible growth. The stock cultures are thereafter stored at 4°C until required for use and subcultured at two weeks interval (de Carvalho Lima *et al*., 2009).

Identification of LAB Isolates

The pure cultures of the LAB were identified via morphological, biochemical and sugar fermentation properties (Yu *et al*., 2013).

Probiotic properties of the LAB Isolates

Bile Tolerance

One milliliter (1mL) cell suspension of 24 hours old culture of LAB strains adjusted to 0.5 Mac Farland's standard was added into 20 mL of freshly prepared sterile MRS media containing 0.5, 1, 1.5 and 2% of No.3 bile salts (sodium di-oxycholate) (Oxoid, England). The broths were incubated and observed for growth via measuring the absorbance (O.D.) after every six hours at 580nm. MRS broth without bile salt served as the control (Neethu *et al.,* 2015).

Acid Tolerance Assay

Freshly grown LAB in MRS broth were centrifuged at 3000g for 15 minutes and pellet was collected in a sterile tube. The pellet was washed twice with phosphate buffer saline (PBS) at pH 7.0 before inoculation in phosphate buffer adjusted to pH 1.0, 2.0, 3.0, 4.0 and 5.0. Serial dilution was done for each LAB strain to ascertain the initial microbial load. Aliquot 1mL sample was taken every 1 hour for 3 hours, and 10-fold serial dilutions were made using peptone water diluents. Samples were plated onto MRS agar, and enumerated for the presence of viable cells after 0, 1, 2, and 3 hours of incubation using pour plate method. Acid tolerance was determined by comparing the final plate count after 3 hours with the initial plate count at 0 hour (Hassanzadazar *et al*., 2012).

Assay for enzyme activity

Lipase Production

Cell free supernatants (obtained after centrifuging 24 hours broth cultures of the isolates) were aseptically inoculated into well-labelled wells of freshly prepared spirit blue agar and the Petri dishes were incubated. The formation of a blue ring around the well indicates a positive result, hence lipase production (Leuschner *et al.*, 1997).

Protease Production

Nutrient agar was supplemented with 1% (w/v) skimmed milk was sterilized by autoclaving at 110^0C for 10 minutes. Upon solidifying, each LAB isolate was streaked on the skimmed milk agar, while uninoculated plates served as control. Upon incubation, a halo zone along the streak line indicated casein hydrolysis, a result of protease production ((Beerens and Luquet, 1990).

Antibacterial Activities of LAB Isolates

The agar well diffusion method was used for detecting antimicrobial activity against indicator strains such as *Escherichia coli, Klebsiella* species, *Pseudomonas* species and *Staphylococcus aureus*, were collected from University College Hospital, Ibadan, Oyo state. The selected LAB isolates were grown in sterile broth for 48 hours at 35 ± 2°C while the pathogens were grown on sterilized nutrient agar plate for 24 hours. Cell free supernatant of the LAB isolates was obtained by centrifuging the broth grown isolate in a cold centrifuge (4°C) at 5000rpm for 15 minutes. Inoculum of the pathogens was suspended in normal saline and was adjusted to 0.5 Mac Farland's Standard. Sterile swab stick was used to inoculate the indicator strains on sterile Mueller Hinton agar plates. Using a sterile cork borer of 5mm in diameter, wells were bore on the inoculated agar plates. The cell free supernatant of the LAB isolates was dispensed into each well and the plates were inversely incubated aerobically for 24 hours at 37°C. Diameter of inhibition around each well indicated the antimicrobial activity of the lactic acid bacteria against the particular pathogen. LAB strains with inhibition zones 0.5-4mm, 5-9mm and 10-15mm were considered as weak inhibition, strong inhibition, and very strong inhibition respectively (de Almeida Júnior *et al*., 2015; Qing *et al.,* 2015).

Quantitative Determination of Bioactive Compounds Produced

Lactic Acid Production

The production of lactic acid was determined by transferring 25mL of supernatant fluid of test organisms into 100mL flasks. This was titrated with 0.1M NaOH and 1ml of phenolphthalein indicator (0.5% in 5/0% alcohol). The titratable acidity was calculated as lactic acid %v/w/v (Sanni and Adesulu, 2013). Each milliliter of 1M NaOH is equivalent to 90.08mg of lactic acid. The titratable acidity was then calculated as stated in A. O. A. C (2010) as :

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

Where ml= volume of NaOH used, N NaOH= molarity of NaOH solution, M. E. = Equivalent factor (90.08)

Hydrogen Peroxide Production

The concentration of hydrogen peroxide produced by the isolate was determined by titration with a standardized (0.1M) Potassium permanganate.

$$H_2SO_4 + 2KMnO_4 + 3H_2SO_4 \longrightarrow K_2MnSO_4 + 4H_2O + O_2$$

Twenty millilitres of dilute H_2SO_4 was added to 25mL of the supernatant fluid of the test organism. Titration was carried out with 0.1M potassium permanganate ($KMnO_4$). Each mL of 0.1M potassium permanganate is equivalent to 1.70mg of Hydrogen peroxide solution. Decolorization of the sample was regarded as the end point. The volume of H_2O_2 produced was then calculated (A.O.A.C., 2010) as:

$$H_2O_2 \text{ produced} = \frac{\text{ml } KMnO_4 \times \text{N } KMnO_4 \times \text{M. E.} \times 100}{\text{ml } H_2SO_4 \times \text{volume of sample}}$$

Where ml $KMnO_4$ = Volume of $KMnO_4$ used, N $KMnO_4$ = molarity of $KMnO_4$, ml H_2SO_4 = volume of H_2SO_4 added, M. E. = Equivalent factor.

Diacetyl Production

Diacetyl production was determined by transferring 25ml of broth cultures of test organisms into 100ml flask. Hydroxylamine solution (7.5ml) of 1M was added to the flask and to a similar flask for residual titration. Both were titrated with 0.1M HCl to a greenish yellow end point using bromocresol blue as indicator (Sanni and Adesulu, 2013). The equivalence factor of HCl to diacetyl is 21.52 mg. The concentration of diacetyl produced was calculated using A.O.A.C. (2010) as:

$$\text{Ak} = \frac{(B-S)(100E)}{W}$$

Where Ak= percentage of diacetyl, B= Number of ml of 0.1M HCl used in the titration of sample

S= Number of ml of 0.1HCl consumed in titration without sample, E= Equivalent factor, W = volume of sample.

Safety assessment of the LAB Isolates

Haemolytic Activity

Blood haemolysis was determined using Nutrient Agar supplemented with 5% sheep blood. Lactic acid bacteria strains were streaked on the blood agar plates and incubated at 37°C for 24 hours. After incubation, the plates were examined for β-haemolysis (clear zones around colonies), α-haemolysis (a green-hued zone around colonies) or γ-haemolysis (no halo around colonies) (Pooja *et al*., 2015).

Production of Gelatinase

Production of gelatinase was determined on Todd-Hewitt agar containing 30 g of gelatin (Difco) per liter as described by Harrigan and McCance (1990). The isolates were sreaked on the agar plate using inoculating loop and incubated at 37^0C for 48 hours. Clear halo around colonies indicate the production of gelatinase (Eaton and Gasson, 2001).

Production of DNase

For DNase testing, a Methyl Green DNAse agar (Difco, Heidelberg, Germany) was used. Isolates were streaked on DNase agar using a sterile inoculating loop. Appearance of a clear halo around colonies after incubation of plates at 37°C for 48 hours indicated DNase production (Gupta and Malik, 2007).

Antibiotic susceptibility Tests

The antimicrobial susceptibility test for the selected lactic acid bacteria strains was conducted by the disc diffusion method (CLSI, 2011 and Prescott *et al.,* 2002). Ampicillin (10µg), Tetracycline (30µg), Clindamycin (2µg), Erythromycin (15µg) and Gentamycin (10µg) antimicrobial discs were used for the test. Inoculum density of the lactic acid bacteria isolates were adjusted to McFarland 0.5 turbidity standard (equivalent to cell density of 10^8 cfu/ml). A sterile cotton swab was dipped in the adjusted suspension and swabbed over the entire surface of pre-dried Mueller-Hinton agar plate. The antimicrobial discs were dispensed onto the surface of the inoculated agar plates. Plates were incubated at 37^0C for 24 hours and the diameter of the zones of complete inhibition were measured.

Antioxidative Properties Assessment

The antioxidative properties of the fermented milk samples were determined using 1,1 – diphenyl-2-picryhydrazyl (DPPH) free radical scavenging activity and ferric reducing power potential (FRAP).

Determination of DPPH Radical Scavenging Ability

The DPPH scavenging assay was performed as reported by Shui (2002) as modified by Kahkoonen *et al.* (2002). The radical scavenging activity of the fermented milk sample was measured based on the principle that DPPH radicals in the presence of antioxidants result in yellow colour change as a result of diphenyl picrylhydrazin production. Freshly prepared 0.1mM of DPPH radicals in methanol was used. 3.5ml of the solution was added to 0.5ml of the *nunu* produced. The mixture was homogenized and left to stand at room temperature for 30 minutes. The absorbance of the solution was measured at 517nm. Methanol was used as blank and distilled water was used for base line correction. Results \were read in duplicate. Free radical scavenging ability was expressed as percentage inhibition.

$$\text{Free radical scavenging ability (\%)} = \frac{\text{Absorbance of control- Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Determination of Ferric Reducing Antioxidant Power (FRAP)

The reducing power assay was performed as described by Wang *et al.* (2008). 2.5ml of the fermented milk was mixed with 2.5ml of 0.2M Sodium phosphate buffer at pH 6.6 and 2.5ml of 1% w/v ferricyanide. Mixture was incubated at 500C for 20 minutes. 2.5ml of trichloroacetic acid (10% w/v) was added to the mixture and centrifuged at 3000rpm for 10 minutes. 5ml of the supernatant was mixed with 2.5ml of distilled water and 0.5ml of 0.1% w/v ferric chloride. The mixture was incubated at room temperature in a dark cupboard for 30 minutes. The absorbance of the mixture was measured using a spectrophotometer at 700nm. Higher absorbance indicates a higher reducing power.

Results

Table 1 shows the total viable counts (TVC) of LAB isolated from milk from the two goat breeds. The TVC of LAB ranged from 0.9 x 10^1 CFU/mL to 6.2 x 10^9 CFU/mL in the milk obtained from Sokoto Red breed whereas for the milk of the West African dwarf, it ranged from 1.2 x 10^1 CFU/mL to 4.7 x 10^{10} CFU/mL after 0 and 24 hours respectively.

Of all the bacterial load, only 34 (34) pure culture of lactic acid bacteria were isolated from both milk source and when subjected to the different morphological, biochemical and sugar fermentation tests, they were identified to belong to 10 species namely; *Lactobacillus plantarum* (26.5%), *Lactobacillus casei* (14.7%), *Pediococcus* spp. (8.8%), *Streptococcus thermophilus* (8.8%), *Lactobacillus acidophilus* (8.8%), *Lactobacillus helviticus* (14.7%), *Lactobacillus brevis* (5.9%), *Lactobacillus delbrueckii* (5.9%), *Lactobacillus bulgaricus* (2.9%) and *Lactobacillus fermentum* (2.9%) (Figure 1).

When subjected to different bile salt concentrations, they all grew profusely at 0.5%-2.0% concentrations as seen in Table 2. Meanwhile, there was an indirect relationship between the growth rate of the isolates and the concentration of bile salts, hence, as the concentration of bile salts increased, the growth of the isolates significantly reduced. For the acidity tolerance, there was a varying survival rate of the isolates especially at pH 1, 2 and 3, while majority grew at pH 4 at 1,2 and 3 hours of exposure but not beyond. Meanwhile, all the isolates grew at pH 5 (Table 3).

The ability of the isolates to produce lipase and protease is shown in Table 4. It was observed that 8 (23.53%) of the 34 isolates S2, S6, S12, S17, M2, M4, M13 and M15 showed protease and lipase production potential, while 7 (20.59%) isolates S1, S9, S14, M5, M8, M11, and M12 showed ability of producing protease only, whereas isolates S4 and M7 produced lipase only while 17 (50%) of the isolates did not produce any enzyme.

Table 5 shows the antimicrobial activity of selected LAB isolates against some test bacteria. It was observed that majority of the LAB had varying inhibitory activity against all the test isolates. Meanwhile, isolate S14 did not show antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. Likewise, isolate M13 did not show any activity against *Salmonella typhi* and *Klebsiella pneumoniae*. More so, isolate M14 did not show antimicrobial activity against *Listeria monocytogenes* and *Staphylococcus aureus*.

The 11 selected LAB isolates showed capability for production of antimicrobial compounds such as lactic acid, diacetyl and hydrogen peroxide. For lactic acid, the highest production (3.6 g/L) was observed in isolate M15 while isolate M13 produced the least amount (0.6 g/L) of lactic acid. However, the highest (1.8 g/L) of diacetyl was by isolate S14 while the least amount (0.9 g/L) was produced by isolate M14. Meanwhile, the highest amount (0.9 g/L) of hydrogen peroxide was produced by isolate M13 whereas the least amount (0.2 g/L) was produced by isolate M14 (Table 6)

Antioxidant analysis showed the consortium had the highest DPPH scavenging ability and reducing power—values comparable to the reference starter—indicating strong functional potential.

Isolates S5, S6 and M15 produced significantly higher antimicrobial compounds and thus were selected for safety assessment tests. All three isolates did not produce DNase, gelatinase, lecithinase and were not haemolytic, that is, did not produce hemolysin (Table 7). These isolates when subjected to different antibiotics were all susceptible to all antibiotics tested against them. However, isolate S6 showed resistance to chloramphenicol (Table 8). These three selected isolates upon characterisation using morphological, biochemical, physiological and sugar fermentation properties were identified as *Lactobacillus acidophilus* (S5), *Lactobacllus plantarum* (S6) and *Lactobacillus acidophilus* (M15).

The antioxidative properties of the isolates were assessed and shown in Table 3.15. The antioxidants present in nunu produced is quantified. Consortium of Isolate S6 and M15 had the highest value while Isolate M15 had the lowest value for DPPH radical scavenging assays with 51.13% and 34.17% respectively. The value of the DPPH reading for the consortium is close to the value for the reference starter, 56.73%. For the assay of reducing power, consortium of Isolate S6 and M15 had the highest value while Isolate M15 had the lowest value for reducing power with

199.038µg and 96.438µg respectively. The value of the reducing power for the consortium is close to the value for the reference starter, 202.718.

Table 1: Total Viable Counts (CFU/mL) of LAB obtained in milk from Sokoto Red and West African Dwarf Goats

Breed/Fermentation Time (Hours)	Sokoto Red	West African Dwarf
0	0.9 x 10	1.2 x 10
6	1.4 x 10^3	2.2 x 10^4
12	3.2 x 10^5	2.4 x 10^6
18	1.8 x 10^8	5.3 x 10^8
24	6.2 x 10^9	4.7 x 10^{10}

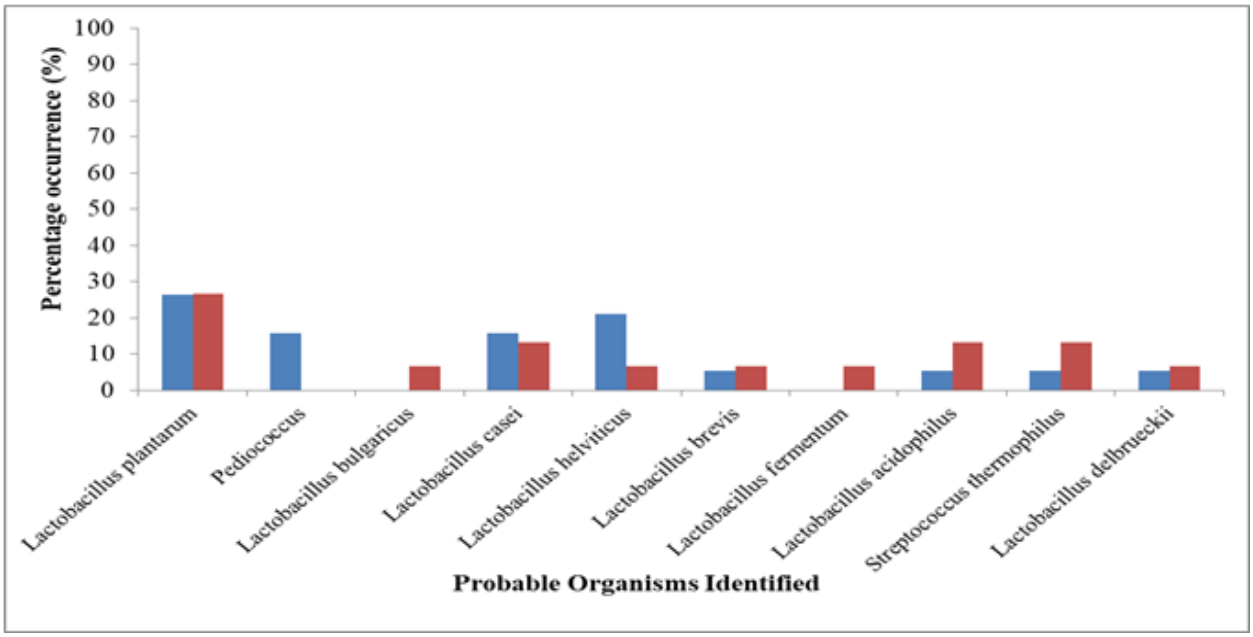

Figure 1: Percentage occurrence of Lactic Acid Bacteria isolated from Milk of Sokoto Red and West African Dwarf goats

Table 2: Percentage resistance of the isolates at various bile salt concentrations

Isolate code	0.5%	1.0%	1.5%	2.0%
S1	76.69± 0.02	65.03±0.05	57.21±0.03	50.15±0.00
S2	96.20±0.00	69.82±0.01	39.14±0.01	15.82±0.21
S3	44.00±0.04	23.00±0.00	13.01±0.01	12.13±0.00
S4	78.18±0.01	39.45±0.00	11.60±0.01	9.96±0.02
S5	49.45± 0.02	34.39±0.00	34.17±0.01	27.02±0.01
S6	90.45±0.00	87.22±0.01	81.33±0.00	74.06±0.00
S7	43.15±0.01	37.82±0.00	37.09±0.01	35.88±0.03
S8	66.10±0.02	35.82±0.00	17.02±0.00	9.04±0.05
S9	83.59±0.02	72.93±0.01	59.05±0.02	51.90±0.00
S10	63.45±0.01	56.37±0.00	56.14±0.07	44.20±0.01
S11	59.66±0.05	36.97±0.02	15.97±0.00	9.78±0.00
S12	40.50±0.00	30.69±0.00	20.25±0.03	15.65±0.01
S13	46.22±0.06	32.01±0.01	29.10±0.04	21.89±0.01
S14	70.73±0.00	67.10±0.05	52.33±0.00	50.78±0.01
S15	53.42±0.01	41.37±0.03	15.85±0.00	10.32±0.03

S16	72.83±0.03	55.19±0.00	49.96±0.01	46.16±0.02
S17	54.59±0.01	51.14±0.00	38.80±0.03	32.28±0.00
S18	57.99±0.01	45.88±0.00	49.95±0.01	40.69±0.03
S19	62.59±0.02	57.09±0.01	11.21±0.02	9.38±0.00
M1	81.44±0.02	61.99±0.00	57.99±0.03	52.84±0.00
M2	42.11±0.01	40.91±0.00	41.55±0.01	25.22±0.01
M3	66.17±0.03	63.45±0.01	51.80±0.02	44.82±0.01
M4	72.98±0.03	18.25±0.01	19.56±0.00	9.58±0.00
M5	82.58±0.00	50.97±0.03	50.52±0.02	47.58±0.00
M6	54.17±0.01	32.54±0.00	19.93±0.00	19.59±0.02
M7	69.06±0.03	24.70±0.01	24. 44±0.01	25.23±0.00
M8	65.78±0.00	41.04±0.02	24.12±0.00	10.10±0.01
M9	65.18±0.01	19.28±0.02	20.10±0.01	16.58±0.02

Isolate code	**0.5%**	**1.0%**	**1.5%**	**2.0%**
M10	36.53±0.00	11.32±0.06	9.68±0.01	8.31±0.00
M11	45.01±0.01	43.74±0.01	10.33±0.00	9.18±0.02
M12	58.11±0.00	30.74±0.02	21.75±0.00	10.17±0.00
M13	96.47±0.00	79.55±0.04	82.51±0.02	65.02±0.00
M14	62.34±0.01	58.16±0.00	57.22±0.01	49.01±0.02
M15	98.49±0.01	71.49±0.02	65.34±0.01	60.34±0.01

Each value represents mean value ± standard deviation from triplicate observations

Table 3: Tolerance of the isolated lactic acid bacteria to low pH

Isolate Code	pH	Viable Counts (log CFU/ml)	Viable Counts (log CFU/ml)	Viable Counts (log CFU/ml)	Viable Counts (log CFU/ml)
Hours of Incubation		0	1	2	3
S1	1	7.89±0.02	7.52±0.05	6.01±0.03	5. 49±0.01
	2	7.89±0.02	7.50±0.09	7.39±0.01	6.30±0.06
	3	7.89±0.02	7.80±0.03	7.64±0.08	7.52±0.02
	4	7.89±0.02	7.87±0.01	7.87±0.07	7.84±0.05
	5	7.89±0.02	7.91±0.01	7.98±0.03	8.26±0.03
S2	1	8.46±0.07	8.40±0.03	8.37±0.01	8.40±0.01
	2	8.46±0.07	8.42±0.09	8.39±0.03	8.45±0.01
	3	8.46±0.07	8.40±0.02	8.36±0.01	8.49±0.03
	4	8.46±0.07	8.47±0.04	8.53±0.08	8.79±0.04
	5	8.46±0.07	8.58±0.07	9.32±0.03	9.55±0.03
S3	1	10.12±0.04	7.40±0.04	6.36±0.03	4.80±0.05
	2	10.12±0.04	7.83±0.02	7.35±0.01	6.81±0.07
	3	10.12±0.04	7.52±0.16	7.29±0.04	7.04±0.04
	4	10.12±0.04	9.38±0.04	8.56±0.09	8.20±0.09
	5	10.12±0.04	9.96±0.05	9.44±0.06	10.02±0.18
S4	1	9.48±0.09	5.59±0.07	4.84±0.02	3.96±0.03
	2	9.48±0.09	6.88±0.04	5.72±0.06	4.97±0.01
	3	9.48±0.09	7.39±0.07	6.82±0.02	6.34±0.02
	4	9.48±0.09	8.63±0.01	8.47±0.04	8.10±0.01
	5	9.48±0.09	9.40±0.03	8.95±0.09	8.63±0.21
S5	1	9.66±0.05	9.50±0.05	9.69±0.05	9.82±0.10
	2	9.66±0.05	9.48±0.07	9.72±0.03	9.94±0.06
	3	9.66±0.05	9.61±0.01	9.70±0.08	9.94±0.01

	4	9.66±0.05	9.63±0.02	9.78±0.01	10.01±0.06
	5	9.66±0.05	9.71±0.03	10.07±0.02	10.33±0.05
S6	1	9.82±0.01	9.76±0.05	9.80±0.06	9.97±0.01
	2	9.82±0.01	9.34±0.09	8.96±0.04	10.02±0.06
	3	9.82±0.01	9.50±0.05	9.68±0.07	9.98±0.02
	4	9.82±0.01	9.64±0.06	9.88±0.03	10.00±0.01
	5	9.82±0.01	9.75±0.03	9.83±0.01	10.06±0.05
S7	1	10.14±0.04	9.01±0.15	8.31±0.08	6.62±0.01
	2	10.14±0.04	8.86±0.04	8.54±0.03	7.89±0.07
	3	10.14±0.04	9.59±0.09	9.38±0.04	9.20±0.06
	4	10.14±0.04	9.89±0.05	9.92±0.07	10.03±0.01
	5	10.14±0.04	10.10±0.01	10.04±0.02	10.09±0.05
S8	1	9.97±0.12	7.37±0.10	7.11±0.09	7.02±0.03
	2	9.97±0.12	8.01±0.09	7.52±0.02	7.18±0.05
	3	9.97±0.12	8.83±0.02	9.01±0.06	8.50±0.09
	4	9.97±0.12	9.55±0.05	9.28±0.03	9.16±0.02
	5	9.97±0.12	9.84±0.02	9.67±0.17	10.02±0.01
S9	1	9.63±0.32	6.52±0.06	4.83±0.01	4.04±0.07
	2	9.63±0.32	6.94±0.08	6.82±-0.05	5.85±0.04
	3	9.63±0.32	6.81±0.03	6.65±0.03	6.36±0.17
	4	9.63±0.32	7.03±0.06	6.80±0.07	6.28±0.03
	5	9.63±0.32	9.16±0.14	8.37±0.04	8.61±0.16
S10	1	8.71±0.09	7.86±0.01	8.41±0.04	8.74±0.10
	2	8.71±0.09	8.53±0.04	8.70±0.01	8.87±0.10
	3	8.71±0.09	8.51±0.02	8.76±0.06	8.93±0.05
	4	8.71±0.09	8.59±0.05	9.05±0.02	9.26±0.01
	5	8.71±0.09	9.02±0.01	9.47±0.06	9.81±0.02
S11	1	8.39±0.01	5.04±0.03	4.39±0.09	3.26±0.02
	2	8.39±0.01	7.44±0.30	6.05±0.10	4.51±0.04
	3	8.39±0.01	7.56±0.01	6.61±0.04	6.25±0.06
	4	8.39±0.01	8.01±0.04	7.83±0.05	7.76±0.09
	5	8.39±0.01	8.20±0.07	8.26±0.00	8.04±0.03
S12	1	9.06±0.07	3.51±0.01	3.32±0.06	3.09±0.02
	2	9.06±0.07	5.86±0.06	3.95±0.03	3.37±0.07
	3	9.06±0.07	5.74±0.05	4.43±0.09	4.11±0.02
	4	9.06±0.07	6.72±0.01	5.60±0.06	5.58±0.01
	5	9.06±0.07	8.81±0.07	8.43±0.19	7.86±0.01
S13	1	8.43±0.03	3.95±0.04	2.83±0.05	2.49±0.01
	2	8.43±0.03	3.79±0.11	3.56±0.02	3.12±0.05
	3	8.43±0.03	5.62±0.03	4.84±0.07	4.20±0.08
	4	8.43±0.03	8.00±0.02	8.28±0.04	8.65±0.03
	5	8.43±0.03	8.30±0.04	8.49±0.09	8.73±0.07
S14	1	9.05±0.01	3.81±0.02	3.60±0.07	2.93±0.03
	2	9.05±0.01	5.84±0.05	4.33±0.01	4.10±0.09
	3	9.05±0.01	6.62±0.02	6.46±0.11	5.52±0.05
	4	9.05±0.01	7.85±0.09	7.27±0.06	7.15±0.08
	5	9.05±0.01	8.57±0.04	9.33±0.02	9.64±0.02
S15	1	8.71±0.12	8.46±0.02	8.61±0.o5	8.72±0.06
	2	8.71±0.12	8.48±0.06	8.55±0.01	8.74±0.01
	3	8.71±0.12	8.67±0.01	9.07±0.04	9.18±0.08
	4	8.71±0.12	8.71±0.03	9.29±0.06	9.50±0.04
	5	8.71±0.12	8.81±0.01	9.58±0.05	10.04±0.01

S16	1	10.62±0.07	7.25±0.09	7.83±0.01	8.09±0.09
	2	10.62±0.07	8.56±0.03	8.21±0.01	8.16±0.05
	3	10.62±0.07	8.94±0.05	9.36±0.04	9.52±0.01
	4	10.62±0.07	9.81±0.07	9.94±0.02	10.14±0.03
	5	10.62±0.07	10.60±0.03	10.79±0.01	10.83±0.07
S17	1	9.49±0.29	4.50±0.02	4.16±0.01	3.39±0.01
	2	9.49±0.29	4.46±0.05	4.45±0.08	4.24±0.03
	3	9.49±0.29	5.40±0.02	5.38±0.06	4.72±0.01
	4	9.49±0.29	7.53±0.07	7.39±0.01	7.61±0.09
	5	9.49±0.29	8.76±0.04	8.81±0.01	9.15±0.02
S18	1	7.58±0.05	3.13±0.07	3.01±0.02	2.64±0.09
	2	7.58±0.05	3.95±0.01	3.47±0.07	3.13±0.05
	3	7.58±0.05	5.69±0.08	4.82±0.04	4.26±0.01
	4	7.58±0.05	6.82±0.03	7.03±0.02	6.77±0.03
	5	7.58±0.05	7.44±0.07	7.21±0.02	7.05±0.05
S19	1	9.19±0.01	2.72±0.04	2.56±0.04	1.94±0.08
	2	9.19±0.01	3.81±0.21	2.97±0.08	2.62±0.06
	3	9.19±0.01	3.73±0.08	3.54±0.03	3.81±0.04
	4	9.19±0.01	5.67±0.05	6.12±0.03	5.93±0.09
	5	9.19±0.01	7.94±0.03	8.88±0.01	8.96±0.04
M1	1	8.60±0.04	4.39±0.04	3.63±0.07	3.18±0.09
	2	8.60±0.04	4.37±0.02	4.12±0.01	4.10±0.05
	3	8.60±0.04	5.52±0.06	4.63±0.01	4.24±0.02
	4	8.60±0.04	8.41±0.05	8.20±0.04	7.95±0.08
	5	8.60±0.04	8.54±0.01	8.86±0.07	9.12±0.02
M2	1	9.21±0.01	8.88±0.03	8.61±0.01	8.40±0.06
	2	9.21±0.01	8.86±0.05	8.70±0.01	8.54±0.05
	3	9.21±0.01	8.90±0.02	8.75±0.04	8.67±0.09
	4	9.21±0.01	8.97±0.01	9.73±0.05	9.98±0.03
	5	9.21±0.01	9.62±0.04	9.94±0.02	10.31±0.06
M3	1	9.35±0.11	9.28±0.02	9.32±0.08	9.55±0.02
	2	9.35±0.11	9.28±0.07	9.39±0.01	10.01±0.04
	3	9.35±0.11	9.31±0.04	9.47±0.01	10.12±0.15
	4	9.35±0.11	9.30±0.06	10.02±0.02	10.09±0.09
	5	9.35±0.11	9.55±0.01	10.11±0.17	10.28±0.10
M4	1	8.72±0.04	7.48±0.02	7.24±0.08	6.85±0.03
	2	8.72±0.04	7.50±0.03	7.31±0.07	7.04±0.03
	3	8.72±0.04	7.81±0.06	7.60±0.02	7.39±0.01
	4	8.72±0.04	8.57±0.05	8.62±0.03	7.17±0.05
	5	8.72±0.04	8.64±0.01	8.83±0.03	9.26±0.09
M5	1	7.39±0.09	6.27±0.05	5.81±0.10	5.00±0.01
	2	7.39±0.09	6.83±0.08	6.69±0.04	6.47±0.01
	3	7.39±0.09	7.26±0.03	7.05±0.07	6.98±0.02
	4	7.39±0.09	7.28±0.09	7.24±0.01	7.11±0.06
	5	7.39±0.09	7.29±0.02	7.14±0.05	8.06±0.01
M6	1	8.06±0.02	4.74±0.08	4.52±0.03	4.31±0.05
	2	8.06±0.02	4.93±0.04	4.66±0.01	4.48±0.07
	3	8.06±0.02	5.28±0.03	4.94±0.01	4.70±0.05
	4	8.06±0.02	6.15±0.04	6.03±0.09	5.82±0.01
	5	8.06±0.02	7.30±0.02	7.75±0.03	7.96±0.07
M7	1	9.74±0.08	9.40±0.02	9.61±0.04	9.88±0.02
	2	9.74±0.08	9.53±0.03	9.60±0.10	10.07±0.06
	3	9.74±0.08	9.66±0.05	10.22±0.02	10.49±0.04

	4	9.74±0.08	9.71±0.03	10.25±0.07	10.52±0.01
	5	9.74±0.08	10.09±0.03	10.28±0.01	10.51±0.08
M8	1	7.52±0.14	5.60±0.04	5.43±0.07	5.14±0.03
	2	7.52±0.14	6.14±0.09	6.07±0.02	5.93±0.05
	3	7.52±0.14	6.21±0.01	6.36±0.08	6.58±0.03
	4	7.52±0.14	6.27±0.27	7.01±0.18	7.37±0.02
	5	7.52±0.14	7.05±0.02	7.30±0.10	7.62±0.03
M9	1	8.66±0.38	8.60±0.06	8.83±0.01	9.10±0.03
	2	8.66±0.38	8.60±0.04	9.21±0.01	9.28±0.03
	3	8.66±0.38	8.63±0.03	9.19±0.06	9.26±0.01
	4	8.66±0.38	8.71±0.01	9.13±0.07	9.37±0.04
	5	8.66±0.38	9.12±0.04	9.40±0.01	10.06±0.01
M10	1	8.03±1.17	7.13±0.08	6.74±0.01	6.64±0.03
	2	8.03±1.17	7.61±0.04	6.70±0.07	6.82±0.02
	3	8.03±1.17	7.77±0.05	7.83±0.10	7.85±0.04
	4	8.03±1.17	7.82±0.06	7.83±0.01	8.20±0.03
	5	8.03±1.17	7.84±0.04	8.19±0.07	8.48±0.02
M11	1	9.42±0.12	9.22±0.01	9.24±0.03	9.29±0.01
	2	9.42±0.12	9.28±0.06	9.34±0.01	9.40±0.032
	3	9.42±0.12	9.31±0.01	9.65±0.03	9.82±0.08
	4	9.42±0.12	9.48±0.06	9.70±0.01	9.98±0.14
	5	9.42±0.12	9.59±0.08	9.84±0.02	10.25±0.01
M12	1	7.75±0.08	7.72±0.02	7.85±0.07	8.09±0.01
	2	7.75±0.08	7.75±0.02	8.02±0.26	8.30±0.19
	3	7.75±0.08	8.05±0.12	8.21±0.05	8.36±0.02
	4	7.75±0.08	8.13±0.01	8.31±0.01	8.48±0.03
	5	7.75±0.08	8.48±0.18	8.59±0.06	8.70±0.02
M13	1	10.08±0.03	10.04±0.07	10.06±0.05	10.06±0.02
	2	10.08±0.03	10.09±0.04	10.09±0.03	10.10±0.09
	3	10.08±0.03	10.15±0.06	10.17±0.01	10.18±0.02
	4	10.08±0.03	10.26±0.02	10.29±0.27	10.33±0.06
	5	10.08±0.03	10.34±0.07	10.77±0.11	10.85±0.02
M14	1	8.19±0.01	8.01±0.01	8.02±0.07	8.04±0.03
	2	8.19±0.01	8.08±0.05	8.17±0.16	8.39±0.01
	3	8.19±0.01	8.15±0.02	8.59±0.03	9.02±0.11
	4	8.19±0.01	8.40±0.05	9.01±0.20	9.17±0.05
	5	8.19±0.01	8.72±0.01	9.17±0.06	9.68±0.01
M15	1	9.26±0.02	9.68±0.01	10.20±0.02	10.51±0.02
	2	9.26±0.02	9.70±0.04	10.26±0.01	10.79±0.02
	3	9.26±0.02	9.77±0.03	10.42±0.03	10.56±0.03
	4	9.26±0.02	10.04±0.00	10.42±0.06	10.66±0.05
	5	9.26±0.02	10.21±0.01	10.54±0.01	10.71±0.02

Each value represents mean value ± standard deviation from triplicate observations

Table 4: Enzyme production by the LAB isolates

Isolate Code	Protease Production	Lipase Production
S1	+	-
S3	-	-
S4	-	+
S5	-	-
S6	+	+
S7	-	-
S8	-	-
S9	+	
S10	-	-
S11	-	-
S12	+	+
S13	-	-
S14	+	-
S15	-	-
S16	-	-
S17	+	+
S18	-	-
S19	-	-
M1	-	-
M2	+	+
M3	-	-
M4	+	+
M5	+	-
M6	-	-
M7	-	+
M8	+	-
M9	-	-
M10	-	-
M11	+	-
M12	+	–
M13	+	+
M14	-	-
M15	+	+

Table 5: Antimicrobial activity of isolated lactic acid bacteria (Diameter of inhibition zone: mm)

Isolate code	*Pseudomonas aeruginosa*	*Salmonella typhi*	*Klebsiella pneumoniae*	*Listeria monocytogenes*	*Staphylococcus aureus*	*Escherichia coli*
S5	17	10	11	13	15	18
S6	8	5	7	11	11	15
S9	15	13	17	18	13	15
S10	5	11	8	12	17	11
S14	8	9	18	15	-	-
S15	11	9	7	11	15	10
M1	18	10	15	9	10	5
M3	18	10	11	18	7	12
M13	5	-	-	10	15	15

M14	13	13	17	-	-	12
M15	12	8	15	20	21	18

Key: - Resistance, + Sensitive, 0.5-4mm Weak Inhibition, 5-9mm Strong Inhibition, 10-15mm Very Strong Inhibition

Table 6: Quantification of Antimicrobial Compounds of the Selected Isolates

Isolate code	Lactic Acid (g/l)	Diacetyl (g/l)	Hydrogen peroxide (g/l)
S5	3.2	1.4	0.5
S6	2.4	1.5	0.8
S9	0.9	1.3	0.7
S10	1.3	1.6	0.5
S14	0.7	1.8	0.4
S15	1.8	1.2	0.4
M1	0.9	1.0	0.3
M3	1.8	1.4	0.6
M13	0.6	1.0	0.9
M14	0.8	0.9	0.2
M15	3.6	1.1	0.6

Table 7: Safety Assessment of Isolated Lactic Acid Bacteria with Desirable Probiotic Traits

Isolate code	DNase	Gelatinase	Lecithinase	Hemolysin
S5	-	-	-	-
S6	-	-	-	-
M15	-	-	-	-

Table 8: Antibiotic Susceptibility of the Lactic Acid Bacteria

Isolate code	Ampicillin	Tetracycline	Clindamycin	Chloramphenicol	Erythromycin	Gentamycin
S5	-	-	-	-	-	-
S6	-	-	-	+	-	-
M15	-	-	-	-	-	-

Key: + Positive; -Negative

Table 9: Antioxidative Properties of the Selected Isolates

Isolates	**DPPH (%)**	**FRAP (µg)**
S6	40.12	193.102
M15	34.17	96.438
S6+M15	51.13	199.038
Control	56.20	202.718

Discussion

The use of microorganisms in the synthesis of useful products especially via fermentation (as probiotics) is no longer a new thing in the field of microbiology. However, in order to ensure the quality and safety of these products, there is need to ensure that the microorganisms to be used are capable of giving the best product quality and are confirmed safe. The study therefore focused on the probiotic properties of lactic acid bacteria (LAB) isolated from goat milk.

From the total viable count of LAB in the dairy milk samples collected, it is indicative that the samples are hugely colonized by lactic acid bacteria. More so, an eventual 34 pure LAB isolates were recovered. This corroborates with reports from different authors regarding the presence of lactic acid bacteria in different varieties of samples, milk inclusive. Gemechu (2015) recovered a total of 50 LAB isolates from goat milk and this discrepancy from the 34 reported in this study may be due to the relatively higher number of goats (11) used in the earlier mentioned study compared to the two used in this study. Additionally, a total of 47 LAB isolates were recovered from different fermented dairy products including feta cheese, manouri cheese and xerotyri cheese purchased from the Lefkada region of the nation of Greece. The presence of such high population of LAB in these fermented dairy products may signify that this group of microorganisms plays vital roles in the fermentation of these products.

Upon identification of the 34 strains of LAB, the most predominant species was *Lactobacillus plantarum* (9) while the least occurring were *Lactobacillus bulgaricus* and *Lactobacillus fermentum* (1). This observation is similar to the reports of Pavli *et al*. (2016) in which the most prevalent LAB strain of all 47 isolated from several dairy products in Greece was *Lactobacillus plantarum* (19). The different species of LAB isolated from this study have been implicated in different studies as the autochthonous organisms during the spontaneous fermentation of dairy products such as milk, cheese, sauerkraut, etc. In ealier studies by Saad *et al*., 2013 and Losio *et al*., 2015, other species such as *Lactobacillus casei*, *Lactobacillus brevis*, etc. were isolated from dairy samples.

One of the features of a potential probiotic microorganism is that it must remain viable while it passes through the gastrointestinal tracts in order to provide its health benefits and also in relatively high population between 10^6 and 10^7 CFU/g (Argyri *et al*., 2013; Kandylis *et al*., 2016). The obtained isolates were subjected to a number of physiological tests; although the level of tolerance to different bile concentrations varied, majority of the isolates when exposed to isolates grew profusely at 0.5-2.0% concentrations; this observation is in line with the reports by Pavli *et al*. (2016) that LAB isolated from cheese showed high resistance to similar concentrations of bile salts for over four hours of exposure. Similarly, Gemechu (2015) reported over 90% survival rate in the LAB strains isolated from raw goat milk when exposed to different concentrations of bile salts; this characteristic of the LAB isolates from this study therefore qualifies them as potential probiotics. It is essential that these potential probiotic isolates survive this condition as biliary salts are vital active agents in the digestive system of humans which performs inhibitory role against unwanted pathogenic bacteria by damaging their DNA and cell membranes.

The survival of LAB strains when exposed to simulated acidic conditions in the human gut is an essential feature for a probiotic microbe. From this study, it was observed that the survival of the isolates varied when exposed to low pH of 1, 2 and 3 with majority surviving up to 3 hours of exposure. This corroborates with the assertions of Kandylis *et al*. (2016) that for a microbe to be considered as a probiotic, it must be able to survive at least 2 hours of exposure under acidic environments as this is most likely what is obtainable in the human gut, hence, when they are ingested, the delivery of their health benefits is assured. The report from the present study is similar to observations from Abd-El Salam & Benkerroum (2006).) in which all the LAB strains isolated from *tchoukou*, a traditional milk cheese produced in Tahoua, Maradi and Zinder regions of Niger.

In this study, a fair percentage (8%) of the isolates showed capability of producing lipase and protease, that is, they showed proteolytic and lipolytic potential. These observations are similar to observations from a number of studies; in a study by Monfredini *et al*. (2012), the different strains of LAB isolated from Grana-Cheese showed weak lipolytic activity. Similarly, only a few number of LAB strains showed weak lipolytic activity, whereas 64% did not produce lipase according to a study by Gemechu (2015) on LAB strains isolated from raw goat milk. Meanwhile, production of lipase by probiotic LAB strains is usually not highly desired especially in dairy products like butter, yoghurt and milk but only cheese where low levels of lipolysis serves to give certain types of cheese good aroma and culture (Monfredini *et al*., 2012). Although proteolytic activity is not a strong or common property of LAB strains, however,

whenever they are produced during fermentation, it helps in development of flavor in fermented dairy products. In addition, proteolytic bacteria could also produce non-epitopic peptides which help in prevention of milk allergy especially in infants as a result of poor digestion of milk proteins (Pescuma *et al*., 2009; Lopes-Kleine and Monnet, 2011).

The LAB strains showed varying inhibitory activity against the test isolates, with majority of the test isolates being susceptible to the activity of the selected LAB strains. This ability of the strains is essential in the prevention of the invasion and colonization of the gastrointestinal tracts by pathogenic microorganisms. This study is similar to the reports of Arques *et al*. (2015) in which majority of the LAB strains isolated from cow milk showed strong but varying antimicrobial activity against test isolates such as *E. coli*, *Listeria moncytogenes*, *Salmonella enteritidis* and *S. aureus*. However, according to reports by Adeniyi *et al*. (2015), significant antibacterial activity is considered when the diameter of inhibition is ≥12 mm but weak when the clearance zones are ≤9 mm, thus only a fair number of the selected LAB strains showed effective antimicrobial activity. In addition, this antimicrobial activity exhibited by these strains is a reflection of their production of bioactive compounds such as lactic acid and hydrogen peroxide. However, the considerable production of diacetyl by these isolates is very much to be desired as it has been reported by other authors (Ferrari *et al*., 2016; Gemechu, 2015) that diacetyl when produced during fermentation of dairy products helps improve the flavor of fermented products while also giving them enticing aroma.

Usually, the inability of LAB strains to produce haemolysin or show haemolytic activity is a highly desirable trait in potential probiotic bacteria. The selected strains from this study did not show haemolytic activity. This observation is consistent with reports from a number of authors; Pisano *et al*. (2014) reported that LAB strains isolated from Sardinian dairy products did not exhibit haemolytic activity. This characteristic is an indication that the LAB strains from this study are avirulent and thus can cause no harm when ingested. More so, their incapability of producing other harmful enzymes such as lecithinase, gelatinase and DNase is an indication of their safety and hence they can be used as starter cultures.

From earlier studies, it is critical that any LAB strain to be used as a probiotic must not be drug-resistant; rather, they should be sensitive to most common antibiotics of choice. This observation corroborates with observations from the report of Zhang *et al*. (2014) that *Lactobacillus delbrueckii* ssp. *indicus* WDS-7 isolated from chinese traditional fermented Buffalo milk was sensitive to similar antibiotics as used in this study.

The antioxidant activity observed in the consortium of isolates S6 and M15, particularly its DPPH radical scavenging value of 51.13%, aligns with earlier research indicating that mixed LAB cultures often exhibit enhanced antioxidative effects compared to single strains, as reported by Lin and Yen (1999) and Kullisaar et al. (2002). Similar to this findings, Kim et al. (2005) demonstrated that LAB with strong DPPH scavenging ability typically show parallel increases in reducing power, reinforcing the close similarity between the consortium and the reference starter culture. The lower antioxidant activity of isolate M15 alone mirrors results from Virtanen et al. (2007), who noted that individual LAB strains often show weaker responses due to limited metabolite diversity. Additionally, the high reducing-power activity shown by the consortium is consistent with observations by Tang et al. (2010) that cooperative fermentation by LAB strains enhances electron-donating capacity. Overall, this data supports earlier conclusions by Giraffa (2014) that synergistic LAB combinations outperform single isolates in functional fermented products, further emphasizing the probiotic and technological relevance of strain consortia.

Conclusion

From this study, only two isolates namely *Lactobacillus plantarum* (S6) and *Lactobacillus acidophilus* (M15) exhibited desirable probiotic traits with antimicrobial properties as well as good technological properties and antioxidant potential, hence are potential probiotic candidates to be used as starter cultures in fermentation of different dairy products or other food substances.

References

Abd-El Salam, M., & Benkerroum, N. (2006). North African brined cheeses. *Brined cheeses*, 139-187.

Abdelbasset, M., & Djamila, K. (2008). Antimicrobial activity of autochthonous lactic acid bacteria isolated from Algerian traditional fermented milk "Raïb." *African Journal of Biotechnology, 7*. https://api.semanticscholar.org/CorpusID:13859234

Adeniyi, B. A., Adetoye, A., & Ayeni, F. A. (2015). Antibacterial activities of lactic acid bacteria isolated from cow faeces against potential enteric pathogens. *African health sciences*, *15*(3), 888-895.

Akabanda, F., Hlortsi, E.H. & Owusu-Kwarteng, J. Food safety knowledge, attitudes and practices of institutional food-handlers in Ghana. *BMC Public Health* **17**, 40 (2017). https://doi.org/10.1186/s12889-016-3986-9

Amaretti, A., Di Nunzio, M., Pompei, A., Raimondi, S., Rossi, M., & Bordoni, A. (2013). Antioxidant properties of potential probiotic bacteria: In vitro and in vivo activities. *Applied Microbiology and Biotechnology, 97*(2), 809–817.*

AOAC (2010). Official Methods of Analysis of Association of Official Analytical Chemists. 18th Edition, Washington, DC.

Argyri, A.A., Zoumpopoulou, G., Karatzas, K.A.G., Tsakalidou, E., Nychas, G.J.E., Panagou, E.Z. and Tassou, C.C. (2013). Selection of potential probiotic lactic acid bacteria from fermented olives by in vitro tests. *Food Microbiology*, 33:282–291. https://doi.org/10.1016/j.fm.2012.10.005.

Arqués, J. L., Rodríguez, E., Langa, S., Landete, J. M., & Medina, M. (2015). Antimicrobial activity of lactic acid bacteria in dairy products and gut: effect on pathogens. *BioMed research international*, *2015*(1), 584183.

Badis, A., Guetarni, D., Boudjema, B.M., Henni, D.E. and Kihal, M. (2004). Identification and technological properties of lactic acid bacteria isolated from raw goat milk of four Algerian races. *Food Microbiology*, 21:579–588. https://doi.org/10.1016/j. fm.2003.11.006.

Beerens, H. and Luquet, F.M. (1990). Practical Guide for Microbiological Analysis of Milk and Dairy Products. Editorial Acríbia, SA, Zaragoza.

Bennama, R., Fernandez, M., Ladero, V., Alvarez, M.A., Rechidi- Sidhoum, N. and Bensoltane, A. (2012). Isolation of an exopolysaccharide- producing Streptococcus thermophilusfrom Algerian raw cow milk. European Food Research and Technology. 234: 119-125.

Birollo, G. A., Reinheimer, J. A., & Vinderola, C. G. (2000). Viability of lactic acid microflora in different types of yoghurt. *Food Research International, 33*(9), 799–805. https://doi.org/10.1016/S0963-9969(00)00101-0

Chassard C, Delmas E, Robert C, Bernalier-Donadille A. 2010. The cellulose-degrading microbial community of the human gut varies according to the presence or absence of methanogens. FEMS Microbiol. Ecol. 74:205–213.

Clinical and Laboratory Standards Institute (CLSI). (2011). Performance Standards for Antimicrobial Susceptibility Testing.

de Almeida Júnior, W.L.G., da Silva Ferrari, I., de Souza, J.V., da Silva, C.D.A., da Costa, M.M. and Dias, F.S. (2015). Characterization and evaluation of lactic acid bacteria isolated from goat milk. *Food Control*, 53:96-103. https://doi.org/10.1016/j. foodcont.2015.01.013.

de Carvalho Lima, K.G., Kruger, M.F., Behrens, J., Destro, M.T., Landgraf, M. and de Melo Franco, B.D.G. (2009). Evaluation of culture media for enumeration of *Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidobacterium animalis* in the presence of *Lactobacillus delbrueckii* subsp *bulgaricus* and *Streptococcus thermophilus*. *Food Science and Technology*, 42:491–495. https://doi.org/10.1016/j.lwt.2008.08.011.

Eaton, T.J. and Gasson, M.J. (2001). Molecular screening of Enterococcus virulence determinants and potential for genetic exchange between food and medical isolates. *Applied and Environmental Microbiology*, 67(4):1628–1635. https://doi.org/10.1128/ AEM.67.4.1628-1635.

Ferrari, I.S., de Souza, J.V., Ramos, C.L., da Costa, M.M., Schwan, R.F. and Dias, F.S. (2016). Selection of autochthonous lactic acid bacteria from goat dairies and their addition to evaluate the inhibition of *Salmonella typhi* in artisanal cheese. *Food Microbiology*, 60:29. https://doi.org/10.3168/jds.2017-14003.

Gemechu, T. (2015). Review on lactic acid bacteria function in milk fermentation and preservation. *African Journal of Food Science*, *9*(4), 170-175.

Guetouache, M., & Guessas, B. (2015). Characterization and identification of lactic acid bacteria isolated from traditional cheese (Klila) prepared from cow's milk. African Journal of Microbiology Research, 9(2), 71-77.

Gupta H. and Malik R. K. (2007). Incidence of virulence in Bacteriocin-Producing Enterococcal isolates. *Lait.* 87:587-601

Haghshenas, B., Nami, Y., Abdullah, N., Radiah, D., Rosli, R. and Khosroushahi, A. Y. (2014). Anti-proliferative effects of Enterococcus strains isolated from fermented dairy products on different cancer cell lines. *Journal of Functional Foods,* 11(C):363–374. https://doi.org/10.1016/J.JFF.2014.10.002.

Harrigan W.F. and McCance M.E. (1990). Laboratory Methods in Food and Dairy Microbiology. Academic Press, London. 8th edition.

Hassanzadazar, H., Ehsani, A., Mardani, K. and Hesari, J. (2012). Investigation of antibacterial, acid and bile tolerance properties of lactobacilli isolated from Koozeh cheese. *Veterinary Research Forum*, 3: 181.

Iranmanesh, M., Ezzatpanah, H. and Mojgani, N. (2014). Antibacterial activity and cholesterol assimilation of lactic acid bacteria isolated from traditional Iranian dairy products. *Food Science and Technology*, 58: 355–359. https://doi.org/10.1016/j.lwt.2013.10.005.

Kandylis, P., Pissaridi, K., Bekatorou, A., Kanellaki, M. and Koutinas, A. A. (2016). *Dairy and non-dairy probiotic beverages. Current Opinion in Food Science,* 7: 58–63. doi:10.1016/j.cofs.2015.11.012. 10.1016/j.cofs.2015.11.012

Kullisaar, T., Zilmer, M., Mikelsaar, M., Vihalemm, T., Annuk, H., Kairane, C., & Kilk, A. (2002). Two antioxidative lactobacilli strains as promising probiotics. *International Journal of Food Microbiology, 72*(3), 215–224.*

Leuschner, R.G., Kenneally, P.M., Arendt, E.K., 1997. Method for the rapid quantitative detection of lipolytic activity among food fermenting microorganisms. Int. J. Food Microbiol. 37, 237–240. https://doi.org/10.1016/S0168-1605(97)00074-3.

Lin, M. Y., & Yen, C. L. (1999). Antioxidative ability of lactic acid bacteria. *Journal of Agricultural and Food Chemistry, 47*(4), 1460–1466.

Lopez-Kleine, L. and Monnet, V. (2011). Lactic acid bacteria. *Proteolytic System*, 49–55.

Monfredini, L.U.C.A., Settanni, L., Poznanski, E., Cavazza, A. and Franciosi, E. (2012). The spatial distribution of bacteria in Grana-cheese during ripening. Systematic and Applied Microbiology, 35:54-63. https://doi.org/10.1016/j.syapm.2011.07.002.

Neethu M. J., Craig R. B. and Malik A. H., 2015. Comparison of Microbiological and Probiotic Characteristics of Lactobacilli Isolates from Dairy Food Products and Animal Rumen Contents. *Molecular Diversity Preservation International and Multidisciplinary Digital Publishing Institute Journals* (MDPI journals) www.mdpi.com/journal/microorganisms. ISSN 2076-2607 doi:10.3390/microorganisms3020198.

Okonkwo, O. I. (2011). Microbiological analyses and safety evaluation of nono: A fermented milk product consumed in most parts of Northern Nigeria. International Journal of Dairy Science, 6, 181–189.

O'Sullivan L, Ross RP, Hill C. Potential of bacteriocin-producing lactic acid bacteria for improvements in food safety and quality. Biochimie. 2002 May-Jun;84(5-6):593-604. doi: 10.1016/s0300-9084(02)01457-8. PMID: 12423803.

Pandya, A. J., & Ghodke, K. M. (2007). Goat and sheep milk products other than cheeses and yoghurt. *Small Ruminant Research, 68*(1–2), 193–206. https://doi.org/10.1016/j.smallrumres.2006.09.007

Pavli, F.G., Argyri, A.A., Papadopoulou, O.S., Nychas, G.J.E. and Chorianopoulos, N.G. (2016). Probiotic potential of lactic acid bacteria from traditional fermented dairy and meat products: Assessment by *In Vitro* Tests and Molecular Characterization. *Journal of Probiotics and Health*, 4:157. doi: 10.4172/2329-8901.1000157

Pescuma, M., Hebert, E.M., Dalgalarrondo, M., Haertle, T., Mozzi, F. and Chobert, J.M. (2009). Effect of exopolysaccharides on the hydrolysis of betalactoglobulin by *Lactobacillus acidophilus* CRL 636 in an in vitro gastric/pancreatic system. *Journal of Agricultural and Food Chemistry*, 57:(12):5571–5577. https://doi.org/10.1021/jf9006505.

Picon, A., Garde, S., Avila, M. and Nunez, M. (2016). Microbiota dynamics and lactic acid bacteria biodiversity in raw goat milk cheeses. *International Dairy Journal*, 58: 14-22. https://doi. org/10.1016/j.idairyj.2015.09.010.

Pisano, M.B., Viale, S., Conti, S. and Fadda, M.E. (2014). Preliminary evaluation of probiotic properties of *Lactobacillus* strains isolated from Sardinian dairy products. *BioMed Research International*, Volume 2014: 1-9.http://dx.doi.org/10.1155/2014/286390

Pooja T., Modi H.A. and Prajapati J.B. (2015). Isolation, Characterisation and Safety assessment of Lactic Acid Bacterial Isolates from Fermented Food Products. *International Journal of Current Microbiology and Applied Sciences* ISSN: 2319-7706. 4(4):713-725 http://www.ijcmas.com

Qing L., Xiaoli L., Mingsheng D., Jianzhong Z. and Ying W., 2015. Adhesion and probiotic properties of *Lactobacillus plantarum* isolated from Chinese traditional fermented soybean paste. *Global Advanced Research Journal of Food Science and Technology*, 4(1): 001-009.

Quigley, L., O'Sullivan, O., Stanton, C., Beresford, T.P., Ross, R.P., Fitzgerald, G.F. and Cotter, P.D. (2013). The complex microbiota of raw milk. *FEMS Microbiology Review*, 37:664–698. https://doi.org/10.1111/1574-6976.12030.

Sanni, A.I. and Adesulu, A.T. (2013). Microbiological and physico-chemical changes during fermentation of maize for *masa* production. *African Journal* of *Microbiology Research*, 7(35): 4555-4362.

Saranraj, P., Naidu, M. A., & Sivasakthivelan, P. (2013). Lactic acid bacteria and its antimicrobial properties: A review. *International Journal of Pharma and Bio Sciences, 4*, 1124–1133.

Sfakianakis P, Tzia C. Conventional and Innovative Processing of Milk for Yogurt Manufacture; Development of Texture and Flavor: A Review. Foods. 2014 Mar 11;3(1):176-193. doi: 10.3390/foods3010176. PMID: 28234312; PMCID: PMC5302305.

Shah, N. P. (2000). Probiotic bacteria: Selective enumeration and survival in dairy foods. *Journal of Dairy Science, 83*(4), 894–907.*

Shah, N., Prajapati, J.B. (2013): Effect of carbon dioxide on sensory attributes, physico-chemical parameters and viability of Probiotic L. helveticus MTCC 5463 in fermented milk, J. food Sci. Technol. DOI 10.1007/s13197-013-0943-9

Wang, Y., Wu, Y., Wang, Y., Xu, H., Mei, X., Yu, D., Wang, Y., & Li, W. (2017). Antioxidant properties of probiotic bacteria. *Nutrients, 9*(5), 521.

Yu, Z., Zhang, X., Li, S., Li, C., Li, D. and Yang, Z. (2013). Evaluation of probiotic properties of *Lactobacillus plantarum* strains isolated from Chinese sauerkraut. *World Journal of Microbiology and Biotechnology*, 29:489–498. https://doi.org/10.1007/s11274-012-1202-3.

Zhang, S., Zhang, L., Jiao, Y., Luo, X., Li, H., Xin, L., ... & Ma, C. (2014). Technological characterization of lactic acid bacteria protease isolated from traditional chinese fermented milk. *Journal of Food Quality*, *37*(6), 395-402.