Isolation, Identification and Screening of Potential Probiotic Bacteria in Milk from South African Saanen Goats

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Abstract

This study aimed to evaluate lactic acid bacteria isolates from Saanen goats' milk for probiotic attributes, thereby determining their potential as direct-fed microbials for goats. Isolates were identified using API 50CH system, 16S rDNA sequencing and matrix-assisted laser desorption ionization-time of flight mass spectrometry. All 17 isolates obtained were identified as *Lactobacillus plantarum* except one identified as *Pediococcus acidilactici*. Four isolates identified as *L*. *plantarum* (Accession numbers KJ026587.1, KM207826.1, KC83663.1 and KJ958428.1) by at least two of the techniques used and isolate 17 differently identified by all the methods used were selected as representatives and then screened for probiotic properties. These isolates displayed phenotypic probiotic attributes including tolerance to acid and bile salts, ability to adhere to intestines and possession of antagonistic activities against *Proteus vulgaris*, *Staphylococcus aureus*, *Salmonella typhimurium, Pseudomonas aeruginosa* and *Escherichia coli*. The lactic acid bacteria isolated from Saanen goats' milk showed potential to be used as sustainable probiotics in goats' industry. Successful use of probiotics in animals depends upon availability of appropriate isolates originating from the specific host animal. This study is a positive contribution towards identification of isolates with potential for formulation as direct-fed microbials for South African Saanen goats.

Keywords

Lactic acid bacteria, Probiotics, *Lactobacillus,* Direct-fed microbials, Saanen goats

Introduction

For years, antibiotics have been included in animal feed at sub-therapeutic levels, acting as growth promoters and to treat or prevent diseases [27]. However, with mounting public concerns about development of antimicrobials resistance and transfer of antibiotic

resistance genes, antibiotics have been banned in some areas of the world. Therefore, the need to find alternative methods to control and prevent pathogenic bacterial colonization has increased. In animal nutrition, probiotics from the three different groups, lactic acid bacteria [40], *Bacillus* spores [1], or yeasts [36] are used as feed additives. The modulation of the gut microbiota with new feed additives, such as probiotics, is a topical issue in animal breeding and creates fascinating possibilities [16].

The Joint Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) working group, defined probiotics as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [21]. The most studied probiotics are lactic acid bacteria (LAB), particularly *Lactobacillus* and *Bifidobacterium*. The definition of probiotic requires that the efficacy and safety of all the strains be verified, and thus, assessment of this constitutes an important part of the characterization. As the probiotic capacities are strain-dependent, methods for reliable identification of LAB are of great importance. Identification of LAB based on carbohydrate fermentation patterns is unreliable and not accurate enough to distinguish closely related strains due to their similar nutritional requirements [34]. In recent years, phenotypic identification is complemented by molecular techniques such as sequencing analysis of the partial 16S rDNA and MALDI- TOF.

Selection of promising probiotic bacteria must fulfil certain standards, and in vitro tests are performed when screening for probiotic candidates. These strains should be generally recognized as safe with minimal possibilities for the antibiotic resistance transfer [45]. Probiotic strains should have the ability to survive through the gastrointestinal tract (GIT), particularly low pH and high bile toxicity prevalent in the upper digestive tract. Furthermore, bacterial strains should have the ability to adhere to intestinal epithelium and produce antimicrobial activities toward potentially pathogenic microorganisms [43]. The aim of the current study was to identify lactic acid bacteria isolated from raw goats' milk and to screen them for probiotic attributes in order to select strains that can be used in goat industry as direct fed microbials.

Materials and Methods

Collection of Milk Samples

A total of 40 raw goats' milk samples were collected from Small-stock Division of the Agricultural Research Council-Animal Production Institute, Irene, South Africa. Milk samples were obtained under hygienic conditions from healthy animals, by hand milking. Aliquots of 200 ml sample per goat were collected into sterile Schott bottles and then transported on ice to the laboratory for analyses within 2 h.

Total Viable Bacteria Counts (TVBC) and Coliform Counts

Each milk sample was vortexed to ensure homogenisation. Then 1 ml of each sample was pipetted aseptically into 9 ml (1:10 dilution) of sterile saline solution (0.85% *w*/*v* NaCl) in a test tube. The mixture was then vortexed (Heidolph REAX 2000, Germany) for 5 min. Thereafter the suspension was serially diluted up to 10^{-6} dilution using sterile saline

solution. Then 1 ml sample from each dilution was plated out onto nutrient agar (Biolab) and violet red bile agar (Biolab) in triplicates for total counts and coliforms, respectively. The plates were incubated at 37 °C for 48 h. The total colony forming units per millilitre (cfu ml^{-1}) of aerobic bacteria and coliforms were recorded.

Isolation and Identification of Lactic Acid Bacteria

One millilitre of each milk sample was aseptically added to 9 ml of sterile 0.85% saline solution and mixed thoroughly. Serial dilutions were performed and 1 ml aliquots from the dilutions were plated out onto De Man, Rogosa and Sharpe (MRS) agar supplemented with 0.05 g l^{-1} cysteine-HCL (MRS-cysHCL) in triplicate by pour plate method [2]. Identification of the isolates at genus level was carried out following the criteria of Sharpe [38], using morphological, phenotypic and biochemical methods. The isolates were examined microscopically for gram reaction and catalase production [19]. In addition, all isolates were tested for CO_2 production from glucose in MRS broth tubes and for their ability to grow at 10 and 45 °C [29]. The ability of isolates to ferment carbohydrates was studied using the API 50CH system (Biomérieux, France), according to the manufacturer's instructions. For genotypic characterization, total genomic DNA of isolates was extracted using the MasterPure DNA Purification Kit (Epicentre, Madison, WI, USA), according to the manufacturer's instructions, with minor modifications. Polymerase chain reaction (PCR) was carried out using Taq PCR Kit (BioLabs, New England), as per manufacturer's specifications. Amplification of the 16 rDNA was performed using both primers 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1525R (5'-AGG GAG GTG WTC CAR CCG CA-3' [26]. For sequence analysis of the 16S rDNA, the PCR products were purified with DNA Clean and Concentrator™ -25 (Zymo Research) according to the manufacturer's instructions, and then sequenced with primer 27F. Sequencing of the amplicons was performed using Big-Dye Sequencer ABIPRISM 313 × l. Sequence homologies were examined by comparing the sequences obtained with those of the GenBank database using BLAST software and identified according to the closest relative. The isolates were also identified using MALDI-TOF analysis in a MALDI BIOTYPER MICROFLEX LT (Bruker Daltonik, Bremen, Germany), according to the manufacturer's instructions.

Screening of Isolates for Probiotic Attributes

Acid Tolerance

The acid tolerance of the isolates was studied using a method described by Liu et al. [24] with minor modifications. Briefly, 1 ml aliquot of the overnight cultures (adjusted to approximately 1×10^8 cfu ml^{-1}) was inoculated into 100 ml of MRS-cysHCL broth and adjusted to pH 1.0, 2.0 and 3.0 using 1 N hydrochloric acid (HCL). The cultures were then incubated anaerobically at 37 °C in anaerobic jars (Oxoid) containing AnaeroGen™ 2.5 L sachet (Thermo Scientific). Then, bacterial growth was examined at 0, 1, 2 and 3 h of incubation using the pour plate technique.

Bile Tolerance

The ability of isolates to grow in the presence of bile salt was determined in MRS-cysHCL broth as described by Walker and Gilliland [46]. Briefly, MRS-cysHCL broths were enriched with 0.3 and 0.5% of ox-gall (Biolab), and then inoculated with 1×10^8 cfu ml^{-1} of each culture. The cultures were incubated anaerobically at 37 °C in anaerobic jars containing AnaeroGen™ 2.5 L sachet (Thermo Scientific). Survival and growth of the cultures were examined at 0, 2, 4 and 24 h of incubation using pour plate technique.

Antibiotic Susceptibility Testing

The antibiotic susceptibility of isolates was assessed using the disc diffusion method according to Charteris et al. [8]. The broth cultures of isolates were prepared in MRS-cysHCL and adjusted to 0.5 McFarland standards (equivalent to 1×10^8 cfu ml^{-1}). Then, 100 µl aliquots of freshly prepared bacterial cultures were each spread onto MRS agar plates. The antibiotic discs were placed on the surface of agar and the plates were incubated anaerobically at 37 °C for 24 h in anaerobic jars (Oxoid) containing AnaeroGen™ 2.5 L sachets. Susceptibility pattern was assessed for vancomycin (30 µg), ampicillin (10 µg), cephalothin (30 µg), co- trimoxazol (25 µg), nalidixic acid (30 µg), gentamycin (10 µg), penicillin G (10 µg), tetracycline (30 µg), erythromycin (15 µg) and oxytetracycline (30 µg). The diameters of inhibition zones were measured from the edge to the border of the zone using a ruler and the results were recorded as average of three readings.

Production of Antimicrobial Activities

The LAB isolates were tested for production of antimicrobial activities against *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus vulgaris* (ATCC 6380), *Salmonella typhimurium* (ATCC 49416), and *Staphylococcus aureus* (ATCC 33591) using agar well diffusion technique according to Mohankuman and Murugalatha [28]. Isolates were separately grown in MRS-cysHCL broth for 48 h at 37 °C. Cell free supernatants were obtained by centrifugation of cultures at 2236 x *g* for 10 min at room temperature. Wells of 6 mm diameters were made on the solidified Mueller Hinton agar plates (Oxoid) evenly spread separately with overnight cultures of each test pathogen. Aliquots of the LAB culture supernatant (100 µl) were dispensed into the wells, and the plates were incubated overnight at 37 °C. The diameters of clear zones of growth inhibition around each well were measured from the edge to the border of the zone using a ruler.

Adhesion Assay

Porcine ileum, collected from pigs immediately after slaughter, were aseptically dissected into 3 cm long sections and kept on ice for a maximum of 9 h. The 5 bacterial isolates were each inoculated into 250 ml MRS-cysHCL broth and incubated at 37 °C to OD_{600} = 1.2, which is equivalent to approximately 1×10^8 cfu ml^{-1}. A section of ileum was added to each of the cultures and incubated for 6 h at 8 °C on a rotary shaker. Subsamples of the cultures were withdrawn every 2 h, serially diluted and plated onto MRS-cysHCL agar plates. Colonies were counted after 24 h of incubation at 37 °C. Furthermore, the ileum sections were aseptically removed from the flasks and mucus layer carefully scraped off with a sterile glass

slide. Preparations of the mucus samples on microscopic slides were treated with the BacLight viability probe (Molecular Probes Inc., Eugene, Oregon, USA) for visualization of adhered bacteria. The slides were incubated for 10 min in the dark at room temperature. Images of adhering bacterial cells were captured using a high-performance CCD camera, mounted on a Nikon Eclipse E400 epi-fluorescence microscope, equipped with a × 60/1.4 Dic H oil objective and filters. Sections of ileum suspended in MRS-cysHCL broth but not inoculated with any bacteria served as control [6].

Results

Total Viable Bacteria Counts (TVBC) and Coliform Counts

The average total viable bacterial count (TVBC) obtained from the raw goats milk was 2.33×10^2 cfu ml^{-1}. This count was within the acceptable standards set in the regulations R.489, which has established the minimum legal standards for raw milk to be <50,000 cfu ml^{-1} for TVBC. No coliforms were detected in any of the raw goats' milk analysed in the current study. This indicated that good herd hygiene, uncontaminated water, proper hygienic milking procedures, and adequately washed and maintained milking equipment were used.

Isolation and Identification of Lactic Acid Bacteria

A total of 17 gram-positive, catalase negative, non-spore forming cocci and rods were isolated and identified using API 50CH system, 16S rDNA sequencing and MALDI-TOF (Table 1). After preliminary phenotypic characterization tests and the interpretation of the API 50CH system, 16 of the isolates were identified as *Lactobacillus plantarum* while one was identi-fied as *Lactobacillus rhamnosus*. Fifteen of the *L. plantarum* isolates were satisfactorily identified (>99%), while for *L. rhamnosus* and one *L. plantarum*, a doubtful identification was obtained. Then, the 17 isolates were further subjected to 16S rDNA sequencing for identification. Based on the 16S rDNA partial sequencing, it was found that the dominant bac-teria found in raw goats' milk were lactobacilli. Ten isolates, including the isolate that was identified as *L. rhamnosus* using API system, were identified as *Lactobacillus pentosus* and seven as *Lactobacillus plantarum*. In an attempt to discrimi-nate between these bacteria we further identified the isolates using their protein profiles using MALDI-TOF analysis. The results of MALDI-TOF analysis enabled reliable identification of the 5 isolates, with biotyper log scores >2.300, which is regarded as highly probable species identification. For the 11 isolates, MALDI-TOF analysis yielded scores of ≥2.000, indicating secure genus or probable species identification. The remaining isolate was probable identified at genus level with score of >1.700. From the 17 isolates obtained from raw goats' milk, 5 representatives were selected for in vitro screening of probiotic attributes.

Table 1. Identification of LAB isolates with API 50CH system, 16S rDNA sequencing and MALDI-TOF

Isolate number	API 50CH Identification	16S rDNA sequencing			MALDI-TOF	
		Phylogenetic affiliation	Accession number	% similarity	Organism best match	BioTyper log score
1	***Lactobacillus plantarum***	***Lactobacillus plantarum***	**KJ026587.1**	**95**	***Lactobacillus plantarum***	**2.307**
2	*Lactobacillus plantarum*	*Lactobacillus pentosus*	AB362714.1	98	*Lactobacillus plantarum*	2.377
3	*Lactobacillus plantarum*	*Lactobacillus pentosus*	AB362714.1	98	*Lactobacillus plantarum*	2.312
4	*Lactobacillus plantarum*	*Lactobacillus plantarum*	KM207826.1	96	*Lactobacillus plantarum*	2.241
5	*Lactobacillus plantarum*	*Lactobacillus pentosus*	AB362714.1	98	*Lactobacillus plantarum*	2.178
6	*Lactobacillus plantarum*	*Lactobacillus pentosus*	AB362714.1	98	*Lactobacillus plantarum*	2.255
7	*Lactobacillus plantarum*	*Lactobacillus pentosus*	KM207826.1	97	*Lactobacillus plantarum*	2.260
8	*Lactobacillus plantarum*	*Lactobacillus plantarum*	KM207826.1	92	*Lactobacillus plantarum*	2.227
9	*Lactobacillus plantarum*	*Lactobacillus plantarum*	KM207826.1	94	*Lactobacillus plantarum*	2.224
10	***Lactobacillus plantarum***	***Lactobacillus plantarum***	**KM207826.1**	**98**	***Pediococcus acidilactici***	**1.986**
11	***Lactobacillus plantarum***	***Lactobacillus plantarum***	**KC83663.1**	**97**	***Lactobacillus plantarum***	**2.231**
12	*Lactobacillus plantarum*	*Lactobacillus plantarum*	KM207826.1	97	*Lactobacillus plantarum*	2.210
13	*Lactobacillus plantarum*	*Lactobacillus plantarum*	KM207826.1	95	*Lactobacillus plantarum*	2.056
14	*Lactobacillus plantarum*	*Lactobacillus plantarum*	KM207826.1	97	*Lactobacillus plantarum*	2.321
15	*Lactobacillus plantarum*	*Lactobacillus pentosus*	AB362714.1	98	*Lactobacillus plantarum*	2.328
16	***Lactobacillus plantarum***	***Lactobacillus plantarum***	**KJ958428.1**	**97**	***Lactobacillus plantarum***	**2.262**
17	***Lactobacillus rhamnosus***	***Lactobacillus pentosus***	**AB362714.1**	**97**	***Pediococcus acidilactici***	**2.060**

Isolates in bold were chosen as representatives of all the isolates found in goats' milk

Screening of Isolates for Probiotic Properties

Acid Tolerance

The mean resident time of food in the stomach is 3 h, and hence, assays are normally run for that long [30]. The effect of acidic conditions of pH 2.0 and 3.0 on the viability of the LAB isolates is shown in Fig. 1. In this study, all *Lactobacillus plantarum* strains and *Pediococcus acidilactici* isolated from goats' milk showed no tolerance to pH 1.0, with no growth

observed after only 1 h of incubation (data not shown). There was a decline of viable cells within an hour of incubation for all the isolates at pH 2.0, with a decrease much higher for *P. acidilactici* (Fig. 1a). No growth (recorded as 1.25 cfu ml^{-1} on logarithmic scale) was observed for all the isolates after 2 h incubation at this pH (Fig. 1a). All the *L. plantarum* strains and *P. acidilactici* isolates showed some resistance during their exposure to pH 3.0. For all the *L. plantarum* strains, there was a decline of two log units in viable counts after 1 h. The viability counts of *P. acidilactici* showed a decline of two logs after 2 h. The residual viable counts for all the isolates were greater than 10^6 cfu ml^{-1} after 3 h at pH 3.0. Furthermore, the results indicate that the isolate identified as *P. acidilactici* was more acid tolerant than all the *L. plantarum* isolates.

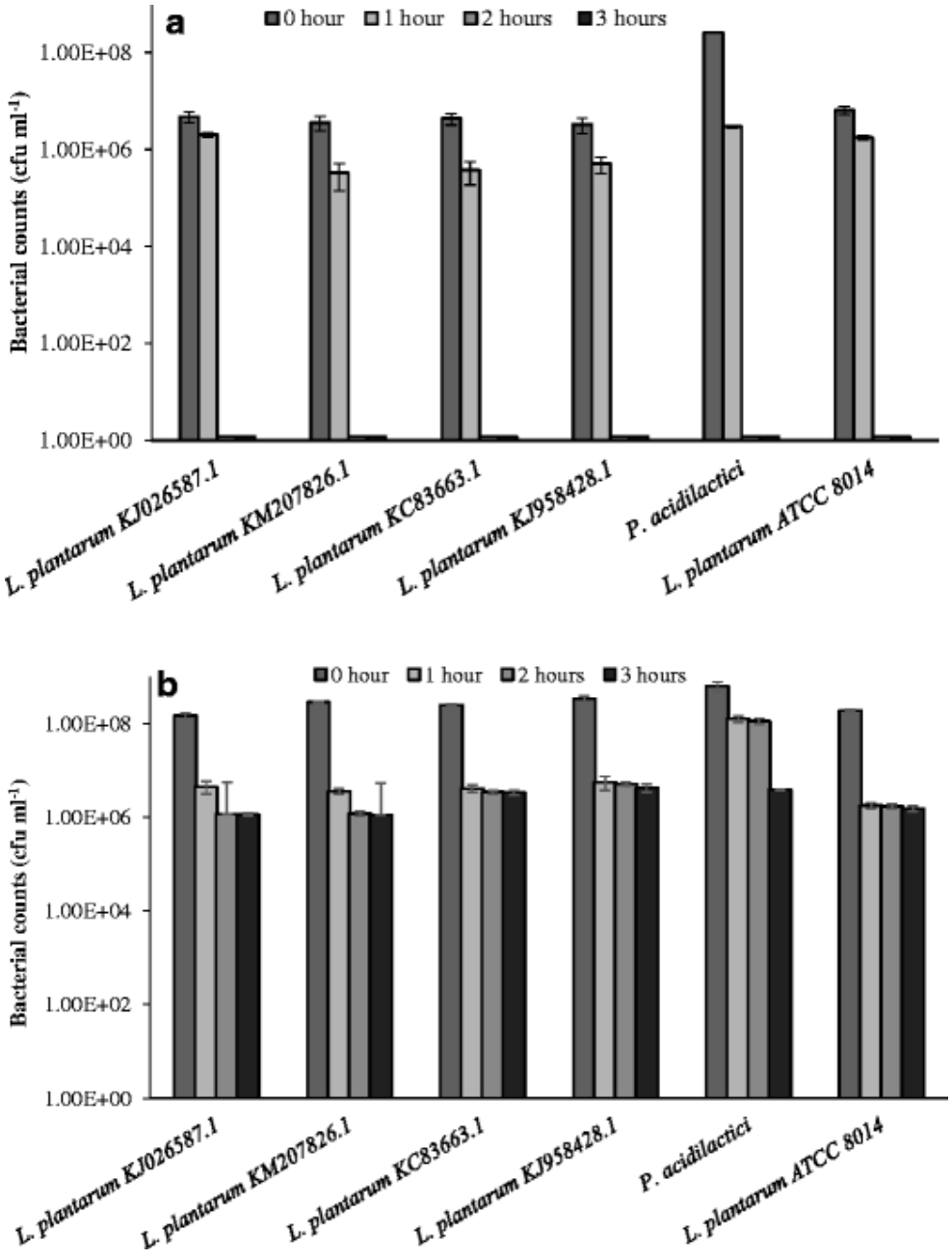

Fig. 1. Survival of LAB isolates after incubation at pH 2.0 (**a**) and pH 3.0 (**b**). Each *bar* represents the average of three independent experiments, *error bars* are standard deviations

Bile Tolerance

Bacteria to be used as probiotics should be able to resist inhibitory factors in the gastrointestinal tract such as bile salts. The ability of all the isolates to resist bile salts was revealed after 24 h of incubation at 37 °C (Fig. 2). There was an increase in viable counts of all the isolates during four hours of incubation in presence of 0.3 and 0.5% ox-gall. This was followed by a decline in viable cell counts after 24 h in 0.3% ox-gall concentration (Fig. 2a). However, viable counts for the cells incubated in 0.5% ox-gall remained the same even after 24 h of exposure (Fig. 2b).

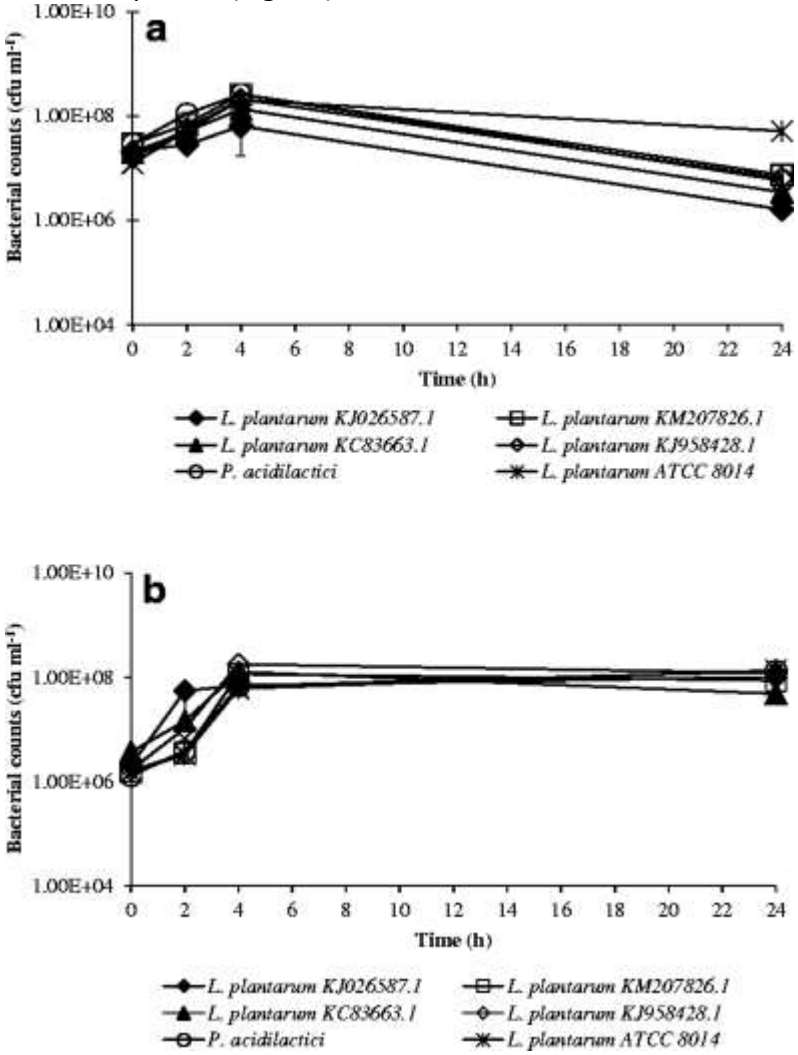

Fig. 2. Survival of LAB isolates in MRS broth supplemented with **a** 0.3% and **b** 0.5% bile salt. Each *point* represents the average of three independent experiments, *error bars* are standard deviations

Antibiotic Susceptibility

A key requirement for probiotic strains is that they should not carry transferable antibiotic resistance genes [3]. In this study, all the 5 isolates from raw goats' milk were assayed for their susceptibility to ten antibiotics, using the disc diffusion method. All isolates displayed resistance to gentamycin, nalidixic acid and vancomycin among all the antibiotics tested (Table 2).

Table 2. Antibiotic susceptibility profiles of the LAB isolates

LAB isolates	Antibiotics									
	TS	**E**	**OT**	**KF**	**NA**	**VA**	**PG**	**T**	**AP**	**GM**
	Concentration (µg)									
	30	**15**	**30**	**30**	**30**	**30**	**10**	**30**	**10**	**10**
	Diameter of inhibition zone (mm*)									
L. plantarum KJ026587.1	5	19	19	15	0	0	17	18.5	15	0
L. plantarum KM207826.1	10	19	19	15	0	0	17	18.5	15	0
L. plantarum KC83663.1	5	19	19	15	0	0	17	18.5	15	0
L. plantarum KJ958428.1	10	19	16.5	15	0	0	17	18.5	22	0
P. acidilactici	10	19	19	15	0	0	17	18.5	22	0
L. plantarum ATCC 8014	5	19	19	15	0	0	17	18.5	15	0

TS co-trimoxazol, *E* erythromycin, *OT* oxytetracycline, *KF* cephalothin, *NA* nalidixic acid, *VA* vancomycin, *PG* penicillin G, *T* tetracycline, *AP* ampicillin, *GM* gentamycin, *0* resistant, *mm** mean of triplicate readings

Antimicrobial Activity Assay

The diameter of inhibition zones showed that all the isolates have antibacterial effects against the tested pathogens (Table 3). The observed inhibitory property of the isolates could be attributed to production of organic acids as it was lost subsequent to neutralization of the culture supernatants with sodium hydroxide. All the isolates showed antibacterial effects against the tested pathogens, with all of them being more antagonistic against *P. aeruginosa* and *S. typhimurium* (Table 3). There was absence of antimicrobial activity in neutralized (pH 7.0) cell free supernatants of all the isolates.

Table 3. Antimicrobial activity of LAB isolates against selected pathogens

LAB isolates	Bacterial pathogens				
	E. coli	***S. aureus***	***P. aeruginosa***	***S. typhimurium***	***P. vulgaris***
	Diameter of inhibition zone (mm*)				
L. plantarum KJ026587.1	6.0	9.8	29.8	30.0	6.0
L. plantarum KM207826.1	5.8	10.0	30.0	29.1	5.0
L. plantarum KC83663.1	6.0	10.0	28.8	30.0	6.0
L. plantarum KJ958428.1	5.8	10.0	28.3	30.0	6.0
P. acidilactici	6.0	10.0	30.0	29.9	5.4
L. plantarum ATCC 8014	6.0	9.1	30.0	28.3	6.0

*mm** mean of triplicate readings

Adhesion Assay

The adhesion of lactobacilli cells to intestinal mucus was used to evaluate the ability of strains to colonize the intestines. Staining with the BacLight viability probe revealed strong adhesion of the isolates to the ileum mucus. Based on the viable counts data obtained (Table 4), most of the isolates adhered to the mucus on the ileum during the 6 h incubation. The negative counts obtained at 2 and 4 h incubation could be an indication of undercounting of bacterial cells caused by chains and clumps formed when growing. Staining of the mucus with the BacLight viability probe indicated that the majority of the adhered cells remained viable.

Table 4. Number of probiotic cells adhering to mucus during incubation period

	Incubation time (hours)		
LAB isolates	**2**	**4**	**6**
	Number of cells adhering (cfu ml^{-1})		
L. plantarum KJ026587.1	-2.3×10^6	1.68×10^7	6.78×10^7
L. plantarum KM207826.1	1.00×10^7	-3.10×10^6	5.54×10^7
L. plantarum KC83663.1	4.60×10^6	4.30×10^6	7.07×10^7
L. plantarum KJ958428.1	-1.25×10^7	0	4.99×10^7
P. acidilactici	3.00×10^5	1.13×10^7	6.37×10^7
L. plantarum ATCC 8014	2.80×10^6	1.20×10^6	5.64×10^7

Expressed as the difference between the number of cells inoculated and the number of cells in suspension after a given incubation period

Discussion

One of the requirements in the production of high quality raw milk is maintaining acceptable bacterial counts which meet the official milk quality standards. The evaluation of results was carried out in accordance with standards set in paragraph 7 of Annex A in the regulations R.489 of 2001 that state total bacterial counts may not exceed 5×10^4 cfu ml^{-1} (raw milk intended for consumption) and 2×10^5 cfu ml^{-1} (raw milk intended for further processing). The presence of coliforms in milk and milk products is an indication of unsanitary production and/or improper handling of either milk utensils [15]. The mentioned legislation further states that, for both the purpose of direct consumption and further processing, coliforms must be below 20 cfu ml^{-1}. Additionally, no *Escherichia coli* is expected in 1 ml of milk intended for direct consumption as well as no colonies must be present in 0.01 ml of milk intended for further processing [39].

The API 50CH kit identified 16 isolates as *L. plantarum* and 1 as *L. rhamnosus*. It is worth mentioning that the isolate identified as *L. rhamnosus,* which indicates it is rod shaped, appeared as round shaped cells that formed clusters under the microscope. This suggested that the API 50CH misidentified this isolate. Based on the 16S rDNA partial sequencing, ten isolates were identified as *Lactobacillus pentosus* and seven as *Lactobacillus plantarum*. However, despite the fact that 16S rDNA sequencing is considered as the 'gold standard' for the identification of anaerobic bacteria, it cannot discriminate *L. plantarum* and *L. pentosus* species because of the high identity value (99%) shared by the two species. Marroki et al.

[26] reported a similar finding stating that *L. plantarum* and *L. pentosus* have very similar rDNA sequences. The correct identification of the 17 isolates was dependent on the presence of the reference strains in the MALDI-bioTyper 3.0 database because the species of the reference strain will give the closest match for the identification of the tested strains. Of the 17 isolates, 15 (88%) were accurately identified at species level as *Lactobacillus plantarum* with scores of ≥2.000, and the remaining 2 (11.76%) as *Pediococcus acidilactici* with scores between 1.700 and 2.000. One of the isolates identified as *P. acidilactici* was isolate 17, which was identified as *Lactobacillus* using API and rDNA sequencing. Since this isolate was observed as cocci shaped, the identification yielded by MALDI TOF was chosen for this isolate. Despite the observed discrepancies, all the techniques used indicated that *L. plantarum* was the dominant LAB in the goats' milk. The results of this study differed from those by researchers elsewhere as most reported lactococci to be the dominant LAB in goats' milk [4, 31, 41]. The disparities in dominant LAB could be attributed to the different goat breeds as none of the previous studies isolated LAB from the milk of South African Saanen goats. The breed has significant effect on the milk composition, which impacts on the microbiota present therein [4]. However, the results were in correlation with those of [12, 32], indicating the presence of high numbers of *L. planturum* in goats' milk. The presence of *P. acidilactici* in goats' milk has also been recently reported [32].

In order to exert their beneficial effects in the host, probiotics must remain alive during both ingestion and their transit prior to reaching the large intestines. They have to pass through the stressful conditions of the stomach with pH between 1.5 and 3.0, and in the upper intestine which contains bile [10, 23]. In this study, all the isolates showed no tolerance to pH 1.0, with a decrease of viable cells in pH 2.0 within an hour of incubation. This phenomenon has been observed for a number of probiotic bacteria where substantial decrease in the viability of strains was often observed at pH 2.0 or below [17]. However, the residual viable counts for all the isolates were greater than 10^6 cfu ml^{-1} after 3 h at pH 3.0. The results observed in the current study correlate with reports from [9], stating that enteric lactobacilli are able to tolerate pH 3.0 for a few hours and pH 2.0 for several minutes, while they are destroyed at pH 1.0. Bile tolerance has been described as an important factor in addition to pH tolerance for survival and growth of probiotics in the gastrointestinal tract. Although the bile concentration in the gastrointestinal tract varies, the mean intestinal bile concentration is believed to be 0.3%, and the staying time is suggested to be 4 h [14, 33]. Resistance of the isolates to ox-gall can most likely be attributed to the expression of bile-resistance related proteins by the bacterial cells [18]. Owing to the high tolerance of ox-gall by all the isolates, it is expected that the strains would likely be effective in bile salt deconjugation.

The overwhelming spread of antibiotic resistance in microbial communities has led to concerns about its possible existence even in beneficial bacterial species, which includes probiotics [37]. The importance of assessing the antibiotic resistance profile pattern of isolates is to restrict the use of probiotic cultures harbouring transferable antibiotic-resistance genes. The isolates displayed resistance to gentamycin, nalidixic acid and vancomycin. Resistance of lactobacilli to these antibiotics has been reported by researchers elsewhere. D'Aimmo et al. [11] reported resistance of *L. acidophilus* and *L. casei* to nalidixic acid while Liu et al. [25] reported resistance of *Lactobacillus* isolates to gentamycin and that of several species of *Lactobacillus* including *L. rhamnosus* and *L. casei,* to vancomycin.

However, this resistance might not pose problems as their genes have been shown not to be transferable. Saarela et al. [35] reported that lactobacilli naturally display a wide range of antibiotic resistance, but in most cases this resistance is not of the transferable type and, therefore, does not usually create a safety concern.

Recent concerns on the rampant and indiscriminate use of antibiotics for disease treatment and growth promotion of livestock, have led to increased interest in the application of probiotics and their antimicrobial metabolites as alternative antimicrobial strategies for treatment and prevention of infections [20]. The most common bacteria that cause mastitis in goats are *Staphylococcus aureus,* followed by minor occurrence of those caused by *Escherichia coli, Clostridium perfringes, Streptococcus, Pseudomonas and Nocardia* genera [5]. Infectious diarrhoea of neonatal animals is one of the most common and economically devastating conditions in the animal agriculture industry. Among the bacterial causes of enteritis in neonatal food animals, *Escherichia coli,* and *Salmonella* spp. are the most common and economically important, but *Clostridium perfrigens* has also been identified as a cause of enteric disease and diarrhoea [22]. In both cases, to prevent the onset of disease, antibiotics have been added to the feedstock. However, the use of antibiotics in animal feed has been regulated and organic methods for livestock have been recommended because of problems such as advent of resistant bacteria and antibiotics residues within livestock products [27]. Hence, antimicrobial activity against pathogens ravaging goats' production is a desirable property of probiotics to be used as direct fed microbial cultures in goats' farming. The diameter of inhibition zones showed that all the isolates have antibacterial effects against the tested pathogens (Table 3). Thus, the LAB isolates from raw goats' milk are potential candidates to be used for control of pathogens responsible for mastitis and bacterial enteritis in goats.

The ability of probiotics to adhere to the target site for colonization is an important trait vital for their expression of optimal functionality. They must adhere to mucosal epithelial cells lining the gut to be designated as probiotic [7, 44], which also depends on the number of bacteria added. The level of adhesion of bacteria positively correlates with the number of bacteria added upon certain point when the saturation of potential binding sites on cell lines probably occurs [13]. Based on the number of viable cells recorded at the end of 6 h of incubation with porcine ileum, all the isolates adhered similarly to the mucus. Tuomola and Salminen [42] also reported similar results where the difference in adhesion of probiotic isolates was small using LIVE/DEAD BacLight viability probe to study the adhesion of 12 different *Lactobacillus* strains. Since the adhesion studies were performed using porcine ileum, future studies can investigate this property using specifically goats' intestines. Nevertheless, the data reported demonstrate the ability of the isolates to adhere to the mucus and thus their potential to successfully colonize the intestinal cells.

Although this study was conducted in vitro, the isolated *L. plantarum* and *P. acidilactici* met most of the criteria used for probiotics. They have shown the ability to tolerate, survive the stressful gastrointestinal conditions and ability to produce antimicrobial activities against some pathogens causing common diseases in goats' industry.

Conclusion

The combination of applied methods for the identification of isolates has shown that *Lactobacillus plantarum* was the dominant species in milk from South African Saanen goats and *Pediococcus acidilactici* to a lesser extent. Although this study was conducted in vitro, the isolated *L. plantarum* and *P. acidilactici* met most of the criteria used for probiotics. They have shown resistance to low pH and tolerance to bile, thus can survive the stressful gastrointestinal conditions. They have also displayed the ability to adhere to the intestinal mucus; as well as the ability to produce antimicrobial activities against some pathogens causing common diseases in goats' industry.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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