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Isolation and Characterisation of Lactic Acid Bacteria from Raw and Fermented Milk

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Authors' contributions

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

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ABSTRACT

The aim of this study was to isolate and phenotypically characterised lactic acid bacteria (LAB) from samples of raw (cow, goat) and traditional fermented milk product (*nono*).The assessed characteristics of LAB as indexed in Bergeys Manual of Determinative Bacteriology are cellular characteristic (Gram staining), growth at pH 4.5 and 9.6, growth in 5% NaCl, production of ammonia from arginine, tolerance to temperature 15 and $45^{o}C$, starch hydrolysis, and fermentation of sugars test. Fifty-five LAB were isolated and identified as *Pediococcus acidilactici* (15), *Lactobacillus plantarum* (29), *Lactobacillus brevis* (4), *Lactobacillus casei* (4), and *Lactobacillus fermentum* (3). Four species of the *Lactobacillus* isolated from *nono* samples were identified as *Lactobacillus casei*, *Lactobacillus brevis*, *Lactobacillus plantarum* and *Lactobacillus fermentum* while *Pediococcus acidilactici* was isolated from raw cow and goat milk. *Lactobacillus plantarum* was the dorminant organism with the highest frequency occurrence of 52.7% while *Lactobacillus fermentum* had the lowest (5.5%). Lactobacillus species are normally found in fermented milk product which could be of great importance in food industry.

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Keywords: Lactic acid bacteria (LAB); isolation and characterisation; Lactobacillus plantarum.

1. INTRODUCTION

Generally, lacteria acid bacteria (LAB) used for preparing fermented milk products are appropriately chosen with some factors such as polysaccharides value, organoleptic quality, improving nutrient, and antimicrobial potential against spoilage or pathogenic organisms [1,2,3]. These organisms are Generally Recognised as Safe (GRAS).

Moreover, screening and selection of starters for production of fermented products like yoghurt have shown a wide spectrum of LAB producing metabolites of different attributes. Starter cultures are also used as natural sources of LAB for production of native cheeses, sourdough and vegetable products mostly in various continents [4,5,6,7]. Yoghurt and other fermented food products are known to be important functional foods, and their beneficial effects are mainly associated with the maintenance of microbial imbalance, and also enhance the healthy gut. Other benefits include prevention of lactose intolerance, treating of gastrointestinal disorders, diarrhoea prevention, and stimulation of immune response [8,7]. However, other immense health benefits of LAB serve as functional and alternative therapy include treatment of tumour, lowering of cholesterol, preventing allergy reactions, and synthesis of the nutrients. Probiotic organisms are also regarded as lactic acid bacteria, and mostly associated with both therapeutic and nutritional benefits since they are known to possess such qualities. Probiotics are often developed as an alternative therapy for both therapeutic and nutritional benefits, and are found at health stores or groceries. They are also attractive for the treatment of infections from challenging and emerging pathogens of food. Furthermore, the use of lactic acid bacteria (LAB) as safe and functional microorganisms had been known for years, and successful investigation has been reported by various researchers that used them as starters for production of fermented food or as probiotics to confer benefits [9,10]. The concept behind great potentials or health enhancing parameters of LAB are known to consumers that consume such probiotic foods. Most researchers concluded that lactobacilli could fight against intestinal disorders, and contribute to increased life's span [9,7]. Krishnendra et al. [11] reported that lactic acid bacteria (LAB) are the mostly known to be safe bacteria whose presence in food are of immense health benefits to humans based on fermentation, preservation, production of nutrients, and prevention of various diseases. These microorganisms are groups of bacteria which reside in the gastrointestinal tract (GIT), and their significance have been noticed over the continents of the world [9,11,12].

Several strains of lactic acid bacteria have been noted to have nutritional benefits like improved lactose utilization, possess tumour lowering properties, and have ability to reduce severity of challenging diseases. Brant and Todd [9] suggested that *Lactobacillus* spp. from dairy origin enhanced health benefits, and can produce antimicrobial substances including bacteriocins whose possess high antimicrobial potential. *Lactobacillus* are classified into different species, which are mostly used as starters. However, an urgent attention should be made due to these great benefits of lactic acid bacteria.Therefore, there is need to isolate and characterize lactic acid bacteria from raw and fermented milk product.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Samples of raw milk from cow, goat and traditional fermented milk product (*nono*) were purchased from a local market known as'*kara'* at Bodija in Ibadan, Nigeria. The samples were brought to Microbial Physiology and Biotechnology Laboratory, Department of Microbiology, University of Ibadan, Ibadan, in sterile bottles for microbiological analysis.

2.2 Culture Medium and Sterilisation Procedures

During the isolation, de Man Rogosa and Sharpe Agar (MRS agar) was used for the isolation of lactic acid bacteria. The components of the isolation medium were weighed using electric weighing machine. MRS agar was poured in one litre Erlenmeyer flask, and 1000 mL of distilled water was added. The solution in the flask was homogenized on hot plates for about 10 minutes to dissolve the components completely. The MRS agar (medium) was sterilized by autoclaving for 15 minutes at 121ºC, and allowed to cooled to 40-45ºC before pouring into plates. All glasswares were sterilised in an oven at 160ºC for two hrs.

2.3 Isolation Procedures

Isolation of lactic acid bacteria was done using pour plate technique. One millilitre of each raw milk samples from goat and cow, and *nono* were taken aseptically and transferred into separate bottles containing 9.0 mL of sterile distilled water, and serial dilutions of the milk samples were made. One millilitre of different dilutions of the samples from raw milk and *nono* was aseptically introduced into sterile Petri dishes containing MRS agar, and were incubated in anaerobic jars at 37°C for 48 hrs. The Petri dishes were checked for bacterial growth and representative colonies were randomly chosen. Isolates were sub-cultured and repeated streaking was done to obtain pure cultures.

2.4 Culture Preservation

A loopful of 18 hrs old pure culture LAB isolates were grown on MRS broth that contained 12% (v/v) glycerol, and was incubated at 30°C in an anaerobic jar for 48 hrs. The MRS medium containing the LAB cultures were stored at -4°C, and sub-cultured at every 4 weeks to get a viable count of the organisms.

2.5 Characterization of Isolates

The cultural morphology such as size, shape, margin, colour, and cellular characteristics (Gram's staining and morphology) of the various isolates were examined and assessed using macroscopic and microscopic technique, respectively [6].

However, biochemical tests including catalase, oxidase, motility, growth at 15ºC and 45ºC, growth at 5% NaCl concentration, production of ammonia from arginine without glucose and meat extract but containing 0.3% arginine and 0.2% sodium citrate replacing ammonium citrate, casein hydrolysis, indole, gelatin hydrolysis, starch hydrolysis, methyl red, and Voges-Proskaeur tests were investigated. The fermentation of carbohydrates (sugars) such as fructose, glucose, galactose, mannitol, sorbitol and raffinose was also carried out.

2.6 Identification of Isolates

The results obtained from the tests carried out were used to phenotypically identified the organism by reference to Bergey's Manual of Systematic Bacteriology and an Approach to the Classification of Lactobacilli [13].

3. RESULTS AND DISCUSSION

Fifty - five presumptive lactic acid bacteria were isolated from raw cow milk, raw goat milk and *nono* samples. On the basis of the cultural and morphological appearances, all the LAB isolates were small colonies, circular, whitish to creamy in colour, raised with entire edges. They were all Gram positive, short to long rods as shown in Table 1.

Table 2 shows the biochemical characteristics of LAB which indicates that all the isolates were negative to catalase, oxidase, nitrate reduction, casein hydrolysis, indole, gelatin hydrolysis, starch hydrolysis, methyl red and Voges-Proskaeur tests. Moreover, all the isolates fermented glucose, fructose and galactose without gas production while *Lactobacillus brevis* (N8, N10, N16, N21) and *Lactobacillus fermentum* (N5, N13, N15) produced gas from glucose. None of the isolates produced ammonia from arginine except for only isolates from *Lactobacillus fermentum* (N5, N13, N15) and *Lactobacillus brevis* (N8, N10, N16, N21). All the isolated LAB also tolerated 4% NaCl except for *Pediococcus acidilactici* (GI, G2, G3, G4, G5, G6, G8, G9, G10, G11, C1, C2, C11, C16, C17). Most of the isolates were able to grow at 15ºC, pH4.5 and 9.6, while some were not able to grow at 45ºC.

The isolates were identified as *Pediococcus acidilactici, Lactobacillus plantarum*, *Lactobacillus brevis, Lactobacillus fermentum* and *Lactobacillus casei* according to Bergey's Manual of Determinative Bacteriology based on their similarities in characteristics with the organisms. Four species of the *Lactobacillus* isolated from *nono* samples were identified as *Lactobacillus casei*, *Lactobacillus brevis*, *Lactobacillus plantarum* and *Lactobacillus fermentum* while *Pediococcus acidilactici* was isolated from raw cow and goat milk. *Lactobacillus plantarum* was also isolated from raw goat and cow milk. *Lactobacillus plantarum* had the highest frequency occurrence of 52.7% while *Lactobacillus fermentum* had the lowest occurrence (5.5%). *Lactobacillus casei* and *Lactobacillus brevis* had 7.3%. In this study, Lactic Acid bacteria were isolated and characterized. The presence of *Lactobacillus plantarum* in fermented foods are in reference to the search of Tannock [14] who reported their occurrence. The cultural, cellular and biochemical characteristics of the isolated LAB were similar with the findings of Rogosa and Sharpe [13]. These microorganisms are found in

Table 1. Cultural and cellular characteristics of lactic acid bacteria (LAB) isolated from raw milk and *nono*

Isolate code	Colour	Margin	Shape	Size	Cellular characteristic
G1	Creamy	Entire	Circular	Small	Gram positive cocci in pairs and tetrads
G2	Creamy	Entire	Circular	Small	Gram positive cocci in pairs and tetrads
G3	Creamy	Entire	Circular	Small	Gram positive cocci in pairs and tetrads
G4	Creamy	Entire	Circular	Small	Gram positive cocci in pairs and tetrads
G5	Whitish	Entire	Circular	Small	Gram positive cocci in pairs and tetrads
G6	Creamy	Entire	Circular	Small	Gram positive cocci in pairs and tetrads
G7	Creamy	Entire	Circular	Small	Gram positive rods in pairs and short chains
G8	Creamy	Entire	Circular	Small	Gram positive cocci in pairs and tetrads
G9	Creamy	Entire	Circular	Small	Gram positive cocci in pairs and tetrads
G10	Creamy	Entire	Circular	Small	Gram positive cocci in pairs and tetrads
G11	Creamy	Entire	Circular	Small	Gram positive cocci in pairs and tetrads
G12	Creamy	Entire	Circular	Small	Gram positive rods in singly and short chains
C1	Creamy	Entire	Circular	Small	Gram positive cocci in pairs and tetrads
C2	Creamy	Entire	Circular	Small	Gram positive cocci in pairs and tetrads
C3	Creamy	Entire	Circular	Small	Gram positive tiny rods in singly and short chains
C4	Whitish	Entire	Circular	Small	Gram positive short rods in singly and short chains
C5	Creamy	Entire	Circular	Small	Gram positive long rods in singly and short chains
C6	Creamy	Entire	Circular	Small	Gram positive rods in singly and short chains
C7	Creamy	Entire	Circular	Small	Gram positive rods in singly and short chains
C8	Creamy	Entire	Circular	Small	Gram positive short rods in singly and short chains
C9	Creamy	Entire	Circular	Small	Gram positive short rods in singly and short chains
C10	Creamy	Entire	Circular	Small	Gram positive rods in singly and short chains
C11	Creamy	Entire	Circular	Small	Gram positive cocciin pairs and tetrads
C12	Creamy	Entire	Circular	Small	Gram positive short rods in singly and short chains
C13	Creamy	Entire	Circular	Small	Gram positive rods in singly and short chains
C14	Creamy	Entire	Circular	Small	Gram positive rods in pairs and short chains
C15	Creamy	Entire	Circular	Small	Gram positive rods in singly and short chains
C16	Creamy	Entire	Circular	Small	Gram positive cocci in singly and tetrads
C17	Creamy	Entire	Circular	Small	Gram positive cocci in singly and tetrads
N1	Whitish	Entire	Circular	Small	Gram positive short rods in singly and short chains
N2	Creamy	Entire	Circular	Small	Gram positive short rods in singly and short chains

N3	Creamy	Entire	Circular	Small	Gram positive short rods in singly and short chains
N4	Creamy	Entire	Circular	Small	Gram positive short rods in singly and short chains
N5	Creamy	Entire	Circular	Small	Gram positive short rods in singly and short chains
N6	Whitish	Entire	Circular	Small	Gram positive short rods in singly and short chains
N7	Creamy	Entire	Circular	Small	Gram positive short rods in singly and short chains
N8	Creamy	Entire	Circular	Small	Gram positive short rods in singly and short chains
N9	Whitish	Entire	Circular	Small	Gram positive short rods in singly and short chains
N10	Creamy	Entire	Circular	Small	Gram positive slender rods in singly and short chains
N11	Creamy	Entire	Circular	Small	Gram positive short rods in singly and short chains
N12	Creamy	Entire	Circular	Small	Gram positive short rods in singly and short chains
N13	Creamy	Entire	Circular	Small	Gram positive short rods in singly and short chains
N14	Creamy	Entire	Circular	Small	Gram positive short rods in singly and short chains
N15	Creamy	Entire	Circular	Small	Gram positive short rods in singly and short chains
N16	Creamy	Entire	Circular	Small	Gram positive short rods in singly and short chains
N17	Creamy	Entire	Circular	Small	Gram positive short rods in singly and short chains
N18	Creamy	Entire	Circular	Small	Gram positive short rods in singly and short chains
N19	Creamy	Entire	Circular	Small	Gram positive short rods in singly and short chains
N20	Creamy	Entire	Circular	Small	Gram positive short rods in singly and short chains
N21	Creamy	Entire	Circular	Small	Gram positive slender rods in singly and short chains
N22	Creamy	Entire	Circular	Small	Gram positive short rods in singly and short chains
N23	Creamy	Entire	Circular	Small	Gram positive short rods in singly and short chains
N24	Creamy	Entire	Circular	Small	Gram positive short rods in singly and short chains
N25	Creamy	Entire	Circular	Small	Gram positive long rods in singly and short chains
N26	Creamy	Entire	Circular	Small	Gram positive short rods in singly and short chains

G= Isolates from goat milk, C=Isolates from cow milk,

Table 2. Biochemical characterization of LAB isolated from raw milk and *nono* samples

Isolates code	Gram's rxn	Catalase	Growth at 4% Nacl	Oxidase test	Motility	Growth at15°C	45°C	pH 4.5	pH 9.6	Methl red test	Indole. Production	Lactose	Glucose	Galactose	Fructose	Arabinose	Raffinose	Rhamnose	Xylose	VP	Sorbitol	Ribose	Mannitol	Maltose	Trehalose	Melibose	Melozitose	Celloboise	Sucrose	Mannose	NH3from arginine	Probable identity
G1	+C	–	–	–	–	+	–	+	+	+	–	–	+	+	+	+	-	–	–	--	–	+	–	+	+	-	–	–	+	+	–	*Pediococcus acidilactici*
G2	+C	–	–	–	–	+	–	+	+	+	–	–	+	+	+	+	-	–	–	--	–	+	–	+	+	-	–	–	+	+	–	*Pediococcus acidilactici*
G3	+C	–	–	–	–	+	–	+	+	+	–	–	+	+	+	+	-	–	–	–	–	+	–	+	+	-	–	–	+	+	–	*Pediococcus acidilactici*
G4	+C	–	–	–	–	+	–	+	+	+	–	–	+	+	+	+	-	–	–	–	–	+	–	+	+	-	–	–	+	+	–	*Pediococcus acidilactici*
G5	+C	–	–	–	–	+	–	+	+	+	–	–	+	+	+	+	-	–	–	–	–	+	–	+	+	-	–	–	+	+	–	*Pediococcus acidilactici*
G6	+C	–	–	–	–	+	–	+	+	+	–	–	+	+	+	+	-	–	–	–	+	+	–	+	+	-	–	–	+	+	–	*Pediococcus acidilactici*
G7	+R	–	+	–	–	+	–	+	+	+	–	+	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	–	+	+	–	*Lactobacilus plantarum*
G8	+C	–	–	–	–	+	–	+	+	+	–	–	+	+	+	+	-	–	+	–	_	+	–	+	+	-	–	–	+	+	–	*Pediococcus acidilactici*
G9	+C	–	–	–	–	+	–	+	+	+	–	–	+	+	+	+	-	–	–	--	–	+	–	+	+	-	–	–	+	+	–	*Pediococcus acidilactici*
G10	+C	–	–	–	–	+	–	+	+	+	–	–	+	+	+	+	-	–	–	--	–	+	–	+	+	-	–	–	+	+	–	*Pediococcus acidilactici*
G11	+C	–	–	–	–	+	–	+	+	+	–	–	+	+	+	+	-	–	–	--	–	+	–	+	+	-	–	–	+	+	–	*Pediococcus acidilactici*
G12	+R	–	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	-	+	+	–	*Lactobacillus plantarum*
C1	+C	–	–	–	–	+	–	+	+	+	–	–	+	+	+	+	-	–	–	–	–	+	–	+	+	–	–	–	+	+	–	*Pediococcus acidilactici*
C2	+C	–	–	–	–	+	–	+	+	+	–	–	+	+	+	+	-	–	–	–	–	+	–	+	+	–	–	–	+	+	–	*Pediococcus acidilactici*
C3	+R	–	+	–	–	+	–	+	+	+	–	+	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	–	+	+	–	*Lactobacillus plantarum*
C4	+R	–	+	–	–	+	–	+	+	+	–	+	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	–	+	+	–	*Lactobacillus plantarum*
C5	+R	–	+	–	–	+	–	+	+	+	–	+	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	–	+	+	–	*Lactobacillus plantarum*
C6	+R	–	+	–	–	+	–	+	+	+	–	+	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	–	+	+	–	*Lactobacillus plantarum*
C7	+R	–	+	–	–	+	–	+	+	+	–	+	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	–	+	+	–	*Lactobacillus plantarum*
C8	+R	–	+	–	–	+	–	+	+	+	–	+	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	–	+	+	–	*Lactobacillus plantarum*
C9	+R	–	+	–	–	+	–	+	+	+	–	–	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	–	+	+	–	*Lactobacillus*

																																plantarum
C10	+R	–	+	–	–	+	–	+	+	+	–	–	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	–	+	+	–	*Lactobacillus plantarum*
C11	+ C	–	-	–	–	+	–	+	+	+	–	–	+	+	+	-	-	-	+	–	-	+	-	+	+	+	-	–	+	+	–	*Pediocococcus acidilactici*
C12	+R	–	+	–	–	+	–	+	+	+	–	–	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	–	+	+	–	*Lactobacillus plantarum*
C13	+R	–	+	–	–	+	–	+	+	+	–	–	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	–	+	+	–	*Lactobacillus plantarum*
C14	+ R	–	+	–	–	+	–	+	+	+	–	–	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	–	+	+	–	*Lactobacillus plantarum*
C15	+R	–	+	–	–	+	–	+	+	+	–	–	+	+	+	+	+	–	+	–	+	+	+	+	+	–	+	–	+	+	–	*Lactobacillus plantarum*
C16	+C	–	–	–	–	+	–	+	+	+	–	–	+	+	+	–	–	–	–	–	–	+	–	+	+	–	–	–	+	+	–	*Pediococcus acidilactici*
C17	+C	–	–	–	–	+	–	+	+	+	–	–	+	+	+	–	–	–	–	–	–	+	–	+	+	–	–	–	+	+	–	*Pediococcus acidilactici*
N1	+R	–	+	–	–	+	+	+	+	+	–	+	+	+	+	+	–	–	–	–	+	+	+	+	+	–	+	+	+	+	–	*Lactobacillus casei*
N2	+R	–	+	–	–	+	–	+	+	+	–	–	+	+	+	+	+	–	+	–	+	+	+	+	+	–	+	–	+	+	–	*Lactobacillus plantarum*
N3	+R	–	+	–	–	+	–	+	+	+	–	+	+	+	+	+	+	–	+	–	+	+	+	+	+	–	+	–	+	+	–	*Lactobacillus plantarum*
N4	+R	–	+	–	–	+	–	+	+	+	–	+	+	+	+	+	+	–	+	–	+	+	+	+	+	–	+	–	+	+	–	*Lactobacillus plantarum*
N5	+R	–	+	–	–	+	–	+	+	+	–	+	+	+	+	+	+	–	+	–	+	+	+	+	+	–	+	+	+	+	+	*Lactobacillus fermentum*
N6	+R	–	+	–	–	+	–	+	+	+	–	+	+	+	+	+	+	–	+	–	+	+	+	+	+	–	+	–	+	+	–	*Lactobacillus plantarum*
N7	+R	–	+	–	–	+	–	+	+	+	–	+	+	+	+	+	+	–	+	–	+	+	+	+	+	–	+	–	+	+	–	*Lactobacillus plantarum*
N8	+R	–	+	–	–	+	–	+	+	+	–	+	+g	+	+	+	+	+	–	–	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus brevis*
N9	+R	–	+	–	–	+	–	+	+	+	–	+	+	+	+	+	–	–	–	–	+	+	+	+	+	+	+	+	+	+	–	*Lactobacillus casei*
N10	+R	–	+	–	–	+	–	+	+	+	–	+	+g	+	+	+	+	+	–	–	–	+	+	+	+	+	+	+	–	–	+	*Lactobacillus brevis*
N11	+R	–	+	–	–	+	–	+	+	+	–	+	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	–	+	+	–	*Lactobacillus plantarum*
N12	+R	–	+	–	–	+	–	+	+	+	–	+	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	–	+	+	–	*Lactobacillus plantarum*
N13	+R	–	+	–	–	+	+	+	+	+	–	+	+g	+	+	+	+	–	+	–	+	+	+	+	+	+	+	+	+	–	+	*Lactobacillus fermentum*
N14	+R	–	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	+	+	+	–	*Lactobacillus plantarum*
N15	+R	–	+	–	–	+	+	+	+	+	–	+	+	+	+	+	–	–	–	–	+	+	+	–	+	+	+	+	+	+	–	*Lactobacillus casei*
N16	+R	–	+	–	–	+	+	+	+	+	–	+	+g	+	+	+	+	+	–	–	–	+	+	+	+	+	+	–	–	+	+	*Lactobacillus brevis*
N17	+R	–	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	–	+	–	+	+	+	+	+	–	–	+	+	+	–	*Lactobacillus plantarum*
N18	+R	–	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	–	+	–	+	+	+	+	+	–	+	+	+	–	+	*Lactobacillus fermentum*

N19	+R	–	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	–	+	–	+	+	+	+	+	–	–	+	+	+	–	*Lactobacillus plantarum*
N20	+R	–	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	–	+	–	+	+	+	+	+	–	–	+	+	+	–	*Lactobacillus plantarum*
N21	+R	–	+	–	–	+	+	+	+	+	–	+	+g	+	+	+	+	+	–	–	–	+	+	+	+	–	+	–	–	+	+	*Lactobacillus brevis*
N22	+R	–	+	–	–	+	+	+	+	+	–	+	+	+	+	+	–	–	–	–	+	+	+	–	+	+	+	+	+	+	–	*Lactobacillus casei*
N23	+R	–	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	–	+	–	+	+	+	–	+	–	–	+	+	+	–	*Lactobacillus plantarum*
N24	+R	–	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	+	+	+	–	*Lactobacillus plantarum*
N25	+R	–	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	+	+	+	–	*Lactobacillus plantarum*
N26	+R	–	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	+	+	+	–	*Lactobacillus plantarum*

Keys: + = positive, - = negative, g= gas production, N= Isolates from Nono samples, R= Rod. All the isolates we negative to voges proskauer test, nitrate reduction test, casein hydrolysis test, gelatin hydrolysis test, starch hydrolysis test, Lactic acid bacteria= LAB

Table 3. Frequency of occurrence (%) of lactic acid bacteria isolated from raw milk and *nono* samples

Sources no of isolates		Total number of isolates	Occurrence (%)	Probable identity
Raw cow milk	10	15	27.2	*Pediococcus acidilactici*
Raw goat milk	5			
Raw goat milk	2	29	52.7	*Lactobacillus plantarum*
Raw cow milk	12			
Nono 1	15			
Nono 2	3	3	5.5	*Lactobacillus fermentum*
	4	4	7.3	*Lactobacillus casei*
	4	4	7.3	*Lactobacillus brevis*
Total		**55**	**100**	

raw milk and fermented milk products which improve the growth and metabolism. Toskoy et al. [15] reported that *Lactobacillus plantarum* occurs normally in raw milk, and other fermented milk products. *Lactobacillus casei* and *Lactobacillus brevis* had been isolated from *nono* samples. However, LAB had been isolated from raw milk and *nono* samples since they have the ability to produce lactic acid. The dominance of *Lactobacillus plantarum* observed in this study could be as a result of decreased pH including production of antimicrobial substances. Shah (2000) shows prevalence of *Lactobacillus plantarum* in *nono* samples.

Steel et al. [16] reported that some strains of *Lactobacillus casei,* and *Lactobacillus brevis* were isolated from raw milk and other diary products in Jordan, that were similar to the samples used in this studies. These organisms were employed to make probiotic yoghurt in order to produce quality, safe product with good antimicrobial and probiotic properties. This experimental results is also similar to the work done by Adesokan et al. [17], they reported that some strains of *Lactobacillus casei* were isolated from raw milk samples, and had good probiotic properties. Moreover, *Lactobacillus* species isolated in this study can also be found in fermented dairy habitat. This organism can also be found in a variety of habitats including fermented foods [17,14].

4. CONCLUSION

The results showed that lactobacillus species are normally found in fermented milk product. The screening of LAB isolates for probiotic potential and molecular identification using 16S rRNA sequencing could be recommended as furtherwork.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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