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Isolation and Identification of Lactic Acid Bacteria from Spontaneously Fermented Kunun-zaki using RAPD-PCR Analysis

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Abstract
Sixty lactic acid bacteria (LAB) were isolated from eighteen types of kunun-zaki produced naturally using millet, sorghum and maize each singly or in combinations with malted grains (millet, sorghum, maize, wheat and paddy rice) and characterized using conventional methods (biochemical and cultural) and molecular analyses. Seven morphotypes (Mt1-Mt7) were revealed among the 60 LAB isolates. Twenty LAB were selected based on the differences in their biochemical and physiological properties and these were subjected to DNA isolation and purification. The Agarose gel electrophoresis of the twenty (20) extracted DNA revealed very pure DNA electrophoretic pattern. The concentration of the DNA sample ranged between 0.7 mg/ml to 4.0 mg/ml and the purity index ranged from 1.5 to 1.9; indicating they were all very pure and can easily be used in Polymerase chain reaction (PCR) analysis. A highly polymorphic primer, OPB20 primer, was selected and used to analyze the 20 LAB DNA of which seven (7) isolates were identified to be Lactobacillus brevis, twelve (12) isolates were Lactobacillus plantarum and one isolate was identified as Lactobacillus acidophilus and these organisms could be potentially useful industrially.
Key words: *Lactic acid bacteria, Kunun-zaki, Randomly amplified polymorphic DNA (RAPD)*

INTRODUCTION

'Kunun-zaki' is an indigenous fermented non-alcoholic beverage widely consumed in Northern Nigeria and is produced from *Pennisetum glaucum* (millet), *Sorghum bicolor* (sorghum) or *Zea mays* (maize). This beverage is believed to be of immense social, economic, nutritional and medicinal importance to its numerous consumers (Beetseh *et al*., 2022). Although cereals are the major raw materials used in its preparation; ingredients (spices) such as: *Zinger officinale* (ginger), *Syzygium aromaticum* (cloves), *Piper guineense* (black pepper) and *Afromonium melegueta* (alligator pepper) are also introduced to give taste. Pulse (2018) reported that millet the key ingredient used in preparing kunun-zaki, is very nutritious and thus beneficial to human health. Other ingredients (cloves and so on) used in kunun-zaki production have been reported to be beneficial to health. Kiran (2018) and Michael (2019) reported that cloves used in kunun zaki production aids in digestion, it is also anti-carcinogenic, protects liver, boosts immune system and controls diabetes.

Kunun-zaki which was usually consumed mainly in the Northern parts of Nigeria is now widely acceptable throughout the Nation and this is due to its refreshing qualities thus serving as food appetizer, refreshing drink and complementary food for infants (Beetseh *et al*., 2022). In most Nigeria cities, the sales and consumption of kunun-zaki is high due to its affordability. Also, this drink is usually hawked at the motor parks, school premises and market places (Ayandele, 2015). However, in spite of its popularity kunun-zaki is currently produced at village technology level (Abiodun *et al*., 2017). Kunun-zaki has a shelf life of about 24h at ambient temperature (28±2°C). The short shelf-life of kunun-zaki drink is however a major problem for producers and consumers (Onyimba and Dishon, 2019).

Lactic acid bacteria play an important role in food productions such as butter, cheese, yoghurt and sauerkraut, also LABs have been employed extensively in fermentation and preservation of foods (Bintsis, 2018). These LABs are reported to cause rapid acidification of the raw materials used through production of organic acids mainly lactic acid and other metabolites. The organic acid production by LABs and a decrease in pH constitute the main mechanism of bio-preservation in fermented foods thereby enhancing the shelf life, microbial safety and sensory characteristics of the end product (Ray and Joshi, 2014). Lactic acid bacteria also increase absorption of nutrients, breaks down toxins, stimulates probiotic activity and produce antioxidants (Paddock, 2019). The aim of this study therefore is to

isolate and identify the lactic acid bacteria responsible for the production of kunun-zaki using Randomly amplified polymorphic DNA-Polymerase chain reaction (RAPD-PCR) analysis.

MATERIALS AND METHODS.

Malting of cereal grains

Five hundred grams (500g) of each of the cereal grains: *Oryza sativa* (paddy rice), *Pennisetum glaucum* (millet), *Sorghum bicolor* (sorghum), *Zea mays* (maize) and *Triticum aestivum* (wheat) were washed separately with tap water and soaked in 1000 ml of tap water (1:2 w/v) for 12 h and then drained. The drained grains were couched separately by covering them with moist cloth for 4 days at ambient temperature (25-30°C) to germinate. After germination, the malted grains were sun dried for 3days and packaged separately in sterile bags (Agarry *et al.* 2010).

Production of kunun-zaki using uncontrolled (natural) fermentation

Kunun-zaki was produced using modified methods of Agarry *et al.* (2010) and Victor-Aduloju *et al.* (2018). The process involved cleaning and steeping of the grains before addition of spices [mixture of cloves (2g), ginger (6g), alligator pepper (2g) and black pepper (2g)] and wet milling. Five hundred gram (500g) of cleaned grains (millet, sorghum or maize) were steeped in 1000 ml tap water (1:2w/v) for 24h at ambient temperature (30-32°C). The water was decanted off and the grains washed with more tap water before wet milling with the mixture of spices in two volume tap water. The paste (900g) was divided into two unequal portions (1:3v/v). One of these was cooked using two volumes of boiling water and allowed to cool to 45°C. Each of the ground malted cereal (either rice, wheat, millet, maize or sorghum) was steeped in 1% Sodium metabisulphite for 5min, washed with fresh water and ground to paste and then mixed separately with the remaining uncooked paste (1:4 w/w) before being added to the cooked paste (i.e. gelatinized starch at 45°C). This mixture was stirred vigorously for about 5 minutes and allowed to ferment for 8-10 h to produce different types of kunun-zaki made with either millet or maize or sorghum. The fermented kunun-zaki was sieved (mesh size approx. 350 µm) and served with or without the addition of sweetening agent. In all, eighteen (18) types of kunun zaki were produced using combinations of cereals singly or in combinations with different malted cereals (Table 1).

Table 1: Production of kunun-zaki using combinations of cereals and cereal malts.

	Formulation/combinations of cereals and malted cereals used for kunun -zaki production							
Kunun zaki types	Malted Millet	Malted sorghum	Malted maize	Malted Wheat	Malted Rice	Millet	Sorghum	Maize
1	-	-	-	-	-	+	+	+
2	+	-	-	-	-	+	+	+
3	-	+	-	-	-	+	+	+
4	-	-	+	-	-	+	+	+
5	-	-	-	+	-	+	+	+
6	-	-	-	-	+	+	+	+
1=no malted grain added; 2= malted millet added; 3=malted sorghum added; 4=malted maize added; 5= malted wheat added; 6= malted rice added;+= Present; -= absent								

Isolation of Lactic acid bacteria from kunun-zaki produced using natural fermentation

The dominant microorganisms associated with kunun-zaki production were isolated using Brain Heart Infusion (BHI)-Sodium azide enrichment technique as described by Agarry *et al.* (2010). Ten (10) ml of kunun-zaki produced in this study was mixed with 100 ml of sterile BHI broth (BHI: LABM, LANCASHIRE, UK) supplemented with 0.02% (w/v) Sodium azide (Sigma, UK) and incubated at 30±2°C for 24h. Sodium azide inhibits cytochrome activity therefore selectively enriched for lactic acid bacteria. Sub-cultures were made from the 24h enriched medium by streaking them on prepared MRS agar (LAB M, Lancashire, UK) plates; these were incubated for the 24h at 30±2°C. Following incubation, discrete colonies (typical pin point) were randomly picked and purified on fresh MRS agar plates. Cultures of the isolates were considered to be pure after three successive subcultures on MRS agar plates. Pure cultures of the microbial isolates were subsequently sub-cultured on MRS agar slants in Bijou bottles; these were covered with sterile mineral oil and kept in the refrigerator for further studies.

Preliminary characterization of lactic acid bacteria isolates

The purified LAB isolates obtained in this study were identified on the basis of standard cultural, morphological and biochemical characteristics (Victor-Aduloju *et al.*, 2018; Helmenstine, 2019). The biochemical test included catalase, sugar fermentation, String (KOH) test (Sagar, 2022).

MOLECULAR ANALYSES

Lactic Acid Bacteria Isolates Genomic DNA Extraction and Purification

DNA extraction was carried out according to the methods of Thottappilly *et al.* (1999) and Ojo *et al.* (2019). A 60 mg of the selected bacterial cells were suspended in 200 µl of water and this was added to ZR bashing beads lysis tubes to which 800 µl of lysis buffer was added to the

mixture; this was mixed briefly by vortexing and left to stand at room temperature for 5 min. The mixture was transferred to a spin column in a collection tube and centrifuged at 10,000 rcf (relative centrifugal field) for one minute and subsequently transferred to a new collection tube to which 400 µl of DNA pre-wash buffer was added to the spin column and centrifuged at 10,000 rcf for one minute. Five hundred (500) µl of g-DNA wash buffer was added to the spin column and centrifuged at 10,000 rcf for one minute. The spin column was later transferred to a clean micro centrifuge tube to which 100 µl DNA Elution buffer was added, this was incubated for 2-5 minutes at room temperature and then centrifuged at top speed for 20 s to elute the DNA. The elute DNA was stored at ≤-20°C for further use.

RAPD-PCR Analysis

The randomly amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) analysis was performed as described by Onasanya *et al*. (2013) and Matsumoto *et al*. (2022) with some modifications. Five random primers were first screened with two pooled genomic DNA from lactic acid bacteria (LAB) to identify the most suitable primers to analyze the 20 LAB isolates genomic DNA. Amplifications were performed in a 25 µl reaction mixture consisting of genomic DNA, reaction buffer (Promega, Madison, Wisconsin, USA), 100 µM each of dATP, dCTP, dGTP, and dTTP, 0.2 µM Operon random primers (OPB-20), 2.5 µM $MgCl_2$, and 1 U of *Taq*polymerase (Sigma- Aldrich). Amplification was performed in a thermowell microtiter plate (ICN Biomedicals, Costa Mesa, USA) using a MJ Research programmable Thermal Controller. The cycling program was (i) 1 cycle of 94°C for 3 min; (ii) 45 cycles of 94°C for 1 min for denaturation, 40°C for 1 min for annealing of primer, and 72°C for 2 min for extension; and (iii) a final extension at 72°C for 7 min. Amplification products were maintained at 4°C until electrophoresis.

Electrophoresis of RAPD-PCR Products

The amplification products were resolved by electrophoresis in a 1.4% Agarose gel (Sigma-Aldrich) using TAE buffer at 100 V for 2 h. A 1 kb ladder (Sigma-Aldrich) was used as the molecular size marker. Gels were visualized by staining with Ethidium bromide solution (0.5 µg/ml; Sigma-Aldrich) and banding patterns were photographed over UV light using a UVP computerized gel photo documentation system (Onasanya *et al*., 2013; Ajayi *et al*.,2023).

Statistical and Cluster Analysis of Morphology

The morphological data were analyzed directly. Pair wise distance matrices were compiled by the NTSYS-pc 2.0 software (Rohlf, 2000) using the Jaccard coefficient of similarity. Phylogenetic tree were created by the unweighted pair-group method arithmetic (UPGMA) average cluster analysis. In order to establish the relationships amongst the LAB isolates, cluster analysis was carried out using numerical taxonomy and multivariate analysis system (NTSYS-PC), version 2.1 (Rohlf, 2000).

RESULTS AND DISCUSSION

Characterization of Lactic Acid Bacteria based on Cultural, Morphological and Biochemical Tests

As shown in Table 2, sixty LABs were isolated from eighteen (18) types of kunun zaki produced in this study. All the isolates were gram positive, catalase negative and showed KOH test negative (data not shown but represented as a footnote in Table 2). The results obtained in this study were in conformity with the findings of Mulaw *et al*. (2019) who isolated and grew Gram positive and catalase negative lactic acid bacteria on MRS agar at 37^{0}C for 24h under anaerobic conditions. The LAB isolates were further subjected to cluster analysis which identified 7 morphotypes (Figure 1).

Table 2: Cultural, morphological and biochemical characteristics of lactic acid bacteria isolated from various types of kunun zaki[1]

			Cultural characteristics[2,3]				Sugar fermentation									
S/N	Isolate code	Source of isolates	Colony type	Color	Elevation	Gram reaction	Sucrose	Arabinose	Xylose	Mannitol	Glucoe	Fructose	Galactose	Lactose	Maltose	Inositol
1	LAB-1	MB-6	moderate	Greyish	Flat	+	-	-	-	+	-	+	-	-	-	-
2	LAB-2	MB-6	moderate	Greyish	Flat	+	-	-	-	-	-	-	-	-	-	-
3	LAB-3	MB-6	moderate	Cream	Flat	+	-	-	-	-	-	+	-	-	-	+
4	LAB-4	MB-5	moderate	Cream	Flat	+	-	-	-	-	-	+	-	-	-	+
5	LAB-5	MB-5	Pinpoint	Cream	Raised	+	-	-	-	-	+	-	-	-	-	+
6	LAB-6	MB-2	Pinpoint	Cream	Raised	+	-	+	-	+	+	+	-	+	+	+
7	LAB-7	MB-3	Pinpoint	Cream	Raised	+	-	-	-	-	-	-	+	-	-	-
8	LAB -8	MB-4	Small	Cream	Raised	+	+	-	-	-	-	-	-	-	+	-
9	LAB-9	MB-4	Small	Cream	Flat	+	-	-	-	-	-	-	-	-	+	+
10	LAB-10	SB-6	Pinpoint	Cream	Raised	+	-	-	-	-	+	-	-	-	+	+

			Cultural characteristics[2,3]				Sugar fermentation									
S/N	**Isolate code**	**Source of isolates**	**Colony type**	**Color**	**Elevation**	**Gram reaction**	**Sucrose**	**Arabinose**	**Xylose**	**Mannitol**	**Glucoe**	**Fructose**	**Galactose**	**Lactose**	**Maltose**	**Inositol**
11	LAB-11	SB-5	Pinpoint	Cream	Raised	+	-	-	-	+	-	-	-	-	-	-
12	LAB-12	SB-2	Pinpoint	Cream	Flat	+	-	-	-	+	-	-	-	-	-	-
13	LAB-13	SB-3	moderate	Cream	Raised	+	-	-	-	-	-	-	-	-	-	-
14	LAB-14	SB-3	moderate	Cream	Raised	+	-	-	-	-	+	-	-	-	-	-
15	LAB-15	SB-3	moderate	Cream	Flat	+	-	-	-	-	+	-	-	-	-	-
16	LAB-16	ZB-6	moderate	Cream	Raised	+	-	-	-	-	+	-	-	-	-	+
17	LAB-17	ZB-2	Small	White	Flat	+	-	-	-	-	+	-	-	-	-	+
18	LAB-18	ZB-3	Pinpoint	Cream	Flat	+	-	-	-	-	+	-	-	-	+	+
19	LAB-19	ZB-4	moderate	Cream	Raised	+	-	-	-	-	+	-	-	-	+	+
20	LAB-20	ZB-6	Small	Cream	Raised	+	-	-	-	-	+	-	-	-	+	+
21	LAB-21	ZB-6	Small	Cream	Raised	+	+	+	-	+	+	-	-	+	+	+
22	LAB-22	ZB-6	Small	Cream	Raised	+	+	+	-	+	+	-	-	+	+	+
23	LAB-23	MB-1	Pinpoint	Cream	Raised	+	+	+	+	-	+	+	+	+	+	+
24	LAB-24	MB-1	moderate	Cream	Flat	+	-	-	-	+	+	-	-	-	+	+
25	LAB-25	MB-1	Pinpoint	Cream	Flat	+	+	-	-	+	-	+	-	+	-	-
26	LAB-26	MB-1	Pinpoint	Cream	Raised	+	-	-	-	+	+	-	+	-	-	+
27	LAB-27	MB-1	Small	Greyish	Flat	+	-	-	-	-	+	-	-	-	-	-
28	LAB-28	MB-1	Small	Cream	Flat	+	-	-	-	-	+	-	-	-	-	+
29	LAB-29	MB-1	Small	Cream	Flat	+	-	-	-	+	+	-	-	-	-	+
30	LAB-30	MB-5	moderate	Cream	Flat	+	-	-	-	+	+	-	+	-	-	-
31	LAB-31	SB-5	Large	Cream	Flat	+	-	-	-	+	+	-	-	+	-	-
32	LAB-32	SB-5	Large	Cream	Flat	+	-	-	-	+	+	-	-	+	-	-
33	LAB-33	SB-5	*Pinpoint*	*Cream*	*Flat*	+	-	-	-	-	-	-	-	-	-	-
34	LAB-34	SB-1	Pinpoint	Cream	Flat	+	+	-	-	-	-	-	-	-	-	-
35	LAB-35	SB-1	moderate	Cream	Flat	+	-	-	-	+	+	-	-	-	-	-
36	LAB-36	SB-1	moderate	Cream	Flat	+	-	-	-	+	+	-	-	-	-	-
37	LAB-37	ZB-1	moderate	Cream	Flat	+	-	-	-	-	-	-	+	-	+	-
38	LAB-38	ZB-1	Large	Cream	Raised	+	+	-	-	-	+	-	+	-	+	-
39	LAB-39	ZB-1	Pinpoint	Cream	Flat	+	-	-	-	-	+	-	-	-	-	-
40	LAB-40	MB-6	Pinpoint	Off white	Flat	+	-	-	-	-	+	-	+	-	-	+
41	LAB-41	MB-6	Pinpoint	Cream	Flat	+	-	-	-	+	+	-	+	-	+	-
42	LAB-42	MB-6	Pinpoint	Cream	Flat	+	-	-	-	+	+	-	+	-	+	-
43	LAB-43	MB-6	moderate	Cream	Flat	+	-	-	-	-	-	-	+	-	-	-
44	LAB-44	MB-6	Pinpoint	Cream	Raised	+	-	-	-	+	+	+	-	-	-	-
45	LAB-45	MB-6	Large	Cream	Raised	+	-	-	-	+	-	-	+	-	-	+
46	LAB-46	MB-1	Small	Pink	Flat	+	-	-	-	+	+	-	-	-	+	-
47	LAB-47	MB-1	Pinpoint	Cream	Flat	+	-	-	-	-	+	+	-	+	-	+
48	LAB-48	MB-1	Small	Cream	Flat	+	-	-	-	-	+	+	-	+	-	+
49	LAB-49	MB-1	Pinpoint	Cream	Raised	+	-	-	-	-	+	-	-	-	-	-
50	LAB-50	MB-1	Pinpoint	Greyish	Flat	+	-	-	-	-	-	-	-	-	-	+
51	LAB-51	MB-1	Pinpoint	Cream	Raised	+	-	-	-	-	-	-	+	-	-	-
52	LAB-52	MB-6	Pinpoint	Cream	Raised	+	-	-	-	+	+	-	-	-	+	-
53	LAB-53	MB-6	Small	Cream	Raised	+	-	-	-	-	-	-	-	-	+	-
54	LAB-54	MB-6	Pinpoint	Greyish	Flat	+	-	-	-	-	+	-	+	-	-	+
55	LAB-55	MB-6	Pinpoint	Off white	Raised	+	-	-	-	-	+	-	-	+	-	+
56	LAB-56	ZB-1	Pinpoint	Off white	Raised	+	-	-	-	-	+	-	+	-	-	+
57	LAB-57	ZB-6	Pinpoint	Greyish	Flat	+	-	-	-	-	-	-	-	-	-	-
58	LAB-58	ZB-6	Pinpoint	Greyish	Flat	+	-	-	-	-	-	-	-	-	-	-
59	LAB-59	MB-1	Pinpoint	Greyish	Flat	+	-	-	-	-	-	-	-	-	-	-
60	LAB-60	MB-1	Pinpoint	Greyish	Flat	+	-	-	-	-	-	-	-	-	-	-

1: MB=millet-based; SB= sorghum-based; ZB= maize_based (1=no malted grain added; 2= malted millet added; 3=malted sorghum added; 4=malted maize added; 5= malted wheat added; 6= malted rice added

2: Cultural characteristics of all LAB isolates: shape (circular), Pigment (none), margin (entire), colony surface (smooth), opacity (opaque)

3: Isolated LABs were catalase negative and KOH negative.

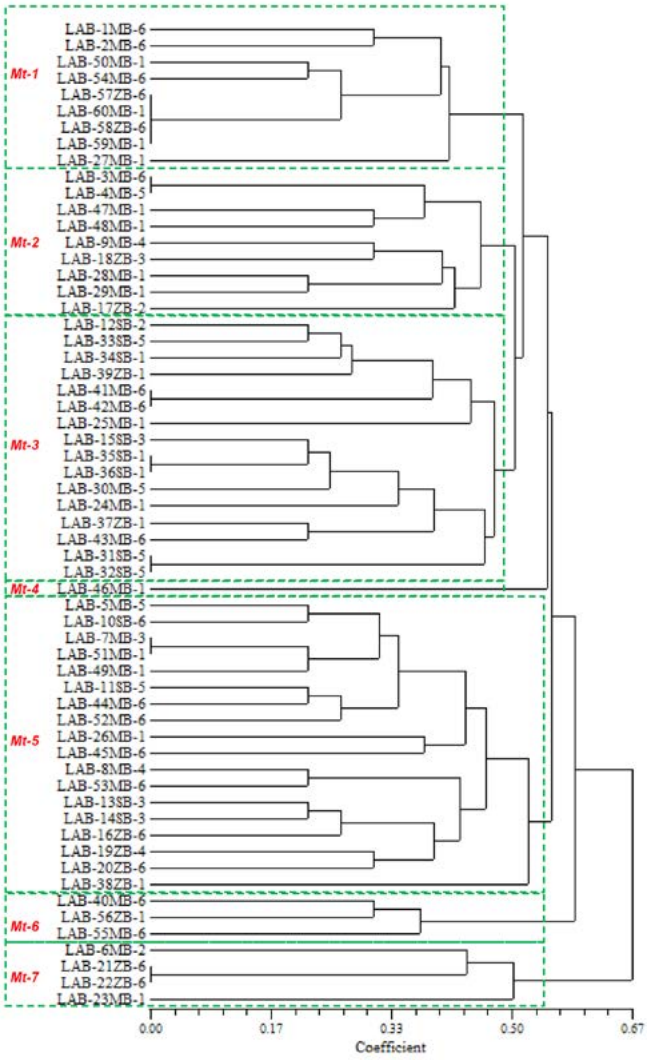

Figure 1: Sixty LAB isolates cluster as revealed by their morphological and biochemical characteristics.

The cluster analysis identified seven (7) morphotypes (Mt-1 to Mt-7) among the 60 isolates as revealed by their morphological and biochemical characteristics (Figure 1). Mt-1 comprised of 9 isolates (LAB-1MB-6 , LAB-2MB-6, LAB-50MB-1, LAB-54MB-6, LAB-57ZB-6, LAB-60MB-1, LAB-58ZB-6, LAB-59MB-1, LAB-27MB-1). Isolates, LAB-57ZB-6, LAB-58ZB-6, LAB-59MB-1 and LAB-60MB-1 were identical. Mt-2 also comprised of 9 isolates (LAB-3MB-6, LAB-4MB-5, LAB-47MB-1, LAB-48MB-1, LAB-9MB-4, LAB-18ZB-3,LAB-28MB-1, LAB-29MB-1, LAB-17ZB-2). One identical group was revealed and the isolates involved were: LAB-3MB-6 and LAB-4MB-5. Mt-3 comprised of 16 isolates (LAB-12SB-2, LAB-33SB-5, LAB-34SB-1, LAB-39ZB-1, LAB-41MB-6, LAB-42MB-6, LAB-25MB-1, LAB-15SB-3, LAB-35SB-1, LAB-36SB-1, LAB-30MB-5, LAB-24MB-1, LAB-37ZB-1, LAB-43MB-6, LAB-31SB-5, LAB-32SB-5). The cluster analysis revealed three different groups in this morphotype.Group1 revealed that LAB-41MB-6 and LAB-42MB-6 were identical; group 2 revealed that isolates LAB-35SB-1 and LAB-36SB-1 were identical and group 3 revealed that isolates LAB-31SB-5 and LAB-32SB-5 were identical.Mt-4 comprised of only one isolate, LAB-46MB-1. Mt-5 had 18 isolates and they were: LAB-5MB-5, LAB-10SB-6, LAB-7MB-3, LAB-51MB-1, LAB-49MB-1, LAB-11SB-5, LAB-44MB-6, LAB-52MB-6, LAB-26MB-1, LAB-45MB-6, LAB-8MB-4, LAB-53MB-6, LAB-13SB-3, LAB-14SB-3, LAB-16ZB-6, LAB-19ZB-4, LAB-1MB-6, LAB-20ZB-6, LAB-38ZB-1. LAB-7MB-3 and LAB-51MB-1 were identical. Mt-6 was made up of 3 isolates (LAB-40MB-6, LAB-56ZB-1 and LAB-55MB-6. Mt-7 was made up of 4 isolates (LAB-6MB-2, LAB-21ZB-6, LAB-22ZB-6 and LAB-23MB-1). The result obtained in this study was in conformity with the reports of Somashekaraiah *et al.* (2019) who reported that different morphotypes existed among lactic acid bacteria isolated from Neera. Similarly, Kyule *et al.* (2022) reported that phylogenetic analysis of the 54 bacterial isolates morphological groups from fish and fish products revealed the genetic diversity of the bacterial isolates from the markets in Kirinyaga County, Kenya. In this study, based on the similarities and differences in the cultural, morphological and biochemical characteristics of the isolates, twenty LAB isolates were selected for molecular analysis (Table 2).

Genomic DNA Isolation and Purification of Twenty Lactic Acid Bacteria Isolates

The Lactic acid bacteria used in this study were isolated and characterized microbiologically and biochemically respectively to arrive at this final isolates (Table 3). The 20 bacteria isolates were subjected to DNA isolation and purification using Zymo Research DNA extraction commercial mini-kit. The prepared and purified genomic DNA were subjected to concentration determination at 260nm and 280nm. The concentration of DNA was particularly determined using the nanodrop equipment. DNA standard curve using Sigma DNA Ladder shows that the standard linear curve equation: y = 0.1511x – 1E-16. and the regression value, R is highly significant ($P \leq 0.01$) (Figure 3). The concentration of the DNA sample range between 0.9mg/ml to 4.0mg/ml (Table 3) The DNA purity index was also estimated to ensure that all the extracted and purified DNA samples will be well utilized in PCR analysis. The range for DNA purity index is between 1.5to 2.0. However, the DNA purity index of the 20 DNA extracted range between 1.5to 1.9 indicating they were all very pure and can be easily used in PCR (Table 3). The Agarose gel electrophoresis of the 20 extracted DNA (DNA fingerprinting) revealed very pure DNA electrophoretic pattern (Plate 1).

Table 3: DNA concentration and purity of genomic DNA from 20 Lactic acid bacteria species and the identification of LAB using specific PCR diagnostic primers

				Detected lactic acid bacteria using specific PCR primers			
S/N	**Code No**	**DNA Concentration (mg/ml)**	**DNA Purity Index (OD260/OD280=1.5-2)**	**Lactobacillus Brevis**	**Lactobacillus fermentum**	**Lactobacillus plantarum**	**Lactobacillus acidophilus**
1	LAB-6MB-2	1.3	1.6	-	-	+	-
2	LAB-10SB-6	1.5	1.7	+	-	-	-
3	LAB-17ZB-2	1.2	1.7	+	-	-	-
4	LAB-18ZB-3	0.7	1.5	+	-	-	-
5	LAB-21ZB-6	1.2	1.7	+	-	-	-
6	LAB-23MB-1	0.9	1.6	-	-	+	-
7	LAB-25MB-1	1.2	1.7	+	-	-	-
8	LAB-26MB-1	1.5	1.5	-	-	+	-
9	LAB-27MB-1	1.7	1.7	-	-	+	-
10	LAB-30MB-5	2.1	1.8	+	-	-	-
11	LAB-31SB-5	2.3	1.7	-	-	+	-
12	LAB-39ZB-1	0.9	1.6	-	-	+	-
13	LAB-42MB-6	1.7	1.7	-	-	+	-
14	LAB-44MB-6	2.4	1.7	-	-	+	-
15	LAB-46MB-1	4.0	1.9	-	-	+	-
16	LAB-49MB-1	0.9	1.7	-	-	+	-
17	LAB-52MB-6	1.1	1.6	-	-	+	-
18	LAB-54MB-6	1.0	1.7	+	-	-	-
19	LAB-55MB-6	3.5	1.7	-	-	-	+
20	LAB-57ZB-6	1.7	1.7	-	-	+	-
+: Presence - : Absence.							

RAPD-PCR Analysis of Genomic DNA from Twenty Lactic Acid Bacteria Isolates

The RAPD PCR analysis was adopted to analyze the genetic diversity among the 20 LAB isolates. The DNA fingerprint of the 20 LAB isolates analyzed using OPB20 primers were very successful (Plate 1). The DNA fingerprint revealed that some of the isolates were similar in their DNA fingerprint while others were different (Plate 1).

Table 4: RAPD primer selected for 20 Lactic acid bacteria PCR analysis.

S/N	Primer Code	Nucleotide Sequence
1	OPB20	GGACCCTTAC

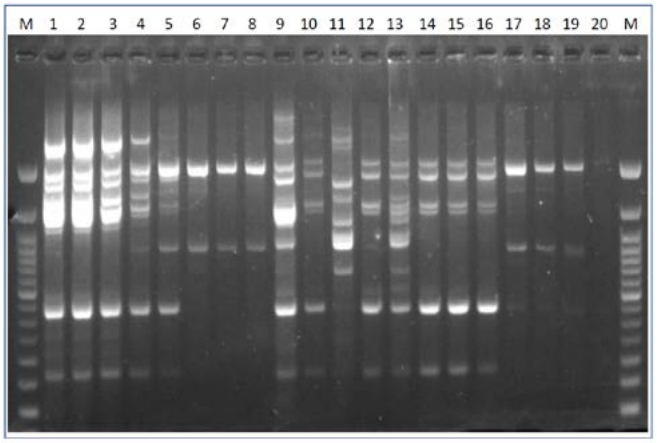

Plate 1: DNA fingerprint of 20 Lactic acid bacteria genomic DNA as revealed by OPB20 RAPD PCR analysis.

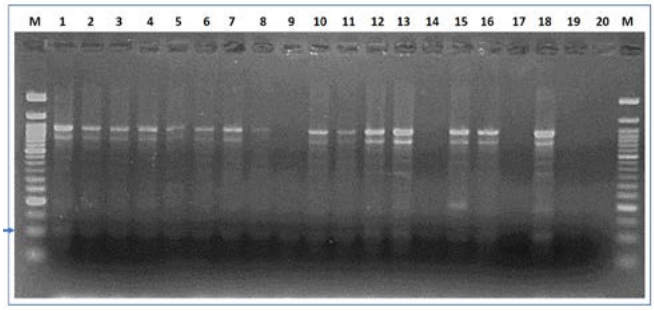

Plate 2: PCR detection of *L.brevis* with primer

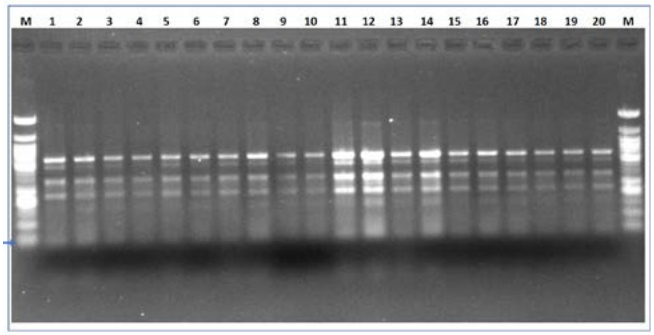

Plate 3: PCR detection of *L. fermentum* with prime

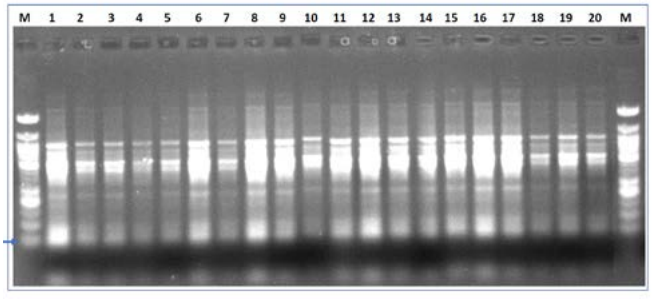

Plate 4: PCR detection of *L. plantarum* with primer

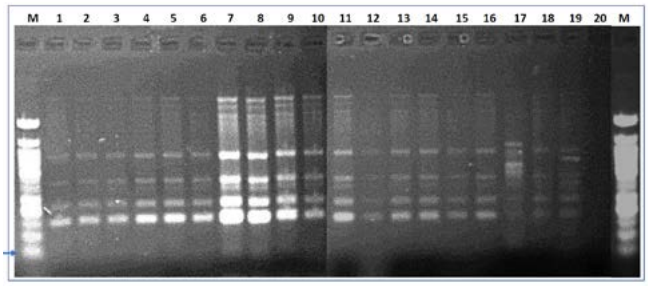

Plate 5: PCR detection of *L. acidophilus* with primer

Table 5: Detection of Lactic acid bacteria species using specific PCR diagnostic primers

+: Presence - : Absence.

S/N	Isolate	*L brevis*	*L fermentum*	*L plantarum*	*L acidophilus*
1	LAB-6MB-2	-	-	+	-
2	LAB-10SB-6	+	-	-	-
3	LAB-17ZB-2	+	-	-	-
4	LAB-18ZB-3	+	-	-	-
5	LAB-21ZB-6	+	-	-	-
6	LAB-23MB-1	-	-	+	-
7	LAB-25MB-1	+	-	-	-
8	LAB-26MB-1	-	-	+	-
9	LAB-27MB-1	-	-	+	-
10	LAB-30MB-5	+	-	-	-
11	LAB-31SB-5	-	-	+	-
12	LAB-39ZB-1	-	-	+	-
13	LAB-42MB-6	-	-	+	-
14	LAB-44MB-6	-	-	+	-
15	LAB-46MB-1	-	-	+	-
16	LAB-49MB-1	-	-	+	-
17	LAB-52MB-6	-	-	+	-
18	LAB-54MB-6	+	-	-	-
19	LAB-55MB-6	-	-	-	+
20	LAB-57ZB-6	-	-	+	-

Four (4) lactic acid bacteria specific PCR diagnostic primers of *L. brevis, L. fermentum, L. plantarum* and *L. acidophilus* were used. *L. brevis* had 260 base-pair at 16S–23S target gene region (Plate 2); *L. fermentum had 129 base-pair* (unique gene) (Plate 3)*; L. plantarum* had147 base-pair (unique gene)(Plate 4) and *L. acidophilus* had 129 base-pair at 16S–23S target gene region (Plate 5). Each base-pair can be seen at the point marked with blue marker. Remarkably, *L. brevis*, *L. plantarum* and *L. acidophilus* were detected in this study while *L. fermentum* was not detected among the 20 isolates. However, seven isolates were identified to be *L. brevis,* 12 isolates were *L. plantarum* and one isolate was identified as *L. acidophilus.* Onyimba *et al.* (2017) isolated *L. fermentum*, *L. plantarum* and *L. acidophilus* from naturally fermented kunun-zaki.

CONCLUSION

This study characterized the dominant LAB in kunun-zaki production using traditional methods and molecular biological techniques. The initial 60 LAB isolates analyzed in this studies revealed seven morphotypes while finalized based on the cultural, morphological and biochemical characteristics, twenty LAB isolates were selected and identified to be *L. brevis*, *L. plantarum* and *L. acidophilus* as revealed by RAPD-PCR assays.

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