Lactic acid bacteria obtained from cereal-based fermented food products at different processing stages

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ABSTRACT

Background: Selective consumption of fermented foods obtained from dairy sources have resulted from problems of lactose intolerance, dairy allergies and strict vegetarian dietary habits. Non-dairy, cereal-based fermented food products undergo different processing stages and it is believed that lactic acid bacteria (LAB), is involved in the fermentation process. However, there are little or no information on the specific LAB that are involved at the various intermediate stages.

Objectives: The aim of this study was to explore indigenous cereal food products as an alternative source of LAB as well as isolate and identify LAB from cereal-based fermented food obtained at different processing stages.

Methods: Two varieties of corn/maize and sorghum (guinea corn) were cleaned, steeped in water and milled. Samples obtained at different processing stages were collected into sterile containers. LAB were isolated on De Man, Rogosa and Sharpe agar and characterized using biochemical, microscopic and molecular methods.

Results: The total viable bacterial cell count ranged from 9.22 to 9.66 log10 CFU/ml. Conventional identification method revealed rod-shaped, Gram positive, catalase negative, non-spore forming bacteria with single, paired and long chain cell arrangements. The 16S rRNA gene sequence analysis identified diverse species of two LAB groups namely: *Lactobacillus* (93.02%) and *Pediococcus* (6.98%) with *L. fermentum* as the dominant *Lactobacillus* spp.

Conclusion: This study revealed the presence of LAB from fermented maize and sorghum at different processing stages. Our findings show that the slurry-processed-stage, which is the commonly consumed fermented food product apparently contains similar diverse LAB as identified in the milled whole product.

Keywords: Lactic acid bacteria; fermented food; maize; sorghum; probiotics

Bactéries lactiques obtenues à partir de produits alimentaires fermentés à base de céréales à différents stades de transformation

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RÉSUMÉ:

Contexte: La consommation sélective d'aliments fermentés obtenus à partir de sources laitières résulte de problèmes d'intolérance au lactose, des allergies aux produits laitiers et des habitudes alimentaires végétariennes strictes. Les produits alimentaires fermentés non laitiers à base de céréales passent par différents stades de transformation et l'on pense que les bactéries lactiques (BL) sont impliquées dans le processus de fermentation. Cependant, il n'existe que peu ou pas d'informations sur les BL spécifiques impliquées dans les différentes étapes intermédiaires.

Objectifs: Le but de cette étude est d'explorer les produits alimentaires céréaliers indigènes en tant que source alternative de BL, ainsi que d'isoler et d'identifier les BL à partir d'aliments fermentés à base de céréales obtenus à différents stades de transformation.

Méthodes: Deux variétés de maïs et de sorgho (maïs de Guinée) ont été nettoyées, trempées dans l'eau et moulues. Les échantillons obtenus à différents stades de traitement ont été collectés dans des récipients stériles. Les BL ont été isolés sur gélose De Man, Rogosa et Sharpe et caractérisés à l'aide de méthodes biochimiques, microscopiques et moléculaires.

Résultats: Le nombre total de cellules bactériennes viables variait de 9,22 à 9,66 log10 UFC/ml. La méthode d'identification conventionnelle a révélé des bactéries en forme de bâtonnet, à Gram positif, catalase négative, non sporulées, avec des arrangements cellulaires simples, appariés et à chaîne longue. L'analyse de la séquence génétique de l'ARNr 16S a permis d'identifier diverses espèces de deux groupes de BL, à savoir : Lactobacillus (93,02%) et Pediococcus (6,98%) avec L. fermentum comme Lactobacillus spp. dominant.

Conclusion: Cette étude a révélé la présence de BL dans le maïs et le sorgho fermentés à différents stades de transformation. Nos résultats montrent que le stade de transformation en bouillie, qui est le produit alimentaire fermenté couramment consommé, contient apparemment des BL diversifiées similaires à celles identifiées dans le produit entier moulu.

Mots-clés : Bactéries lactiques ; aliments fermentés ; maïs ; sorgho ; probiotiques

INTRODUCTION

A relationship exists between food product characteristics, functionality, food microbiota, microbial diversity and food health benefits. Food fermentation promotes food preservation, enhances food safety, functionality, organoleptic properties, nutritional and consumer acceptability of final food product.[1,2,3] Conventional sources of health-promoting fermented foods have been dairy-based (milk and milk products). However, consumption of fermented foods from dairy sources is associated with problems of lactose intolerance, dairy allergies and strict vegetarian dietary habits.[4,5]

Cereal-based fermented foods are widely consumed in many developing countries. They are non-dairy food source but also serve as important energy source and contribute to socio-economic development of the region.[6] In Nigeria, a notable cereal-based fermented food is known as Akamu by the Igbos or Ogi, by the Yorubas. Akamu is made from fermented cereal gruel or porridge of maize or corn (Zea mays, yellow or white varieties), red/brown guinea corn/sorghum (Sorghum vulgare), white guinea corn/sorghum *(Sorghum bicolor)* and millet *(Pennisetum americarum)*.

Cereal-based fermented foods undergo different processing stages during their production to obtain the final food product. It is believed that each of these processing stages contain microbes responsible for their fermentation. They are employed for several beneficial purposes. The cooked slurry is commonly consumed both by adults as breakfast meals and as a weaning food for infants.[7,8] However, the consumption of uncooked slurry with or without the liquor (Omidun)/Omi Ogi or Omikan) have been attributed to have anti-diarrhoeal properties.[9] The residue/chaff is used as poultry and other livestock feed by local farmers. The processed residues also have potential use as livestock feed and in energy generation.[10]

Spontaneous fermentation of cereal-based foods involve a competitive activities of endogenous and contaminating microorganisms with lactic acid bacteria (LAB) as a dominant microbiota.[11,12,13] Among LAB genera are *Lactobacillus, Lactococcus, Leuconostoc, Bifidobacteria* and *Pediococcus.* They are either microaerophilic or anaerobic, producing lactic acid as the major metabolic end product of carbohydrate fermentation.[14,15] Lactic acid bacteria have known probiotic health benefits as well as some proteolytic and lipolytic properties. They can also convert carbohydrates to several organic acids and a wide range of other products.[16]

There are documented reports of LAB identified from cereal-based fermented products. Molecular identification of LAB in effluents generated during Ogi production identified *L. plantarum, L. fermentum, L. reuteri, Enterococcus faecium, Pediococcus acidilactici, Pediococcus pentosaceus, Enterococcus faecalis,* and *L. brevi*[17]. In the indigenous fermentation of three varieties of maize, molds and bacteria including *L. plantarum, L. fermentum, Leuconostoc mesenteroides, Staphylococcus aureus, Saccharomyces cerevisiae* were associated with maize fermentation.[18] Similarly, *Weissella paramesenteroides, L. brevis, L. rossiae, L. fermentum, L. plantarum, Acetobacter pasteurianus, Paenibacillus spp*. and Bacillus spp. were identified from Omidun uncooked and cooked slurry of Ogi gruel.[9]

However, there is paucity of scientific information on microbes involved in fermentation at the different processing stages of these cereal-based food products. The aim of this study therefore was to explore our indigenous cereal food products as an alternative source of LAB, isolate and identify specific LAB responsible for fermentation at various intermediate stages during the production of cereal-based food.

MATERIAL AND METHODS

Sample collection

Two different varieties of corn and sorghum (guinea corn) cereals were used for the study.

Processing of samples

Methods described by Evans *et al*. (2013)[7] and Afolayin *et al*. (2017)[9] were adopted with slight modification. A 1000 g weight of each cereal sample (white and yellow corn; white and brown guinea corn) was cleaned, steeped in 3 L of portable water in a sealed container. This was allowed to soak undisturbed and fermented for 72 h at 28 ± 2 °C after which it was washed thoroughly and wet milled. The wet mass was filtered using a muslin cloth and the filtrate was allowed to stand for 2-3 h after which the supernatant was decanted and the slurry, allowed draining completely in a bag to form a semi-solid mass. A 250 g weight of each sample at different processing stages: wet-milled material (M), residue (R), slurry (S) and 250 ml of liquor (L) were collected in a sterile sample container, stored in the refrigerator maintained at 8 °C and used within 7 days.

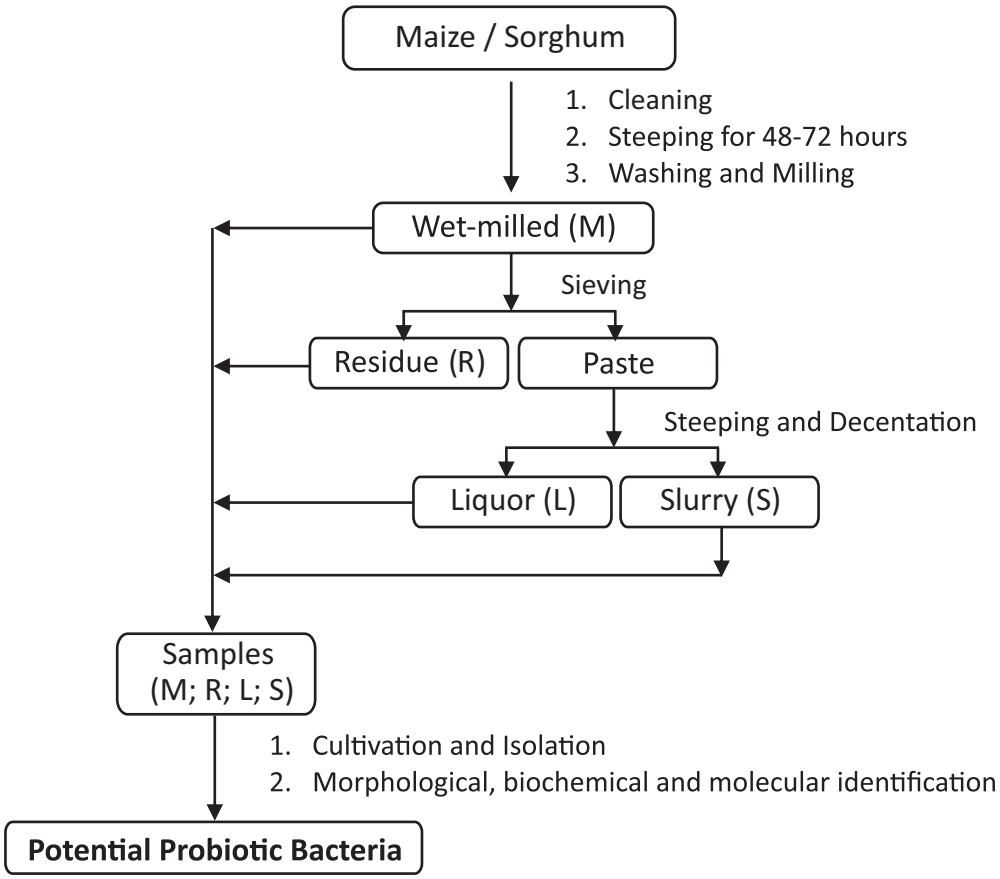

Fig.1: Process flow for processing and identification of LAB in cereal-based fermented food

Cultivation, enumeration and isolation of lactic acid bacteria

A 10% (w/v or v/v) concentration of each fermented semi-solid or liquid samples were prepared in 0.1% peptone water (Oxoid, UK) and homogenized for 30 mins. A 10-fold serial dilution of samples was carried out 7 times in 0.1% peptone water. 1 ml of appropriate dilutions of each sample was inoculated into a previously prepared de Man-Rogosa-Sharpe (MRS) Agar (Hi-Media, India) supplemented with cycloheximide (final concentration, 0.1g/L) and spread-plated using sterile glass beads. The plates were incubated at 37 °C for 48 h and examined for microbial growth. The number of colonies were counted, average taken and the colony-forming unit (CFU) per ml or g was calculated. Triplicate determinations were undertaken. Discrete colonies were selected based on morphological characteristics such as colour, size, margin, elevation and lustre. The selected colonies were repeatedly streaked on MRS agar without cycloheximide to obtain a pure microbial colony of bacteria. Pure cultures were maintained in MRS agar slants and stored at 4 °C for further analysis.

Characterization and identification of isolated LAB from fermented cereals

Morphological Identification

Pure bacterial isolates obtained at different processing stages of corn and guinea corn varieties were macroscopically examined based on colour, shape, elevation and margin. Other phenotypic identifications included surface (smooth, coarse; lustre or dull); mucoid (slimy or sticky) and murkiness (transparent or opaque) of the colony were observed.

Gram staining: Gram staining was performed in order to differentiate bacterial isolates into Gram positive or Gram negative and present them as either rods, cocci or coccobacilli.[19] A smear of isolated organisms was made on a clean glass slide by mixing a microbial colony with a loopful of water. It was air-dried and heat-fixed. The prepared and heat-fixed smear was flooded with a solution of 1% crystal violet for 1 min, after which the excess stain was rinsed off using distilled water. The smear was then flooded with Lugol's iodine for I min,

while the excess iodine was rinsed off with distilled water. The smear was further decolourized with 98% alcohol and quickly rinsed with distilled water. Safranin red was added, allowed for 1 min, rinsed off with distilled water and examined under oil immersion (100x) objective.

Spore staining

A method of Mulaw *et al*. 2019[20] was adopted with some modifications. A smear of the test isolates was made on a clean glass slide and air-dried. It was heat fixed, covered with blotting paper and flooded with malachite green. The slides were then heated for 5 mins, cooled, washed with water, flooded with safranin and rinsed. The drained and blotted slides were examined microscopically under oil immersion for the colours of vegetative cells and spores.

Biochemical Identification

Biochemical identification of isolates obtained from fermented foods were conducted using standard protocols.[21,22]

Catalase test: Slide method was used for this test. A drop of sterile normal saline was placed on a clean grease free slide. It was mixed with a colony of bacterial isolate and emulsified with a drop of 3% hydrogen peroxide (H_2O_2) and observed for presence or absence of froth or gas bubbles.

Oxidase test: A colony of pure bacterial isolate was smeared on a piece of Whatman (No. 1) filter paper previously soaked with 2 drops of freshly prepared oxidase reagent (1% aqueous tetramethyl-p-phenylene diamine hydrogen chloride) with the aid of flamed and cooled clean grease-free glass slide. Presence or absence of purple colour within 5 sec was observed.

Citrate utilization test: A slope of melted and autoclaved Simmon's citrate agar was prepared in bijou bottles. Pure isolates were thereafter streaked on the slope and the butt stabbed using a sterile straight wire. It was incubated at 37 °C for 48 h and observed for colour change.

Indole test: A colony from the pure bacterial isolate was suspended in peptone water and incubated at 37 °C for 24 h. After the period of incubation, 3 drops of Kovac's reagent was added, mixed properly and observed for colour change.

Urease test: A streak was made on the surface of a urea agar slant with a portion of the well-isolated colony of the test organism and incubated at 37 °C for 48 h. It was examined for the development of a pink colour.

Triple sugar iron (TSI) test: The butt of TSI agar slant was aseptically stabbed and the surface streaked with a touch from the top of the isolated colony. Tubes were loosely capped, incubated at 35 °C for 18-24 h and examined for colour change in the medium.

Molecular characterization of lactic acid bacteria isolates

Colony PCR and amplification

Discrete and small colonies of pure isolates obtained from respective samples were subjected to 16S rRNA colony PCR.[23] It was appropriately mixed in 10 µl of DNA free water and microwaved (Inventum, Netherlands) twice at 800W for 1 min each to release the total genomic DNA. Amplification of 16S rRNA gene segment with expected PCR product size of 1500bp was undertaken using universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'- GGTTACCTTGTTACGACTT-3').[24,25] Forward primer is complementary to the 5'end of 16S rRNA, and the reverse primer is complementary to the 3' end of 16S rRNA region. A total of 25 µl PCR reaction mixtures were used to perform PCR amplification using Thermocycler PCR machine (Eppendorf, Germany). PCR amplification cycling conditions was set as follows: Initial denaturation at 95 °C for 2 min), final denaturation at 95 °C for 30 sec, annealing at 55 °C for 30 sec, extension at 72 °C for 90 sec and final extension at 72 °C for 5 mins.

Agarose gel electrophoresis of LAB gene segment

The quality and purity of PCR product was verified to be the expected 1500bp using Agarose gel electrophoresis in 1% Agarose, 1 x Tris-acetic EDTA (TAE) buffer and 0.1 µl/ml of Gel Red dye. A 6 µl of solution consisting 5 µl of diluted loading dye and 1 µl of PCR product was pipetted and carefully loaded into each of the wells on the Agarose gel while 6 µl of Gene ruler ™ (ladder) and negative control were loaded into the wells at both ends of the Agarose gel respectively. The gel electrophoresis was set to run at 100 V for 30 mins and the bands were viewed using UV trans-illuminator, gel documentation system.

Sanger DNA sequencing

From the gel electrophoresis, the PCR products with expected size of 1500bp were selected and prepared for Sanger sequencing. A 10 µl of each of the selected PCR product and 10 µM of forward and reverse primers were carefully loaded into 96 well plates. The DNA sequencing was carried out using Applied Biosystems 3730XL DNA analyser (BiomekR), (Beckman coulter, USA) to obtain the

full sequence of the amplicons. DNA data set were opened and assembled using the Finch TV sequence alignment editor software, version 1.4.0. They were blasted based on sequence similarity values determined using the basic alignment search tool (BLAST) of the National Centre of Biotechnology Information (NCBI).[26] Sequences with ≥88% similarity to the previously published sequences (https://www.ncbi.nlm.nih.gov) were used as the criteria to indicate species identity.

Statistical analysis

Data obtained from the study were analysed using the IBM(R) Statistical Package for Social Sciences (SPSS(R)) version 23, USA software package by one-way analysis of variance (ANOVA).

RESULTS

Morphological and biochemical identification

A total of 77 discrete colonies were isolated from 16 samples of cereal-based fermented products at different processing stages. Colony identification on MRS agar plates revealed small to medium sized, cream to white colour, mostly circular, flat or raised, smooth, shiny or dull colonies with entire margin. Isolates were rod-shaped, non-spore forming, Gram-positive bacteria with single, paired and long chain cell arrangements.

Biochemical identification revealed catalase negative, indole negative, urease negative and citrate negative isolates. The isolates are able to ferment sugar but did not produce hydrogen sulphide.

Bacterial enumeration

The result of total bacterial count obtained from fermented cereals at various processing stages is as shown in Fig. 2. Similar amounts of bacteria were obtained from the various cereal varieties and processing stages and there was no statistical difference observed ($p>0.05$). The bacterial count ranged from 9.22 to 9.66 $\log_{10}$ CFU/ml or g. Slurry of yellow maize had the highest bacterial count (9.66 ± 0.05 $\log_{10}$ CFU/g) while the least bacterial count was obtained from white maize residue sample (9.22 ± 0.07 $\log_{10}$ CFU/g).

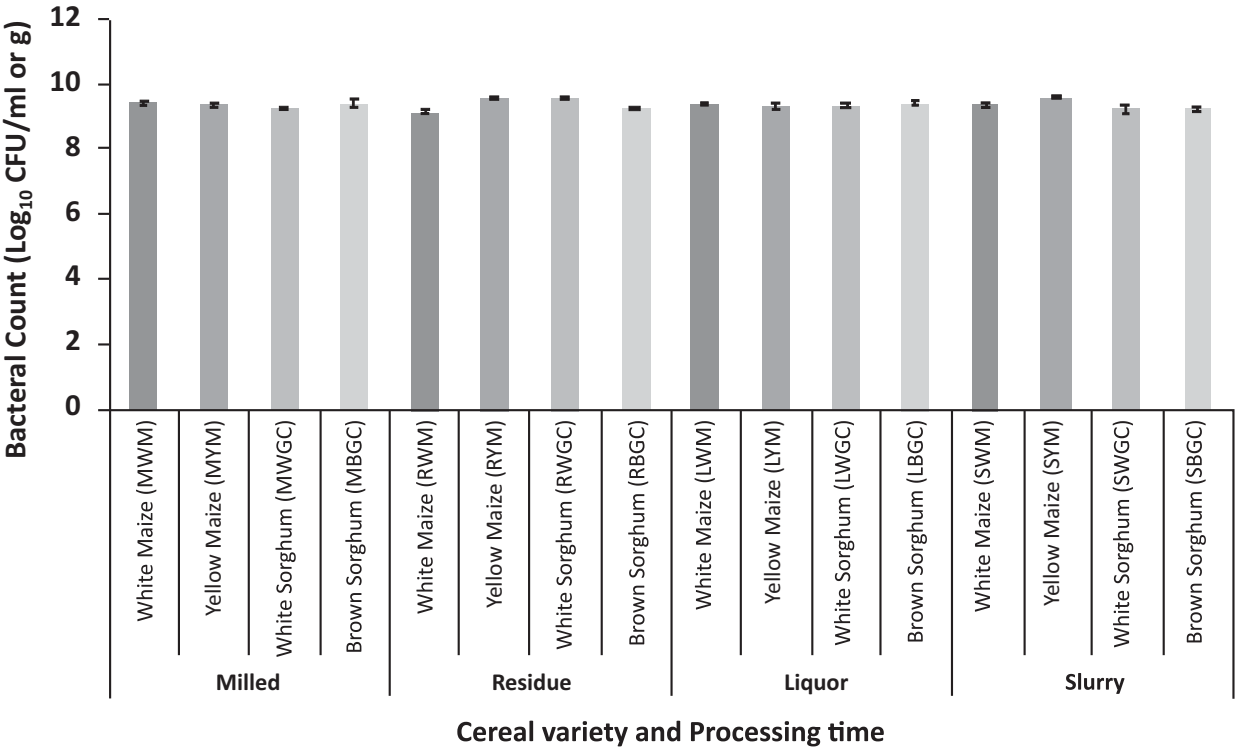

Fig.2: Total bacterial count from various fermented cereals obtained at various processing stages

The result of the bacterial diversities at the various processing stages (Table 1) shows that Lactobacillus fermentum dominated all the processing stages of the studied samples while L. plantarum were most abundant in the wet-milled stage. Wet-milled samples exhibited highest percentage occurrence of bacteria across all cereal varieties studied. Milled (crude) sample produced the most diverse bacteria. Least diverse bacteria were obtained from the waste products (residue and liquor).

Table 1: Bacterial diversity at various processing stages

Bacterial isolates	% Occurrence of diverse bacterial species produced at different processing stages				
	Wet-Milled	Residue	Liquor	Slurry	Total
Lactobacillus fermentum	16.3	16.3	16.3	14	62.9
Lactobacillus pentosus	2.3	0.0	0.0	0.0	2.3
Lactobacillus plantarum	23.3	0.0	0.0	2.3	25.6
Lactobacillus hilgardii	0	2.3	0.0	0.0	2.3
Pediococcus pentosaceus	2.3	0.0	2.3	2.3	6.9

Values calculated as percentage of the mean value of data obtained from triplicate determinations

Molecular identification

Following amplification of the bacterial 16S rRNA gene by PCR, the result of the gel electrophoresis of PCR products for every bacterial colony harvested revealed a band of about 1500bp. Representative samples are shown in Fig. 3. This result is consistent with the size of the 16S rRNA gene. Gene sequencing of the PCR products and subsequent alignment of partial 16S rRNA sequences against organisms in the NCBI database, revealed the identity of LAB isolated from the various samples of the cereal-based fermented food products (Tables 2-5).

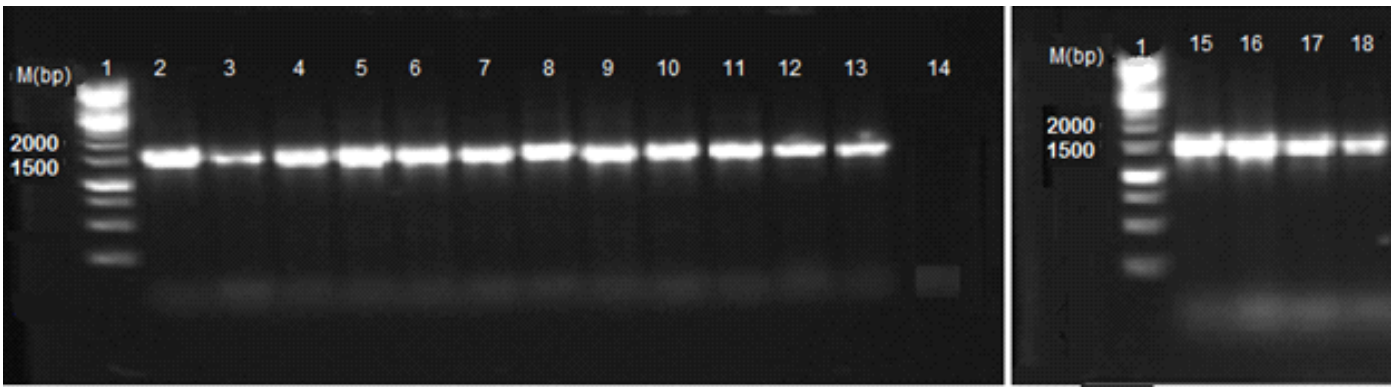

Fig. 3: Representative of 16S rRNA gene amplification products of different samples at various processing stages.

1= Ladder (Gene Ruler (TM)), 2= MWGC, 3= LYM, 4= MYM, 5= MWM, 6= RYM, 7= SWM, 8= MBGC, 9= LWM, 10= SBGC, 11= SYM, 12= RWM, 13= SWGC, 14= Negative control without DNA, 15= RWGC, 16= LWGC, 17= RBGC, 18= LBGC

Table 2: LAB identified at wet-milled processing stage

Cereal type	Colony ID	Organism	Strain	% Similarity
White Guinea corn	MWGC-1	*Lactobacillus fermentum*	SMVDUDB4	97%
	MWGC-2	*Pediococcus pentosaceus*	FB145	97%
	MWGC-3	*Lactobacillus plantarum*	CQ11-2	94%
	MWGC-5	*Lactobacillus plantarum*	LY21	98%
Brown Guinea corn	MBGC-1	*Lactobacillus plantarum* subsp. plantarum	FM3-1	100%
	MBGC-2	*Lactobacillus fermentum*	2-28	94%
	MBGC-3	*Lactobacillus plantarum*	GG3	95%
	MBGC-4	*Lactobacillus fermentum*	HT4	96%
	MBGC-5	*Lactobacillus plantarum* subsp. plantarum	BNH17	88%
White maize	MWM-1	*Lactobacillus plantarum*	PKRSU4	98%
	MWM-2	*Lactobacillus fermentum*	GERU1	97%
	MWM-3	*Lactobacillus pentosus*	SB7	96%
	MWM-4	*Lactobacillus plantarum*	A	98%
	MWM-5	*Lactobacillus plantarum*	Gene	98%
	MWM-7	*Lactobacillus plantarum*	NL6	93%
Yellow maize	MYM-2	*Lactobacillus plantarum*	X3-4B	100%
	MYM-3	*Lactobacillus fermentum*	PGC145	100%
	MYM-5	*Lactobacillus fermentum*	PGC148	100%
	MYM-6	*Lactobacillus fermentum*	GERU1	97%

Similarity values determined using BLAST of the GenBank. Sequence similarity values =88% to the published sequence in database were used to determine species identity.

Table 3: LAB identified in the residue samples

Cereal type	Colony ID	Organism	Strain	% Similarity
White guinea corn	RWGC-1	*Lactobacillus fermentum*	CAU7532	93%
	RWGC-2	*Lactobacillus fermentum*	2-28	97%
Brown guinea corn	RBGC-2	*Lactobacillus fermentum*	CAU1576	92%
White maize	RWM-1	*Lactobacillus fermentum*	SBM	96%
	RWM-2	*Lactobacillus fermentum*	TAD47	98%
	RWM-4	*Lactobacillus fermentum*	APBSMLB166	94%
Yellow maize	RYM-1	*Lactobacillus fermentum*	SMVDUDB4	97%
	RYM-3	*Lactobacillus hilgardii*	CAU10223	94%

Similarity values determined using BLAST of the GenBank. Sequence similarity values = 92% to the published sequence in database were used to determine species identity.

Table 4: LAB identified in liquor samples

Cereal type	Colony ID	Organism	Strain	% Similarity
White guinea corn	LWGC-2	*Lactobacillus fermentum*	GERU1	96%
	LWGC-3	*Lactobacillus fermentum*	4-1	98%
Brown guinea corn	LBGC-1	*Lactobacillus fermentum*	GERU1	98%
	LBGC-2	*Lactobacillus fermentum*	APBSMLB166	97%
White maize	LWM-1	*Lactobacillus fermentum*	4-17	95%
	LWM-3	*Pediococcus pentosaceus*	CSCWL2-4	97%
Yellow maize	LYM-1	*Lactobacillus fermentum*	F53	98%
	LYM-2	*Lactobacillus fermentum*	SMVDUDB4	98%

Similarity values determined using BLAST of the GenBank. Sequence similarity values = 95% to the published sequence in database were used to determine species identity.

Table 5: LAB identified at slurry processing stage

Cereal type	Colony ID	Organism	Strain	% Similarity
White guinea corn	SWGC-3	*Lactobacillus fermentum*	HBUAS51148	100%
	SWGC-4	*Lactobacillus plantarum*	IAH_19	100%
Brown guinea corn	SBGC-3	*Lactobacillus fermentum*	2-28	95%
White maize	SWM-1	*Lactobacillus fermentum*	APBSMLB166	95%
	SWM-3	*Lactobacillus fermentum*	GERU1	98%
Yellow maize	SYM-1	*Lactobacillus fermentum*	SMVDUDB4	98%
	SYM-2	*Lactobacillus fermentum*	2-24	97%
	SYM-4	*Pediococcus pentosaceus*	IMAU50390	98%

Similarity values determined using BLAST of the GenBank. Sequence similarity values = 95% to the published sequence in database were used to determine species identity.

As shown in Fig.4, the taxonomic positions of the various LAB isolated with their relative abundance are compared with closely related taxa in the family Lactobacillaceae. The evolutionary distances calculations were done with the aid of ClustalW sequence alignment editor software, the units being the number of base substitutions per nucleotide position/site.

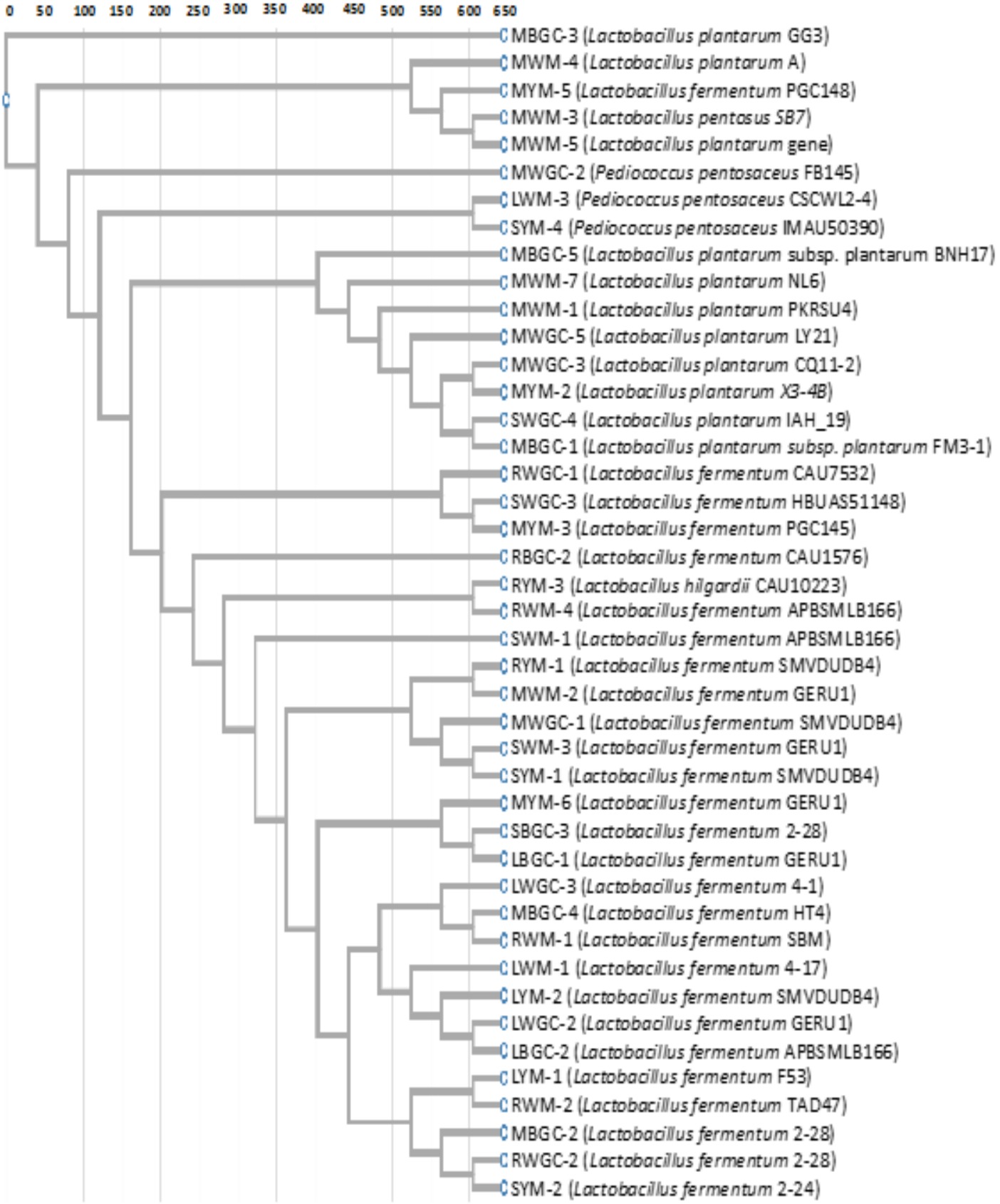

Fig. 4: Phylogenetic tree of LAB isolated generated based on multiple sequence alignment of 16S rRNA gene sequences using ClustalW

DISCUSSION

In this study *Lactobacillus* and *Pediococcus* groups of lactic acid bacteria were present at various processing stages of cereal-based fermented foods. These processing stages are necessary steps towards production of final, consumable cereal product. Howbeit, these stages commonly disregarded during processing, may contain some fermenting and useful bacteria that may have probiotic potentials. Evaluating the presence and amount of LAB in samples obtained at different processing stages will give insights into the bacterial diversity in the different samples. Wet milling involves the process of breaking down the cereal kernel into various component parts. At the wet-milled processing stage, the whole, steeped cereal kernel was grinded with water using a clean mill. Wet-milled stage is the requisite step towards the separation of various components of the cereal. The sample obtained at this stage therefore contains all components of the cereal before it is fractionated via filtration. During filtration process using a clean muslin cloth, the component that remains on the filter is termed the residue as it is often discarded or used in livestock feeding and energy generation.[10] Thereafter, the filtrate passes through the filter where it settles to yield the slurry and liquor samples. Both slurry and liquor samples are the consumed fractions after processing.

Microbial enumeration of the isolates from the different processing stages showed that slurry of yellow maize had the highest bacterial count while the white maize residue gave the least bacterial count. This is in agreement with the report of Afolayan *et al*. 2017.[9] The obtained result could be attributed to the documented report that the yellow maize supports microbial growth due to higher nutritional content. Yellow maize contains β-carotene - the precursor of Vitamin A and that gives them some level of nutritional advantage.[27,28]

As revealed from morphological and biochemical tests, LAB are indole negative, urease negative and citrate negative, rod-shaped, non-spore forming Gram-positive bacteria having single, paired and long chain cell arrangements typical of the family *Lactobacillaceae*. They are catalase- and oxidase-negative due to the absence of cytochrome and porphyrins within the cells.[29] The use of 16S rRNA gene sequencing was employed in the molecular characterization of PCR products because it has been acclaimed as the gold standard for identifying bacterial species.[30,31] It has revolutionized identification as it is a preferred method over biochemical tests because it can identify organisms to species levels. It leverages on the presence of housekeeping genes to classify bacteria according to their phylogeny, genus and/or species.[32,33] It has been successfully used to identify novel bacterial species and genera.[34,35]

The 16S rRNA gene sequences of amplified PCR products from the different cereal types at various processing stages and assembly using Finch TV, identified *Lactobacillus fermentum* (62.9%), *Lactobacillus plantarum* (25.6%), *Lactobacillus pentosus* (2.3%), *Lactobacillus hilgardii* (2.3%) and *Pediococcus pentosaceus* (6.9%). It was not surprising to note that the wet-milled cereal sample contained the most diverse bacteria as this sample consists of all components of the cereal kernel. *Lactobacillus plantarum* and *Lactobacillus fermentum* were the dominating species isolated. There has been similar reports on the abundance of these species in plant-based fermented foods.[36,37,17] The predominance of *Lactobacillus fermentum* in various samples obtained at all the processing stages studied depicts the nature of this LAB species. *L. fermentum* are Gram-positive bacteria commonly present in fermenting plant and animal materials.[37,2,18] They are reportedly key microorganism in many fermentation technology including sourdough making, where they contribute to flavor, texture and provide health-promoting dough ingredients.[38] They have been linked to many health benefits such as probiotic abilities, production of potent and diverse antimicrobial peptides; enhancing immunologic response, cholesterol lowering abilities and promotion of gastrointestinal and respiratory health benefits.[37] Similarly, *Lactobacillus plantarum* was reported to dominate fermented Ogi samples and most cereal-based products. *Lactobacillus plantarum* has sodium chloride, acid and bile salt tolerant ability and are commonly found in fermented foods and in the gastrointestinal tract.[39] *Lactobacillus hilgardii* is most abundant in fermentation of wine, dairy products and wine musts. It has however been reported that natural Gamma-aminobutyric acid (GABA), a neurotransmitter which improves relaxation and sleep was produced in fermentation process that utilizes *Lactobacillus hilgardii.*[40] *Pediococcus pentosaceus* are used as probiotics commonly added as beneficial microbes in fermentation in the making of sausages, cheese and yoghurts.[41]

However, residue and liquor samples revealed the least bacterial diversity, which could be attributed to the nature of the samples. Both samples are devoid of cereal endosperm which is the soft, internal component of the cereal.[42] The rich, starchy endosperm of cereals is

therefore a good substrate for fermentation bacteria. It is noteworthy from this study, that the slurry, which is the generally consumed portion of the fermented cereal, contained apparently as rich, diverse LAB as identified in the whole product (wet-milled) and other fractions of fermented food product. Incidentally, *Lactobacillus pentosus* and *Lactobacillus hilgardii* were not identified from slurry processing stage. This result could be occasioned by duration of fermentation undergone at each processing stage of the samples. Different LAB were identified from effluent generated from first and second fermentation times in Ogi production.[17]

The nucleotide sequences of PCR products revealed LAB strains with varied percentage similarity to LAB species and strains already existing in NCBI non-redundant database. Phylogenetic tree constructed from the 16S rRNA sequences of the identified LAB and subsequent evolutionary analyses indicated the cluster of strains with similar sequences. This grouping revealed the relatedness of species and are therefore considered to be close relatives of *Lactobacillus plantarum* and *Lactobacillus fermentum*. Hence, further analyses will be conducted to determine the full genome sequences and evaluate whether they possess probiotic capabilities.

CONCLUSION

LAB have been found to be involved in the fermentation of commonly consumed cereal-based foods. The presence of LAB in the various processing stages of the product as revealed by 16S rRNA gene sequence analysis shows diverse species of two LAB groups namely: *Lactobacillus* and *Pediococcus* with *L. fermentum* as the dominant Lactobacillus spp.

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