Isolation and Molecular Characterization of Some *Lactobacillus* Species Isolated from Three Locally Fermented Foods

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ABSTRACT

This study investigated the isolation and molecular characterization of lactic acid bacteria (LAB) from three locally fermented Nigerian foods; West African soft cheese (Wara), fermented yellow maize cereal porridge (*Ogi solo*), and fermented white maize cereal porridge (*Ogi funfun*). Samples were obtained from Waso market in Ogbomosho, Oyo State, and processed according to standard microbiological techniques on MRS medium under anaerobic conditions. Total LAB counts ranged from 6.0×10^6 to 11.0×10^6 CFU/g, with the highest count in the yellow maize cereal porridge. Five distinct LAB isolates were obtained and molecularly characterized via 16S rRNA gene sequencing. From the BLAST analysis, with a similarity of 99-100% to the reference strains, the isolates were identified as *Lactococcus lactis* FYM1, *Lactobacillus acidophilus* FYM2, *Lactobacillus paracasei* WAC1, *Lactobacillus delbrueckii* WAC2, and *Lactobacillus iners* FWM. All the isolates have been deposited in NCBI GenBank under accession numbers PQ758598, PQ555634, PQ346814, PQ555635, and PQ555636, respectively. The phylogenetic analysis showed that the isolates clustered within the *Lactobacillaceae* family and had a close relationship to other known probiotic strains. This shows the very high diversity that exists among the beneficial LAB in traditional Nigerian fermented foods and their availability for further studies in food biotechnology and probiotic development.

Keywords: Fermented food, *Lactobacillus*, Lactic acid bacteria, probiotics, molecular characterization.

INTRODUCTION

Fermented foods have been reported to be highly nutritious and therapeutic not only because of the macro/micronutrients present in them but also due to the presence of microorganisms that have functionalized the food through their metabolism and secondary metabolites. These beneficial microorganisms confer nutritional and medicinal benefits on the food in which they are found (Adesemoye *et al*., 2025). The metabolic activities of the beneficial microorganisms found in foods can help improve the bioavailability of phytochemicals, macro and micro nutrients in a biochemical process called fermentation. Through the presence or inoculation of microorganisms like bacteria or yeasts into food, fermentation occurs by breaking down carbohydrate into organic acids and alcohols in anaerobic environments (Punia *et al*., 2022).

In Nigeria, some fermented foods can be classified based on their raw materials, these fermented food categories include sorghum-based fermented foods e.g. brown pap locally called *ogi baba* by the Yoruba tribe in southwestern part of Nigeria, maize-based fermented foods e.g. yellow corn meal also locally called "*Ogi"* by the Yoruba tribe. Also, millet-based fermented foods locally called "*burukutu*" "*kunu-zaki*, "*masa*", and *pito*. Cassava-based fermented foods e.g. *fufu*, *gari*, and *lafun* also belong to fermented foods classified on the bases of their raw materials which are highly consumed in Nigeria and other parts of the world (Adesulu-Dahunsi *et al*., 2020). Furthermore, West African soft cheese locally called *Wara* is another fermented food which is locally processed using fresh cow milk curdled with juice extract of *Calostropis procera* leaves which can be consumed raw after fermentation or as snacks when fried. The unripe soft cheese curd is commonly consumed in the western and northern part of Nigeria. And due to its nutritional composition, a plethora of beneficial microorganisms are found in it (Adesemoye *et al*., 2025)

Among the beneficial microorganisms found in fermented foods are lactic acid bacteria (LAB) (Wang *et al*., 2021). Lactic acid bacteria are rod or cocci, non-spore forming, Gram-positive bacteria with strong tolerance to pH, capable of carbohydrate fermentation to produce lactic acid primarily as the end product (Oladipo *et al.*, 2025a; 2025b); they are also known to biodegrade proteins and lipids. Lactic acid bacteria are also capable of producing alcohols, esters, aldehydes, and some compounds like sulfur compounds to produce flavors in various fermented foods (Bintsis, 2018; Oladipo and Oyewumi, 2024). Lactic acid bacteria constitute more than 61 genera, but the genera frequently found in fermented foods are *Lactococcus, Lactobacillus, Leuconostoc, Streptococcus* and *Pediococcus* (Wang *et al*., 2021). Recently *Leuconostocaceae* and *Lactobacillaceae* have been merged into family *Lactobacillaceae* while the Lactobacillus genus was reclassified into 25 genera (Zheng *et al*., 2020; Wang *et al*., 2021).

Lactic acid bacteria that are beneficial and edible are called probiotics (Oladipo and Ogunleke, 2023), although not only LAB are found in this category, but LAB constitute the larger part of it. Lactic acid bacteria are highly beneficial to man in many ways as reported by Oladipo *et al.* (2013). This researched work focused on the isolation and molecular characterization of lactic acid bacteria isolated from West African cheese (*Wara*), yellow corn meal (*Ogi solo*) and white corn meal (*Ogi funfun*) collected from a local market within Ogbomosho metropolis.

MATERIALS AND METHODS

Sample Collections and preparation

Fermented yellow maize cereal porridge (locally called *Ogi solo*), fermented white maize cereal porridge (locally called *Ogi funfun*) and West African soft cheese (locally called *wara*) samples were collected from *Waso* market (8° 8' 31.7940" N and 4° 14' 42.6696" E) in Ogbomosho, Oyo State. The samples were collected in sterile screw cap bottle and conveyed to the laboratory in ice packs to retain the microbial integrity of the samples. One gram of the sample was aseptically added to 9 mL of sterile MRS (De-Man, Rogossa and Sharpe) broth, carefully shaken and were left to homogenize for 30 minutes. Ten-fold serial dilutions were prepared from the sample homogenate, and were streaked onto duplicate sterile petri dishes of MRS (De-Man, Rogossa and Sharpe). The streaked plates were incubated anaerobically at 30˚C for 2 - 3 days in an anaerobic incubator. The incubated isolate were checked after 72 hours for visible colonies and the colonies were further subjected to sub-culturing to acquire a pure culture. Fresh medium were prepared and sterilized at 121^0C for 15 minutes and allowed to cool and then pour aseptically in the sterilized petri dishes. The isolates inoculums were sub cultured on new plate by streaking in other to get a pure distinct colony. The plates were incubated at 37^0C for another 72 hours. The plates were observed for pure and distinct colony after 72 hours

Molecular Characterization of the Isolates

Fresh culture of the isolates was subjected to DNA extraction using the EZ-10 Spin Column Bacterial Genomic DNA Miniprep Kit (Bio Basic, Markham, Canada). The DNA integrity and concentration of the isolates were determined by taking optical density measurements using a Nano Drop spectrophotometer (Thermo Fisher Scientific, Waltham, USA) (Romero-Luna *et al*., 2020).

Amplification and Sequencing of 16S rRNA Gene

The amplification of the desired DNA template was carried out using PCR with universal primer sets: forward primer 3′-AGAGTTTGATCCTGGCTCAG-5′ and reverse primer 5′-GGCTGCTGGCACGTAGTTAG-3′. The thermal cycler was programmed with a pre-incubation period of 2 min at 94 °C and then 35 cycles, each consisting of denaturation for 30 s at 94 °C, annealing for 30 s at 55 °C, and elongation for 1 min at 72 °C. A final elongation of 10 min at 72 °C followed the 35 cycles. The amplicons were visualized through 1% agarose gel electrophoresis with reference to a 1 kbp DNA ladder. The PCR product was quantified using a Nano Drop spectrophotometer and purified using the QIAquick (Qiagen, Germantown, USA) purification kit (Romero-Luna *et al*., 2020).

RESULTS AND DISCUSSION

Isolation and Molecular Identification of the Isolates

The total *Lactobacillus* counts of the fermented yellow maize cereal porridge (*Ogi solo*), fermented white maize cereal porridge and West African soft cheese (*Wara*) samples used in the study (Figure 1) are shown in Table 1, it was observed that the highest colony count was observed on fermented yellow maize cereal porridge plate with colony forming unit of 11.0 x 10^6, while West African Soft cheese and fermented white maize cereal porridge had the lowest colony count of 6.0 x 10^6 respectively. The result is in agreement with the report of David *et al.* (2019) who reported the colony count within the range of 6.0 x 10^2 CFU/ml to 2.0 x 10^4CFU/ml in fermented foods. Also, Eji *et al.* (2023) reported the isolation of lactic acid bacteria from 20 locally fermented foods with CFU/g ranges from 2.0 to 2.5 x 10^4 in all the samples. According to Wang *et al*. (2021), lactic acid bacteria are regarded to be hetero-lactic fermentative because they are capable of fermenting carbohydrate to produce lactic acid and thus abundant and highly resourceful in fermentation, which lend credence to the presence of lactic acid bacteria in fermented foods used in this study.

The isolates were molecularly characterized and were identified using targeted unique sequence (1500 bp length) that were aligned using BioEdit sequence alignment editor version 7.2.5.0. The 16S rRNA gene sequences obtained showed 99%–100% similarity to reference strains in the NCBI database after the BLAST search, the isolates were identified as *Lactococcus lactis* FYM1, *Lactobacillus acidophilus* FYM2, *Lactobacillus paracasei* WAC1, *Lactobacillus delbruecki* WAC2, and *Lactobacillus iners* FWM (Table 2), the gel (1500bp) picture of the amplicons are illustrated in Figure 2. After the BLAST search in NCBI data base, it was observed that *Lactococcus lactis* FYM1 has 99% homology with *L. lactis* IPLA1064 (CP184693) isolated from raw milk cheese in Denmark (Lopez-Gonzales *et al*., 2025). *Lactobacillus acidohilus* FYM2 has 9.15% homology with *L. acidophilus* ATCC 4356 (CP139575) isolated from sour milk in Bioscience, Los Alamos National Laboratory, USA (Kumar, 2023). *Lactobacillus paracasei* WAC1 has 100% homology with *L. paracasei* 77FHE (MT383743) isolated from raw cow milk sample in Faculty Of Women For Arts, Science and Education, Asmaa Fahmy, Naser City 11757, Egypt (Abo-Safe *et al*., 2020). *Lactobacillus delbrueckii* WAC2 has 99.5% homology with *Lactobacillus delbrueckii* A281 (MN437943) isolated from diary product in College of Food Science and Nutritional Engineering, China Agricultural University (Ren, 2019). *Lactobacillus iners* FWM has 99% homology with *L. iners* C025C1 (CP049231) isolated from human vagina in Institute for Genome Sciences, University of Maryland Baltimore, USA (France *et al*., 2020).

The isolates: *Lactococcus lactis* FYM1, *Lactobacillus acidophilus* FYM2, *Lactobacillus paracasei* WAC1, *Lactobacillus delbruecki* WAC2, and *Lactobacillus iners* FWM were registered in NCBI Genbank and were given accession numbers PQ758598, PQ555634, PQ346814, PQ555635 and PQ555636 respectively. Based on molecular characterization of the isolates, the result obtained highlighted *Lactobacillus* sp as the highest occurring organisms and this is in agreement with the report of Eji *et al*. (2023) who also reported the isolation and characterization of *Lactobacillus plantarum, Lactobacillus acidiophilus, Lactobacillus fermentum* and *Lactobacillus casei* from traditionally fermented food such as *ogi*, and soy milk. Furthermore, Hamad *et al*. (2022) and Latif *et al*. (2023) reported the isolation and molecular identification of *Lactobacillus* sp. from milk based fermented foods. Oladipo *et al.* (2025b) also reported the isolation and molecular identification of *Lacticaseibacillus casei, Lactobacillus delbrueckii, Lactobacillus johnsonii, Lactobacillus brevis* and *Lactobacillus jensenii* from fermented African oil bean seeds, fermented *Citrullus colocynthis* seeds, fermented maize cereal porridge, and West African soft cheese respectively.

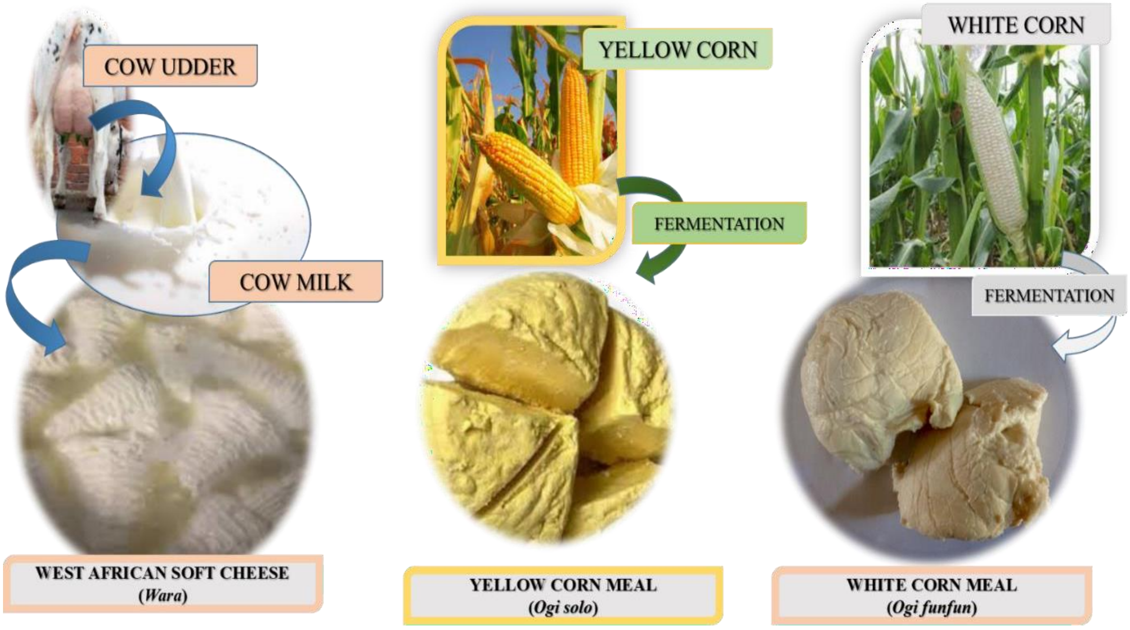

Figure 1: Fermented Foods Used in the Study

Table 1: The Microbial Load of the Samples

Samples	**Total Lactic Acid Bacteria Count CFU/g**
West African Soft Cheese	6.0×10^6
fermented white maize cereal porridge	6.0×10^6
fermented yellow maize cereal porridge	11.0×10^6

Key: CFU/ml: colony forming unit per gram.

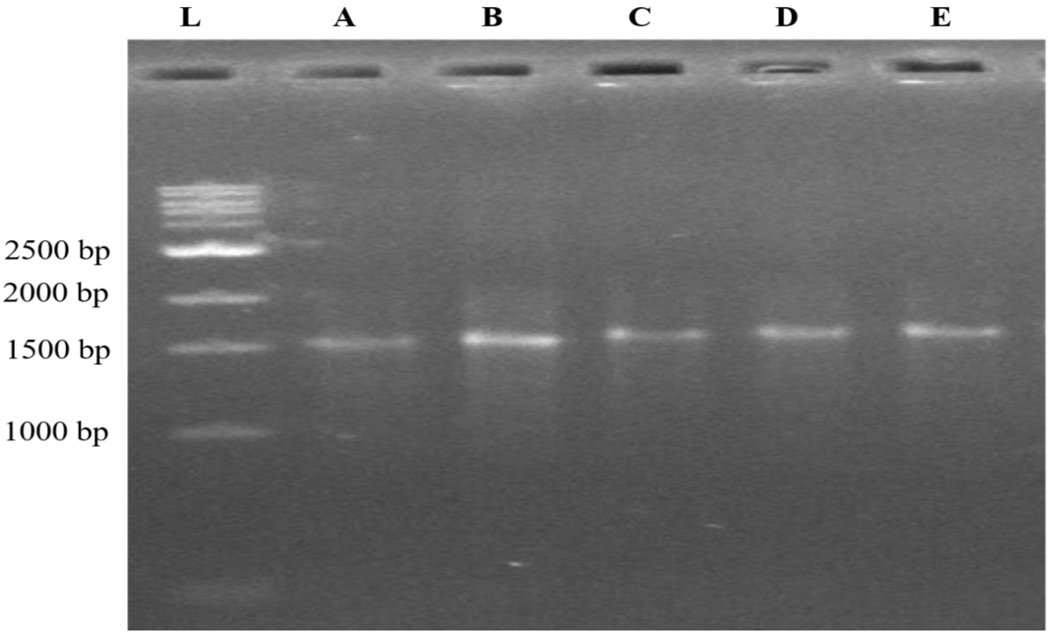

Figure 1: Polymerase chain reaction amplification of the isolates DNA

Key: A: FYMI, B: FYM2, C: WAC1, D: WAC2, E: FWM. FYMI: fermented yellow maize cereal porridge, FWM: fermented white maize cereal porridge, WAC: West African soft cheese.

Table 2: Molecular Identity and Accession Numbers of the Isolates

Code	Sources	Identity	Accession Number
FYM1	fermented yellow maize cereal porridge	Lactococcus lactis	PQ758598
FYM2	fermented yellow maize cereal porridge	Lactobacillus acidophilus	PQ555634
WAC1	West African soft cheese	Lactobacillus paracasei	PQ346814
WAC2	West African soft cheese	Lactobacillus delbrueckii	PQ555635
FWM	fermented white maize cereal porridge	Lactobacillus iners	PQ555636

Key: FYM1 and FYM2: isolates from fermented yellow maize cereal porridge, FWM: isolate from fermented white maize cereal porridge, WAC and WAC2: isolates from West African soft cheese.

The phylogenetic relationship among the isolates is shown in Figure 3; the isolates principally cluster with the *Lactobacillaceae* family, related to *Lactobacilus delbrueckii* (AB008207.1), *Lactobacillus acidophilus* (AB680529), *Lactobacilus iners* (NR036982.1), *Lactobacillus paracasei* (LC638743.1), *Lactococcus lactis* (NR113960). The isolates from the fermented food samples form a well-supported clade that is distinct but closely related to known reference strains obtained from NCBI. According to the phylogenic analysis, FYM1 showed 99% homology with *Lactococcus lactis* NBRC strain, FYM2 showed 99.6% homology with *Lactobacillus acidophilus* NBRC 13951 strain (Igbal *et al*., 2009), WAC1 showed 99% homology with *Lactobacillus paracasei* R094 strain (Mulaw *et al*., 2019), WAC2 showed 99% homology with *Lactobacillus delbrueckii* YIT 0080 strain (Igbal *et al*., 2009) and FWM showed 99% homology with *Lactobacillus iners* DSM 13335 (Mulaw *et al*., 2019). Previous studies have highlighted the significance of fermented food as probable vehicles that play essential role in the continuous isolation of Lactic acid bacteria due to their capacity to not only generate tremendously elevated levels of lactic acid but also thrive consistently in extreme acidic state (David *et al*., 2019).

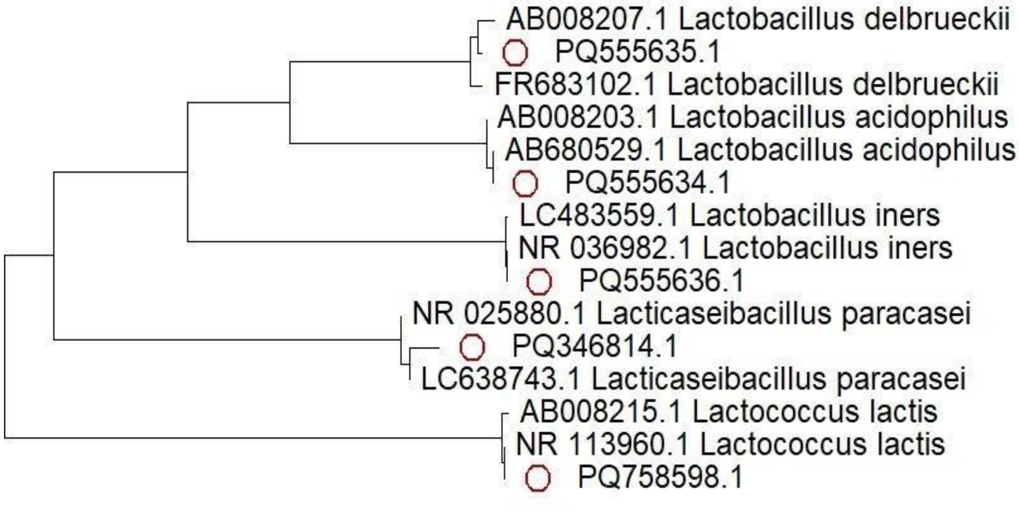

Figure 2: The phylogenetic tree showing the relationship between the isolates

CONCLUSION

The isolation and molecular characterization of 5 different LAB species from 3 Nigerian fermented foods commonly consumed were achieved. The characterized isolates, which have been deposited in NCBI GenBank, constitute valuable genetic resources demonstrating the immense microbial biodiversity existing in traditional

Nigerian fermented foods. The results demonstrate the importance of indigenous fermented foods as sources of beneficial microorganisms and therefore form the basis for further benefits from their therapeutic and commercial exploitation. This work contributes to food security through the isolation and molecular identification of probiotic lactic acid bacteria from locally fermented foods, which are crucial for nutritional enhancement, and shelf life extension. Understanding these LAB can help improve food availability and safety thus addressing SDG 2 (Zero hunger). The study also contributes to SDG 3 (Good health and well-being) as probiotic lactic acid bacteria are known for their health benefits which include antimicrobial properties, digestive health, and immune system support. Isolating and characterizing local strains of LAB could lead to innovative health-promoting applications.

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