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# Molecular Identification And Probiotic Properties Of Lactic Acid Bacteria Isolated From Selected Nigerian Fermented Food Products

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***Abstract***
*Lactic Acid Bacteria (Lab) Are The Foremost Industrial Prerequisite For The Sustainable Production Of Wide-Ranging Dairy Foods, Probiotic Centered Foods, Processed Functional Foods, And Fermented Foods Across The Globe. Currently, Their Application As Health-Promoting Microorganisms Makes It More Vivacious As Compared To Other Microbes. Many Food Industries Are Cognizant Of The Fact That The Diet Preferences Among The World Community Have Been Shifting Towards Products With Desirable Health Aid. Hence This Study Aims At Isolating, Screening And Molecular Identification Of The Lactic Acid Bacteria That Could Serve As A Potential Probiotics. Isolation Of Lactic Acid Bacteria Was Carried Out Using Standards Method After Which The Selected Isolates Were Molecularly Identified And Screened For Probiotic Characteristics. A Total Of Fifty Potential Lactic Acid Bacteria Were Isolated However Four Of The Isolates Were Selected For Further Studies. The Isolates Were Identified As Lactobacillus Casei, Lactobacillus Fermentum, Lactobacillus Plantarum And Lactobacillus Rhamnosus. Lactobacillus Casei Had The Highest Frequency Of Occurrence With 42%, Lactobacillus Plantarum Had The Frequency Of Occurrence Of 30%, Lactobacillus Fermentum Had 18% While Lactobacillus Rhamnosus 10%. All The Selected Isolates Survived At Ph 2.0 And Ph 3.0, Tolerated Bile At 0.2 And 0.4%, Survived At Sodium Chloride At Concenteration 0f 1% To 5% And All Isolates Were Found To Utilized Lactose.*

***Key Words:*** *Lactic Acid Bacteria, Lactose, Bile*

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## I. Introduction

Since antiquity, microorganisms have represented, and still are, an indispensable part of human nutrition with high levels of their consumption via naturally microbial fermented products with massive amounts of viable beneficial microbes, such as fermented fruits, their juices, fermented animal products, and other food products of diverse origins. In history, humans, unconsciously, and without even having a clue about the existence of the microbial world, have used microbes in order to initiate numerous food processes. Nowadays, a massive assortment of fermented food products and liquids probiotics are the health promoting viable microorganisms that exhibit a beneficial effect on the health of human being by improving the intestinal microbial balance.

Probiotics are living microorganism that stimulates the health of the host by maintaining the intestinal microflora. (Asmita and Koirala, 2019).

Lactic acid bacteria (LAB) are a group of Gram positive, non-spore forming, cocci or rods which produce lactic acid as major end product from fermentation of carbohydrates. Majority of microorganisms used as probiotics belong to the LAB and bifidobacteria. Within the group of LAB, *Lactobacillus* species are most commonly utilized group of microorganisms for their potential beneficiary properties as probiotics. The antagonistic activity of such bacteria is known to inhibit a large number of enteric and urinary pathogenic bacteria. Lactic acid bacteria including *Lactobacillus*, *Leuconostoc*, *Lactococcus*, *Pediococcus* and *Bifidobacterium* are found throughout the gastrointestinal tract. *Lactobacillus* and *Bifidobacterium* spp. are prominent members of the intestinal flora and are the commonly studied probiotics bacteria (Ram Kumar *et al*., 2013).

Probiotic bacteria can inhibit pathogens by the production of antagonistic compounds/by competitive exclusion (competition for nutrients attachment sites). Probiotic bacteria directly take up or decompose the organic matter and improve the water quality of an aquatic ecosystem. Beneficial microbial cultures produce a variety of exoenzymes such as amylase, protease, and lipase, which help to degrade the unconsumed feed feces in the pond, in addition to the possible role of these enzymes in the nutrition of the animals by improving feed digestibility and feed utilization. Among all the microbial interventions to augment the production, use of probiotics is in the central dogma. The modes of action of probiotics include the inhibition of a pathogen through the production of bacteriocin-like compounds, competition for attachment sites, competition for nutrients (particularly iron in

marine microbes), alteration of the enzymatic activity of pathogens, immunostimulatory functions, and nutritional benefits such as improving feed digestibility and utilization ( Vijayaram and Kannan, 2018).
Hence this study aims at isolating and screening lactic acid bacteria of probiotic potential

## II. Materials and methods

**Sample collection**

Yoghurt, Wara, Nunu, Kunu, Ogiri, Ogi, Palmwine,Fura And Pito (Nigeria fermeted drinks) Were Purchased From Bodija and Sango Markets In Ibadan Oyo State Nigeria.

**Isolation of Lactic Acid Bacteria:** One ml (1ml) of liquid samples and one gram (1g) of solid sample were added to 9ml of sterile distilled water, subsequently a serial dilution of the isolates was prepared up to dilution five. An aliquot (0.1Ml) of the dilution were spread plated on de Mann, Rogosa, and Sharp (MRS) agar plates and then incubated anaerobically at 37ºC for 24 hours colonies were isolated sub cultured and purified by repeated streaking. Further, the isolated bacterium was subjected to biochemical characterizations. Bacterial morphology was examined using Microscope. The strain was kept in MRS agar slant at 4°C for further analysis.

**DNA extraction and gene amplification**. The chromosomal DNA was extracted from freshly grown bacterial culture following the phenol-chloroform extraction protocol. The 16S rRNA genes were amplified. The reaction was carried out for 35 cycles with the following standard PCR cycle parameters: DNA Pre-heating at 94ºC for 3min; denaturation at 94ºC 1min for 35 cycles, annealing at 55ºC for 45s, 72ºC for 1min of primer extension, and elongation step at 72ºC for 10 min. To analyze PCR products, 2 µl of reaction mix (10 mm TE buffer and 2 µl) was electrophoresed at 120v for about sixty minutes to get the existing DNA fragments. Latterly, the purity analysis of DNA was conducted comparing the A260/280 ratio.

**Sequence data analysis and phylogenetic study**: The amplified rRNA sequence was translated using the Sangers' Sequencing technique. The sequence was processed for quality analysis, subsequently submitted to the Gene Bank database. Post-submission, the sequence was tallied with the available nucleotides from the NCBI-database using the blast algorithm.

**Evaluation of probiotic potentials of isolated bacterial cultures**

Bacterial cultures were selected for further determination of probiotic potentials

**pH tolerance**

The isolated bacterial cultures were inoculated into sterile MRS broth tubes of varying pH, of 2,and 3, and incubated at $37^0$C for 2-3 days. Then 0.1ml inoculums from each tube was poured to MRS agar medium by pour plate method and incubated at $37^0$C for 48hrs. The growth of LAB on MRS agar was used to designate isolates as pH tolerant (Tambekar and Bhutada, 2010).

**Bile salt tolerance**: The medium with varying concentrations of bile salt (0.2% and 0.4.0%) was inoculated with each selected bacterial culture and incubated at $37^0$C for 48hrs. Then 0.1ml inoculums was transferred to MRS agar by pour plate method and incubated at $37^0$C for 48hrs. The growth of LAB cultures on agar plates was used to designate isolates as bile salt tolerant (Tambekar and Bhutada, 2010).

**Lactose utilization:** The acid production by selected bacterial cultures was detected by observing the change in colour of the medium. Sterilized fermentation medium (10g peptone, NaCl 15g, phenol red 0.018g, lactose 5g, for 1L distilled water and final pH 7.0) was inoculated with different cultures and incubated at $35^0$C for 24-48 hrs. Change in colour from red to yellow indicates the production of acid (Ahmed and Kanwal, 2004).

**NaCl tolerance:** Salt tolerance of selected bacterial cultures was assessed after 3days of incubation at concentration of 1-5% NaCl in MRS broth (Adebayo-tayo and Onilude, 2008).

## III. RESULTS AND DISCUSSION

A total of fifty lactic acid bacteria strains identified as *Lactobacillus casei*, *Lactobacillus fermentum*, *Lactobacillus plantarum* and *Lactobacillus rhamnosus* were isolated from the food samples. Table 1 shows the bacteria distribution in the samples. Figure 1.0 shows the percentage frequency of occurrence of the lactic acid bacteria strains.

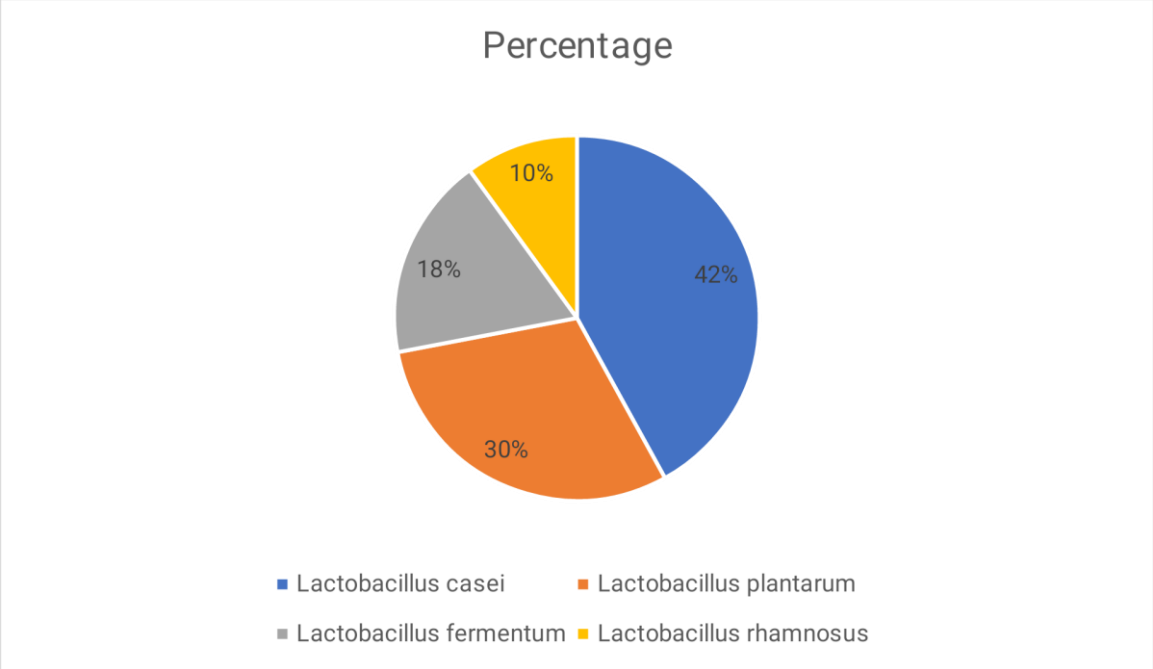


**Figure 1.0: Frequency of occurrence of LAB strains from the samples**

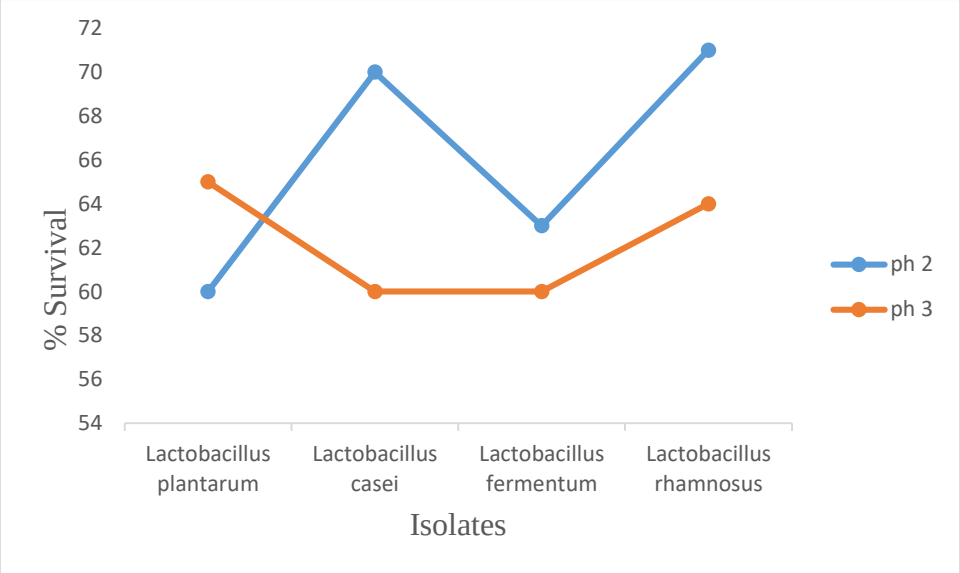


**Figure 2.0**: **The survival percentage of lactobacilli isolates in pH 2 and 3**

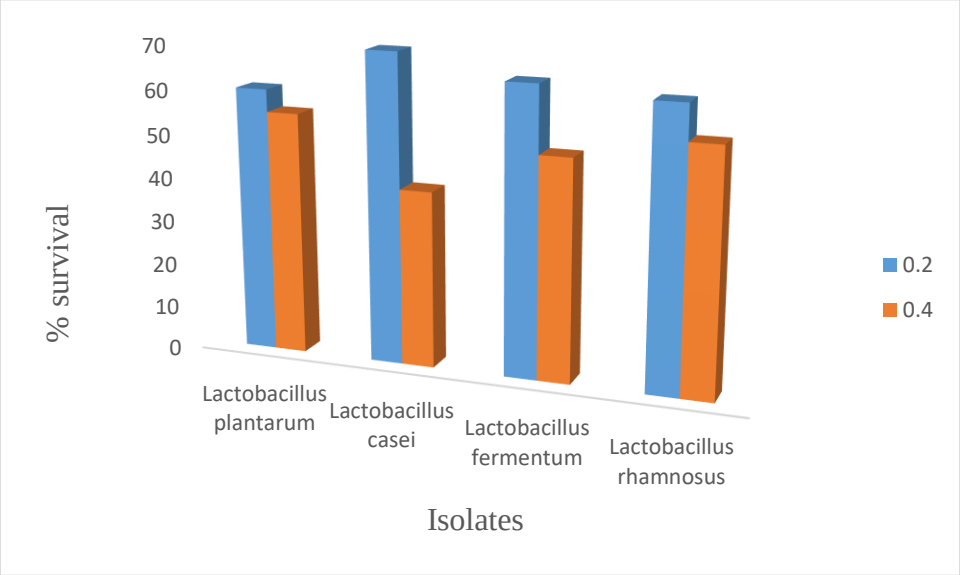


**Figure 3.0**: **Survival percentage of lactobacilli isolates in 0.2 and 0.4% bile salt concentrations**

**Table 2: Sodium chloride tolerance among the selected isolates**

| Isolates | 1% | 2% | 3% | 4% | 5% |
|---|---|---|---|---|---|
| Lactoacillus plantarum | + | + | + | + | + |
| Lactoacillus fermentum | + | + | + | + | + |
| Lactoacillus casei | + | + | + | + | + |
| Lactoacillus rhamnosus | + | + | + | + | + |

**Table 3:**

| Isolate | Acid production | Gas production |
|---|---|---|
| Lactoacillus plantarum | + | + |
| Lactoacillus fermentum | + | + |
| Lactoacillus casei | + | + |
| Lactoacillus rhamnosus | + | + |

## IV. Isolation and identification

Lactic acid plays an important role in the food, pharmaceutical, chemical, feed and cosmetic industries. Currently, reported sources of lactic acid bacteria are extensive, in-cluding plants, animals and the environment (Guanghui *et al*., 2022).

A total of fifty (50) bacterial isolates were isolated, screened and were found belonging to the genus *Lactobacillus*. The isolates were found to be *Lactobacillus casei*, *Lactobacillus fermentum*, *Lactobacillus plantarum* and *Lactobacillus rhamnosus*. *L. casei* had the highest frequency of occurrence of 42%, *L. plantarum* had a frequency of occurrence of 30%, *L. fermentum* had 18% and *L. rhamnosus* had the least frequency of occurrence of 10% as shown in figure 1.0

Table 1.0 shows the morphological and cultural characteristics of the isolates while table 2 shows the distribution of the isolates in the samples while table 3 shows the molecular identification of the isolates.

**Table 1.0: Morphological Characteristics of the Lactic Acid Bacteria Isolates**

| Isolate code | Cultural characteristics | Morphological Characteristics | Gram staining | Catalase | Endospore | motility |
|---|---|---|---|---|---|---|
| YOG 1 | | Rod | + | - | - | - |
| YOG 2 | | Rod | + | - | - | - |
| YOG 3 | | Rod | + | - | - | - |
| YOG 4 | | Cocci | + | - | - | - |
| YOG 5 | | Rod | + | - | - | - |
| YOG 6 | | Cocci | + | - | - | - |
| YOG 7 | | Cocci | + | - | - | - |
| WAR 1 | | Cocci | + | - | - | - |
| WAR 2 | | Rod | + | - | - | - |
| WAR 3 | | Rod | + | - | - | - |

| | | | | | | |
|---|---|---|---|---|---|---|
| WAR 4 | | Rod | + | - | - | - |
| WAR 5 | | Rod | + | - | - | - |
| WAR 6 | | Rod | + | - | - | - |
| WAR 7 | | Rod | + | - | - | - |
| WAR 8 | | Cocci | + | - | - | - |
| NUN 1 | | Cocci | + | - | - | - |
| NUN 2 | | Rod | + | - | - | - |
| NUN 3 | | Rod | + | - | - | - |
| NUN 4 | | Rod | + | - | - | - |
| NUN 5 | | Cocci | + | - | - | - |
| NUN 6 | | Rod | + | - | - | - |
| NUN 7 | | Rod | + | - | - | - |
| OGIR 1 | | Cocci | + | - | - | - |
| OGIR2 | | Rod | + | - | - | - |
| OGIR3 | | Rod | + | - | - | - |
| OGI 1 | | Cocci | + | - | - | - |
| OGI 2 | | Rod | + | - | - | - |
| OGI 3 | | Rod | + | - | - | - |
| OGI 4 | | Cocci | + | - | - | - |
| OGI 5 | | Rod | + | - | - | - |
| OGI 6 | | Rod | + | - | - | - |
| PAW 1 | | Cocci | + | - | - | - |
| PAW 2 | | Rod | + | - | - | - |
| PAW 3 | | Rod | + | - | - | - |
| PAW 4 | | Rod | + | - | - | - |
| PAW 5 | | Cocci | + | - | - | - |
| PAW 6 | | Rod | + | - | - | - |
| FUR 1 | | Rod | + | - | - | - |
| FUR 2 | | Cocci | + | - | - | - |
| FUR 3 | | Rod | + | - | - | - |
| FUR 4 | | Rod | + | - | - | - |
| FUR 5 | | Rod | + | - | - | - |
| FUR 6 | | Cocci | + | - | - | - |
| PIT 1 | | Rod | + | - | - | - |
| PIT 2 | | Rod | + | - | - | - |
| PIT 3 | | Cocci | + | - | - | - |
| PIT 4 | | Rod | + | - | - | - |
| PIT 5 | | Rod | + | - | - | - |
| PIT 6 | | Cocci | + | - | - | - |
| PIT 7 | | Cocci | + | - | - | - |

**Ph**

Probiotic LAB candidates should be capable of withstanding the extreme conditions in the digestive tract, from the mouth to the intestines, and should be able to subsequently colonize the intestinal surface. Gastric acidity serves as a precondition prior to conducting microbial selection before entering the intestines. The acid resistance of LAB is of great importance not only for their own growth but also for the fermentation and preparation of probiotic products. Several mechanisms are involved in the acid resistance regulation of LAB, including central metabolic pathways, proton pumps, changes in cell membrane composition and cell density, DNA and protein damage (Harnentis *et al*., 2020).

The transit through the acidic surroundings of the stomach represents a primary defense mechanism that all ingested microorganisms must deal with, including pathogens and beneficial probiotics. According to FAO/WHO (2002), the potential probiotic microorganism must be alive to confer a health benefit on the host. Generally, the acid resistance of isolates are strain- and species-dependent with general decrement below pH 3 (Ilyanie *et al.,* 2021).Before reaching the intestinal tract, probiotic bacteria must pass through acidic stomach condition where the pH can be as low as 1.5 during fasting and rises to pH 3 or even higher after a meal (Adesulu *et al*., 2018).

The pH factor directly affects the bacterial growth. Probiotic bacteria are mostly delivered in a food system so these must have to tolerate acid in order to survive in the human gastrointestinal tract.

In this preset work LAB isolates survived well at pH 2 and 3 with a percentage survival ranging from 60 to 70%. In a similar study Adesulu *et al*., 2018 investigated lactic acid bacteria strains showing more than 65% survival rate at pH 2 2.5. ).

**Bile salt**

The ability of a potential probiotic strain to tolerate or withstand intestinal bile salt is of immense importance to their survival and growth in the GIT; thus, it is a major requirement for probiotic selection. In the

poultry GIT, the duodenum and cecum have a total bile salt concentration of 0.175 and 0.008% and the bile salt concentration for humans' ranges from 0.14 to 0.93 mM . However, the average level of 0.3% bile salt has been considered in many studies as the threshold for bile salt tolerance of a potential probiotic (Harnentis *et al*., 2020). Tolerance to bile salts is a prerequisite for colonization and metabolic activity of bacteria in the small intestine of the host (Havenaar et al., 1992). This will help Lactobacilli to reach the small intestine and colon and contribute in balancing the intestinal microflora (Tambekar and Bhutada, 2010). Resistance of bacteria to the intestinal bile salts is also an important factor to be considered during probiotic selection. In this present work all the LAB isolates survived at 0.2 and 0.4% bile concentration at varying survival rates.

According to Bustos *et al*., (2018), LAB are able to survive in bile salts because they contain bile salt hydrolase (BSH), an enzyme which is active in the form of bile acids. Bacterial membranes are the main targets of bile acids. For bacteria to survive bile salts, they produce BSH by conjugation into free bile acids. Free bile acids can participate in a variety of metabolic processes, including the regulation of fat absorption; cholesterol metabolism; the creation of homeostatic conditions in the bacterial membrane; and regulating nitrogenous bases, fats, and amino acid biosynthesis, which allow changes in fat, resulting in exopolysaccharide (EPS) production.

**Tolerance to sodium chloride**

All the selected isolates tolerated sodium chloride concenteration ranging from 1 to 5%. Tolerance against sodium chloride is a property for a probiotic organism to survive in extreme Gastrointestinal tract (GI) tract conditions. The high sodium chloride concentration provides an advantage to LAB as compared to the less tolerant bacteria. Since, it promotes the LAB to begin metabolism, which leads to acid production and further inhibits the growth of nondesirable bacteria (Vikas *et al*., 2022).

Probiotic organism have to stand in a high salt concentration in a human gut. Vikes *et al.,* 2022 reported lab isolates surviving at 7% salt concentration.

## V. Conclusion

The isolated Lactobacillus spp. from the samples fulfills some of the required criteria of a probiotics. These isolated strains were able to survive in a favourable environment of human gastrointestinal tract such as high salt, low pH and high bile concentration. Therefore, these Lactobacillus isolates show their potential to be used as probiotic. This spp. is hence a good candidate for further investigation under in vitro as well as in vivo conditions to elucidate their potential beneficial health effects and their possible application as novel probiotic species in the food industry

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