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Characterization and Probiotic Potential of Lactic Acid Bacilli Isolated from Gari and Palm Wine in Ekpoma, Nigeria

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Abstract: **Traditionally fermented foods play an essential role in the diets of many cultures, often offering health benefits beyond basic nutrition. Gari and Palm wine, two staples in West African cuisine, are rich in lactic acid bacteria (LAB), which are known to contribute to both fermentation processes and potential health benefits. This study focuses on the isolation and characterization of LAB from these fermented products to evaluate their probiotic potential. LAB cultures were grown on MRS agar under both aerobic and anaerobic conditions, with growth observed at 24 and 48 hours, in line with similar studies (Arjun et al., 2012; Amoa-Awa et al., 2007; Nwachukwu et al., 2016). Some variations in incubation times across studies were noted, possibly due to differences in sample preparation and LAB strains used (Ramdass et al., 2014; Olaoluwa et al., 2013). The isolated LAB, identified as Gram-positive, catalase- and oxidase-negative cocci and bacilli, were consistent with previously documented LAB characteristics (Arjun et al., 2012; Amoa-Awa et al., 2007; Nwachukwu et al., 2016). In addition, these LAB showed antimicrobial activity against pathogens such as Staphylococcus aureus and Escherichia coli, supporting findings from prior research (Guo et al., 2009; Dunne et al., 2001). The LAB's survival in acidic environments was confirmed, as they remained viable in diluted hydrochloric acid. These findings emphasize LAB's role in traditional fermentation and their potential health benefits, underscoring the need for further research into the probiotic properties of LAB in Gari and Palm wine. It is recommended to encourage public education on the probiotic benefits of LAB in traditionally fermented foods and to enhance support for large-scale LAB production for health applications.**

Keywords: **lactic acid bacteria, probiotic potential, Gari, Palm wine, antimicrobial properties, fermentation.**

1. INTRODUCTION

1.1 INTRODUCTION

Elie Metchnikoff first suggested the possibility of colonizing the gut with beneficial flora in the early 20th century; since then, microorganisms with probiotic potentials have been marketed and consumed with intended health benefits (Ramdass *et al*., 2014).

World Health Organization (WHO) defines probiotic as "live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host". The word probiotic appears to be a composite of the Latin preposition pro ("for") and the Greek adjective βιωτικός (biōtikos), "fit for life" (Hatis, 2007). Though the WHO defines the probiotic as microorganism with the ability to confer health benefits, some school of thoughts such as the European Food Safety Authority however have a contrary opinion, as they believe that their benefit to health has not been sufficiently scientifically proven (Mohammed *et al*., 2015).

There are numerous claimed benefits of using commercial probiotics, such as reducing gastrointestinal discomfort, improving immune health, relieving constipation, biopreservation of food or avoiding the common cold. Probiotics are generally safe, but they may cause bacteria-host interactions and unwanted side effects in rare cases (Emiliano *et al*., 2014).

There are numerous microorganisms with proven ability to act as probiotic, among these are the Lactic acid Bacteria (LAB). Lactic Acid Bacteria (LAB) constitute a diverse group of microorganisms associated with plants, meat, and dairy.

The LAB have been characterized primarily by their ability to form various isomers of lactic acid from the fermentation of glucose (Mahdieh *et al*., 2012).

In general, the LAB may be characterized as Gram-positive, aerobic to facultatively anaerobic, asporogenous rods and cocci which are oxidase, catalase, and benzidine negative, lack cytochromes, do not reduce nitrates to nitrite, are gelatinase negative, and are unable to utilize lactate (Anupama and Nivedita, 2017).

The monograph published by Orla-Jensen is the base of the present classification of lactic acid bacteria (LAB) using the following criteria: cellular morphology, mode of glucose fermentation, range of growth temperature, and sugar utilization patterns. Four genera were recognized as LAB: *Lactobacillus sp*, *Leuconostoc sp*, *Pediococcus sp*, and *Streptococcus sp* (Emiliano *et al*., 2014).

The LAB can be isolated from several sources, including traditionally fermented food such as Gari and drinks such as Palm wine. These food and drinks are obtained from the natural process of microfloral fermentation (Nwachucku *et al*., 2016).

This fermentation process or technology is known and used by many people as one of the oldest forms of food conservation and preservation in different parts of the world, with lactic microflora playing an important role in the preparation of these traditional food and drink.

Gari is a common staple in West Africa, which is produced from the fermentation of Cassava, a plant first introduced into Africa from America by the Portuguese in 1558 (Onoja, 2014).

Palm wine on the other hand, is an important alcoholic beverage resulting from the spontaneous fermentation of the sap of the palm, which has been attributed to yeast and bacteria. It consists mainly of water, sugar, vitamins and many aroma and flavor components in very small amount (Nwachukwu *et al*., 2016).

The concept of probiotic microbes that Metchnikoff introduced has led to the widespread consumption of food preparations containing LAB and/or bifidobacteria, with the expectation that they will confer health benefits including reduction of cholesterol levels, improvement of immune function, resistance to infectious diseases, and prevention of colon cancer (Nwachukwu *et al*., 2016).

Fermented food and drink containing LAB are traditionally used everyday in many cultures. Although some of these food and drink are believed to have probiotic potentials, only few studies have however documented the probiotic potential of LAB isolated from them, and even fewer documentation exist on the probiotic potential of LAB isolated from Gari and Palm wine which are almost ubiquitous to every home in Western Africa.

This study is therefore undertaken to evaluate the probiotic potential of LAB isolated from Gari and Palm wine.

1.2 AIM

This study is aimed to isolate and characterize the LAB in traditionally fermented Gari and Palm wine and assess for their probiotic potential.

1.3 OBJECTIVES

1. Characterization of the LAB in Gari.

2. Characterization of the LAB in Palm wine.

3. Assessment of the probiotic potential of LAB isolated from Gari.

4. Assessment of the probiotic potential of LAB isolated from Palm wine.

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1.4 LITERATURE REVIEW

Probiotics are defined as "living micro-organisms, which upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition" (Guarner and Schaafsma, 1998; Tannock, 2002) but interest in this area was initiated by Metschnikov 100 years ago (Metschnikoff, 1907). Most probiotic microorganisms belong to Lactic Acid Bacteria (LAB), such as *Lactobacillus sp*, *Bifidobacterium sp* and *Enterococcus sp* (Klein *et al*., 1998). The yeast *Saccharomyces boulardii* has been studied extensively (Elmer *et al*., 1999) and other bacterial species, like *Bacillus sp* (Senesi *et al*., 2001) and *Clostridium butyricum* (Takahashi et al., 2004) have also been studied for their probiotic potential. In some countries, the use of *Enterococcus sp* as a probiotic has been questioned because of safety aspects regarding transfer of genes conferring antibiotic resistance (Lund and Edlund, 2001).

Most scientists agree that probiotic strains shall be able to survive transit through the gastric acid environment as well as exposure to bile and pancreatic juice in the upper small intestine to be able to exert beneficial effects in the lower small intestine and the colon, although there are convincing data on beneficial immunological effects also from dead cells (Mottet and Michetti, 2005).

Best effect is achieved if the microorganisms colonize the intestinal surface mucus layer since they then can affect the intestinal immune system, displace enteric pathogens, provide antioxidants and antimutagens, and possibly other effects by cell signaling. That intake of LAB influences multiple systems was elegantly shown for Lactobacillus using microarray analysis (Di Caro *et al*., 2005). One month treatment resulted in up-regulation of 334 genes and down regulation of 92 genes involved in inflammation, apoptosis, cell-cell signaling, cell adhesion and differentiation and signal transcription and transduction (Di Caro *et al*., 2005).

In recent years, multiple reports have described beneficial effects from various aspects on important diseases, like intestinal infections, inflammatory bowel disease (IBD), and allergy by addition of selected strains to food products, often together with fiber or a prebiotic substance. In many countries, there are now several probiotic products on the market, but the documentation is often based upon case reports, animal studies or uncontrolled small clinical trials. Furthermore, there is no general acceptance on how to characterize probiotic microorganisms, and few products declare the actual content of live microorganisms (Asa, 2016).

Metchnikoff was the first to associate lactic acid bacteria (LAB) in fermented yogurt with health and longevity of certain Balkan communities. The concept of probiotic microbes that he introduced has led to the widespread consumption of food preparations containing LAB and/or bifidobacteria, with the expectation that they will confer health benefits (Ramadass *et al*., 2012).

The term 'probiotic' was however firstly used in 1965 by Lilly and Stillwell to describe substances which stimulate the growth of other microorganisms. After this year the word 'probiotic' was used in different meaning according to its mechanism and the effects on human health. The meaning was improved to the closest one we use today by Parker in 1974. Parker defined 'probiotic' as 'substances and organisms which contribute to intestinal microbial balance'. In 1989, the meaning we use today was improved by Fuller. Then this definition was broadened by Havenaar and Huis Veld in 1992 to include mono or mixed culture of live microorganisms which applied to both animal and man (Hatis, 2007)

1.4.1 LACTIC ACID BACTERIA

Lactic acid bacteria (LAB) are well known as commensal in gastrointestinal tract of any animal, and are typical probiotic bacteria utilized in various types of fermented foods. For more than ten thousand years, LAB have contributed to improve flavor, preservation, and nutrition of fermented foods and drink. Moreover, it is indicated that these fermented foods promote health through modulation of multi-dimensional biological mechanisms, such as immunological and neurological function (Tomonori *et al*., 2016).

A typical lactic acid bacterium grown under standard conditions is gram-positive, nonsporing, catalase negative in the absence of porphorinoids, aerotolerant, acid tolerant, organotrophic, and a strictly fermentative rod or coccus, producing lactic acid as a major end product. It lacks cytochromes and is unable to synthesize porphyrins. Lactic acid bacteria are characterized by the production of lactic acid as a major catabolic end product from glucose fermentation. They are grouped in one order and six families. From the 32 described genera, only 22 species belonging to five genera have been isolated from food and wine. The homofermentative species produce lactic acid (<85%) as the sole end product, while the

heterofermentative species produce lactic acid, CO_2 and ethanol/acetate. At least half of the end product carbon is lactate (Helmut and Jurgen, 2009).

The monograph published by Orla-Jensen is the base of the present classification of lactic acid bacteria (LAB) using the following criteria: cellular morphology, mode of glucose fermentation, range of growth temperature, and sugar utilization patterns. Four genera were recognized as LAB: *Lactobacillus sp*, *Leuconostoc sp*, *Pediococcus sp*, and *Streptococcus sp* (Briggs *et al.*, 1953).

1.4.2 ISOLATION OF LAB

LAB have been isolated from various sources, including Gari, and Palm wine, among others. Quite a few Agars have been used for this purpose, as there is no single Agar that can isolate all the strains of the LAB. Different studies have employed different methods in the isolation of LAB.

In an Indian study, isolation of LAB and their identification was carried out in the Department of Gastrointestinal Sciences, Vellore, Tamil Nadu, India between 2008 and 2010. Samples of freshly made curd were obtained from 16 households within a 50 km radius of Vellore and transported immediately to the laboratory. The households chosen belonged to laboratory researchers who made curd each day at home using a small portion of curd to inoculate milk which was then left covered overnight at room temperature. Curd samples were transported to the laboratory in the morning and were plated on MRS agar and incubated overnight at 37°C. Microbial colonies that grew out in culture were identified by Gram stain, catalase test and biochemical characterization. Strains identified were subjected to growth curve analysis to determine the period during which exponential growth was seen. LAB were cultured on Lactobacillus MRS broth for 16 h at 37°C with 10 percent CO_2 under anaerobic conditions and used for testing (Ramdass *et al*., 2014).

In a 2011 Taramani study, sample of 1 mL was taken from several serial dilutions and poured into separate petri plates. Nutrient agar (20 mL) was poured into 6 petri plates. It was then solidified and kept in incubator at 37ºC for 48 h in inverted position. Similarly, 20 mL of MacConkey agar was solidified and kept for incubation at 37ºC for 48 h in inverted position. Appropriate serial dilutions of the blended mixture were plated onto PCA (Plate count agar) and MRS (de Man Rogosa and Sharpe) agar and incubated at 37°C for 48 h. The translucent/opaque colonies with 2-3 mm in diameter having entire margins were taken and suspended in nutrient broth and incubated at 37°C for 48 h. The process was repeated until pure cultures were obtained. These isolated organisms were maintained in nutrient agar slants, by sub-culturing them periodically and stored at 37°C (Arjun *et al*, 2012).

In a 2007 Ghana study, ten milliliter of sample was homogenized in 90 mL sterile salt peptone solution containing 0.1% bacteriological peptone and 0.9% NaCl as the 1:10 dilution. After serial dilution, aerobic mesophilic bacteria were enumerated by pour plate on Plate Count Agar (PCA) incubated aerobically at 37°C for 3 days. Then it was enumerated on MRS agar supplemented with cycloheximide (0.005%). Plates were incubated at 30°C for 2 d under aerobic conditions. The organisms were phenotypically characterized by Gram staining. Determination of morphology was done by phase-contrast microscopy. Only gram-positive, catalase negative, non-motile rod and cocci isolates strains were selected. The presence of catalase activity was assessed by the formation of gas bubbles after the suspension of bacterial cells in a droplet of 3% hydrogen peroxide on MRS. Stock cultures of the isolates were stored in MRS broth containing 15% glycerol at 80°C. Carbohydrate fermentation pattern of lactic acid bacteria used for sap fermentation were determined according to the manufacturer's instructions (Amoa-Awwa *et al.*, 2007).

Isolates on MRS were examined by Gram reaction and catalase test. Isolates which were gram-positive catalase negative regular rods, coccoid or cocci were assumed to be lactic acid bacteria and further examined by gas production in MRS broth with Durham tube and also in MRS broth in which glucose was replaced with gluconate as sole carbon source, growth at 10°C and 45°C, growth at pH 4 and 9, growth in 6 and 18% NaCl, and Hugh and Leifson test (Hugh and Leifson 1953). The species of lactic acid bacteria were tentatively identified by determining their pattern of carbohydrate fermentation (Amoa-Awua *et al*., 2007).

In the Amoa-Awua 2007 study, MRS agar (0.56 g) was dissolved in 10 ml of distilled water, and autoclaved at 121^0C for 15 min, then the agar was poured in two petri plates, one for aerobic growth and the other for the anaerobic growth of the organism. The test organisms were taken from the agar's land and was streaked on the MRS agars plates.

The Aerobic plate was incubated at 48^0 C and the anaerobic plate was incubated at 72^0C for 48 hours in an anaerobic chamber (This slows down the growth). The colony morphology was then identified (Amoa-Awua *et al*., 2007).

In another study done in Ghana, about 15 colonies from a segment of the highest diluted or suitable GYC (Glucose, Yeast extract, Calcium carbonate) and MRS plates were subcultured by streaking repeatedly on agar till pure cultures were obtained. On GYC, only colonies which were able to produce clear zones around their colonies at one stage or other were isolated. All isolates were examined by colony and cell morphology.

Also, in a study done in Ibadan on traditionally fermented food, twenty five milliliter portions were aseptically removed at different stages of fermentation processes. Each sample was homogenized with 200 ml sterile 0.1% peptone water. This was then serially diluted and 0.1 ml from appropriate dilutions were spread plated on MRS agar plates in duplicates and incubated in Gas Pak jars at 30˚C for 72 h. Colonies with distinct morphological differences such as colour, size and shapes were randomly picked from MRS agar plates as presumptive lactic acid bacteria isolates and repeatedly streaked on fresh MRS agar plates to purify the isolates. They were then maintained on appropriate slants at 4˚C (Olaoluwa *et al*., 2013).

More so, in a study done in Abia state on Palm wine, 1 ml aliquot of each palm wine was taken aseptically and serially diluted up to 10-fold using 0.1% bacteriological peptone. 1.0 ml dilutions were plated out in duplicate using spread plate method according to Cheesbrough (2000), for total heterotrophic bacterial count, Mac-Conkey agar for the total coliform count and Sabouraud dextrose agar containing 0.05 mg/ml streptomycin for yeast count. The inoculated plates were incubated aseptically at 30^{o}C for 24 h for bacteria and 24 – 48 h for the yeast. Subcultures of discrete colonies were made and were stored on agar slants for characterization (Nwachukwu *et al*., 2016).

1.4.3 PROBIOTICS

The word ‘probiotic’ comes from Greek language ‘pro bios’ which means ‘for life’ opposed to ‘antibiotics’ which means ‘against life’. The history of probiotics began with the history of man consuming fermented foods. In 1908 a Russian researcher Ellie Metchnikoff, who has a Nobel prize, firstly proposed the beneficial effects of probiotic microorganisms on human health. Metchnikoff hypothesized that Bulgarians are healthy and long-lived people because of the consumption of fermented milk products which consists of rod shaped bacteria (*Lactobacillus sp*). Therefore, these bacteria affect the gut microflora positively and decrease the microbial toxic activity. The term ‘probiotic’ firstly used in 1965 by Lilly and Stillwell to describe substances which stimulate the growth of other microorganisms. After this year the word ‘probiotic’ was used in different meaning according to its mechanism and the effects on human health. The meaning was improved to the closest one we use today by Parker in 1974. Parker defined ‘probiotic’ as ‘substances and organisms which contribute to intestinal microbial balance’. In 1989, the meaning use today was improved by Fuller. Thus, probiotic is a live microbial supplement which affects host’s health positively by improving its intestinal microbial balance. Then this definition was broadened by Havenaar and Huis in’t Veld in 1992 to include mono or mixed culture of live microorganisms which applied to animal and man (Hatice, 2007).

Probiotics are growth promoting factors produced by microorganisms (Lilly and Stillwell, 1965). Parker (1974) defined probiotic as “Organisms and substances with beneficial effects on animals by influencing the intestinal microflora”.

Maintaining balance of bacteria residing in the intestine is necessary to healthy intestine. Many factors may change the balance away from potentially beneficial, health promoting bacteria like *Lactobacilli sp* and *bifidobacteria sp* to potentially harmful or pathogenic microorganisms like *clostridia sp*, sulphate reducers and *bacteroides* sp (Arjun *et al.*, 2012).

Use of probiotics help to protect the host from various intestinal diseases and disorders while increasing the number of beneficial bacteria and making the balance steady (Fooks *et al.*, 1999). It is believed that most probiotics do not permanently adhere in the intestine but exert their effects as they metabolize and grow during their passage through the intestine (colonization). Thus, daily consumption of these bacteria is probably the best way to maintain their effectiveness (Arjun *et al.*, 2012).

With the current focus on disease prevention and the quest for optimal health at all ages, the probiotics market potential is enormous. Health professionals are in an ideal position to help guide their clients toward appropriate prophylactic and therapeutic uses of probiotics that deliver the desired beneficial health effects (Arjun *et al.*, 2012).

Coconut palm (*Cocos nucifera*) produce fermented sap called toddy is popular among Asian countries. Toddy is a natural fermented food which contains microorganisms that are probiotic in nature. These probiotic microorganisms could act as antimicrobial agent so it can be used as natural food additive in order to increase immunity without side effects (Ashraf

and Shah, 2011). The microorganisms on toddy fermentation produce lactic acid and CO_2 that make the toddy anaerobic and leaven the product (Arjun *et al.*, 2012).

It is believed that Probiotics affect intestinal bacteria by increasing the numbers of beneficial anaerobic bacteria and decreasing the population of potentially pathogenic microorganisms. Probiotics affect the intestinal ecosystem by stimulating mucosal immune mechanisms and by stimulating nonimmune mechanisms through antagonism and competition with potential pathogens. These phenomena are thought to mediate most beneficial effects, including reduction of the incidence and severity of diarrhea, which is one of the most widely recognized uses for probiotics. Probiotics reduce the risk of colon cancer in animal models, probably due to their role in suppressing the activity of certain bacterial enzymes that may increase the levels of procarcinogens, but this has not been proven in humans (Francisco *et al.*, 2011).

Some presumed benefits of Probiotics include:

IMMUNE SYSTEM

• Maintain optimal health and wellness.

• Provide a natural defense or immune system for the body.

• Prevent growth of harmful bacteria.

• Strengthen the immune system towards allergies and other autoimmune diseases.

• Help the body to produce vitamins.

DIGESTIVE SYSTEM

• Support healthy digestion.

• Increase defecation and reduce constipation.

• Help control the illness-causing bacteria in the intestinal tract.

• Reduce the effects of Candidal infection.

• Improve digestion of lactose, especially for those who are lactose-intolerant.

• Reduces the cholesterol level.

• Reduces blood pressure.

• Improving the body's absorption of minerals, especially calcium.

• Decreasing dental-caries-causing microbes in the mouth.

Probiotics To Prevent Disease

• Cure vaginal yeast infections.

•Cure Urinary tract infections.

• Prevent diarrhea after having treatment with certain antibiotics.

• Prevent diarrhea caused by virus or Salmonella.

• Manage the signs and symptoms of irritable bowel syndrome (IBS).

• Strengthen the immune system to combat allergies and other immune diseases.

• Reduce amounts of cancer-causing substances in the intestine.

• Reduce the effects of a Candidal infection.

• Prevent and/or reduce colon cancer.

• Reduce the development of allergy in the children.

• Reduce infections and inflammation.

• Fights eczema (Vijaya *et al*., 2013).

The view of probiotic as being beneficial to health is not one universally held. Some are of the opinion that the purported benefits have not been sufficiently scientifically proven (Nadja *et al*., 2016).

1.4.4 PROBIOTIC POTENTIAL OF LAB.

A few *Lactobacillus sp*, *Bifidobacterium sp*, *Saccharomyces boulardii*, and some other microbes have been proposed as and are used as probiotic strains (live microorganisms as food supplement) in order to benefit health. The health claims range from rather vague as regulation of bowel activity and increasing of well-being to more specific, such as exerting antagonistic effect on the gastroenteric pathogens *Clostridium difficile*, *Campylobacter jejuni*, *Helicobacter pylori* and *rotavirus*, as well as neutralizing food mutagens produced in colon, shifting the immune response towards an effective response, and thereby alleviating allergic reactions, and lowering serum cholesterol (Tannock, 2002).

Unfortunately, most publications are case reports, uncontrolled studies in humans, or reports of animal or in vitro studies. In most research however, the probiotic strains employed are of human origin and were able to survive the transport in the human gastrointestinal (GI) tract and to colonize the human large intestine. The properties they exhibit include survival in the stressful environment of the stomach – acidic pH and bile – with induction of new genes encoding a number of stress proteins. Since the availability of antioxidants decreases rostrally in the GI tract production of antioxidants by colonic bacteria provides a beneficial effect in scavenging free radicals (Tannock, 2002).

LAB strains commonly produce antimicrobial substance(s) with activity against the homologous strain, but LAB strains also often produce microbicidal substances with effect against gastric and intestinal pathogens and other microbes or compete for cell surface and mucin binding sites. This could be the mechanism behind reports that some probiotic strains inhibit or decrease translocation of bacteria from the gut to the liver. A protective effect against cancer development can be ascribed to binding of mutagens by intestinal bacteria, reduction of the enzymes-glucuronidase and glucosidase, and deconjugation of bile acids, or merely by enhancing the immune system of the host. The latter has attracted considerable interest, and LAB have been tested in several clinical trials in allergic diseases (Nadja *et al*., 2016).

Characteristics ascribed to a probiotic strain are in general strain specific, and individual strains must be tested for each property. Survival of strains during production, packing and storage of a viable cell mass has to be tested and declared (Nadja *et al*., 2016).

In the 2012 Tranani study, sodium chloride tolerance test was done with one gram of 5% Sodium Chloride salt mixed with 5 mL of MRS broth in a test tube, subsequently tube with 5 mL of MRS broth without salt was taken. With a circular loop, the test cultures were inoculated into the broth (with and without salt) and incubated at 37^0C for 48 h. In the same study, bile tolerance test was done where isolates were grown in MRS broth containing 2% of bile salts mixture at 37°C for 24 and 48 h. The growth was checked using the pour plate technique wherein 1 mL of culture of appropriate dilutions was overlaid with MRS agar. The plates were incubated at 37°C for 48 h and the cell count was compared with that of the control MRS agar plates (containing cultures grown in MRS medium without bile salts mixture). Bacterial growth was expressed as colony forming units per ml (CFU/ml) and the survival percentage (% ± SD) of strains to bile salts was calculated (Arjun *et al.*, 2012).

Also, isolates were grown in MRS broth at 37°C for 48 hours so as to ascertain tolerance to acidic pH. The cultures were centrifuged at 8,000 rpm for 10 min at 4°C. The pellets were washed twice in sterile phosphate buffered saline (PBS, pH 7) and re-suspended (1:100) in PBS to achieve a cell density of 1×1012 cells/ml. This was employed for setting up the experimental control and studying survival of isolates at low pH (pH 1, 2 and 3 prepared in PBS). The suspensions were incubated at 37°C and samples were removed after every 1 hour to 4 hours. Counts of surviving cells were determined by plating on MRS agar using the procedure followed in bile tolerance assay (Arjun *et al.*, 2012).

In the vellore India study done on curd, it was observed that only one of the strains studied survived exposure to pepsin although several survived exposures to acid for varying periods of time. However, it is unlikely that bacteria ingested in curd will be stressed to the extent as those bacteria in the study, because the food with which curd is eaten is likely to buffer gastric acid and to limit pepsin effect. Most of the examined strains were resistant to pH 3.0 even after 3 h of exposure. These results are consistent with reports of ability of lactobacilli to retain their viability when exposed to pH values of 2.5-4.0. None of the lactobacilli survived in pepsin at pH 2.0, in consonance with published literatures. In contrast to pepsin, all the strains examined in the study survived in the presence of pancreatic or bile salts simulating the

near neutral small intestine environment. All LAB tested fulfilled the safety requirement that a probiotic strain should not have β-hemolytic activity. The LAB strains exhibited resistance to a wide variety of antimicrobials. It is most likely that this reflects intrinsic resistance (rather than genetic resistance which can be transferred) to the specified antimicrobials, due to lack of targets in the microbes for these antimicrobials. This property also makes them useful in combination with antibiotics, to prevent antibiotic-induced diarrhea (Ramdass *et al.*, 2014).

In another study, the existence of two beneficial microorganisms were confirmed by different tests namely gram's staining, motility, catalase, oxidase tests, biochemical tests, indole methyl red, trible sugar iron, lactic acid confirmatory test, sugar fermentation, aerobic, anerobic, NaCl tolerant, probiotic confirmatory tests, bile salt tolerant and bile tolerant (Arjun *et al.*, 2012).

Gram's staining results showed the isolated culture was purple colored, non-sporulating and rod shaped. Gram's staining test showed positive for organisms 1 (*Lactobacillus*) and 2 (*Streptococcus*). A total of 10 organisms were isolated from different samples. Two bacterial strains which were clear, round, opaque, white to yellow color colonies, 2-3 mm in diameter having entire margins were taken for the study. The isolates were tested for the gram nature and catalase negative (Arjun *et al.*, 2012). The isolates which are gram positive in nature and catalase negative were studied further. Some of the cultures were bacilli (short rods), the others were cocci, and one was a coccobacilli. Based on the Gram nature, morphology and catalase test, the cultures were observed and subjected to biochemical tests (Arjun *et al.*, 2012).

The isolates grown on MRS broth were treated with 5% of NaCl at 37°C for 48 h. Both the isolates showed good resistance to 5% NaCl even after exposure for 48 h. (Arjun *et al*, 2012).

One of the important criteria to be fulfilled and can be used as a probiotic is its ability to resist the effect of bile salts in the gastrointestinal tract (Lee and Salminen, 1995). However, there are no reports on the exact concentration to which a selected strain should be tolerant.

On the other hand, in the study done in Andhra, India, it was observed that all the Lactic acid bacterial strains survived and tolerated bile salts concentrations of 0.3 to 2.0 % quite effectively. But a marginal decrease in the viability of all the strains was found when a bile salt concentration was increased from 0.3. Also, among six Lactobacillus spp. tested, majority showed resistance to most of the antibiotics they were tested against. Likewise, the antimicrobial activity of LAB was exhibited by most of the LAB used in the study. It was therefore concluded that *Lactobacillus fermentu*m and *Lactobacillus casei* had good probiotic characteristics in terms of acid tolerance, bile tolerance, antibiotic sensitivity and antibacterial activity against different pathogens and could be used as potential functional probiotics in the food and dairy industry for commercial use (Srinu *et al.*, 2013).

A probiotic strain should survive transit through the stomach where the pH is low around 1.5 to 3. Hence, tolerance to extremely acidic conditions is another important feature of probiotic strain (Dunne *et al*., 2001; Guo *et al*., 2009). It was observed that at pH 3.0, lactobacillus showed better survival, even after 4 h of incubation. However, it was noted that the percentage of survival decreased with decrease in pH (Dunne *et al*., 2001; Guo *et al*., 2009).

Another important feature of probiotic culture is its ability to kill pathogens which infect the gastrointestinal system. The isolates were checked for their antimicrobial activity against *B. cereus, L. monocytogenes* and *E. coli* which are common food borne pathogens that infect the gastrointestinal tract. The results showed that two of the ten isolates could inhibit the indicator organisms, however, at different inhibition levels (Dunne *et al*., 2001; Guo *et al*., 2009).

Several researchers have observed that strains which can produce antimicrobial substances are active against pathogenic bacteria (Topisirovic *et al*., 2006). The differences in inhibition potential among the selected isolates could be due to different intrinsic factors induced by food origins (Klayraung *et al*., 2008).

In a similar study done in Egypt, tolerance of tested lactic acid strains were demonstrated, variable susceptibility to bile salts concentrations was noted. The survival of all examined LAB gradually decreased by increasing the concentrations of bile salts. The results showed that the survivals of isolated lactic acid strains grown in modified MRS broth (pH 2) were enumerated in MRS agar after anaerobic incubation at 37^{o}C for 48 h. The survival of all tested strains under acidic conditions also decreased with increasing incubation time and a wide change in the survival of the LAB strains. Generally, all tested strains were affected by exposure to the pH 2 and there was not any growth after 24 h in all examined

strains. After the examination of all tested strains, the strains that survive well in pH 2 were taken to use as aciduric strains and to have good probiotic potential (Mohammed *et al.*, 2015).

1.4.5 FOOD FERMENTATION

It is estimated that 25% of the European diet and 60% of the diet in many developing countries consist of fermented foods (Stiles, 1996; Ogunbanwo et al., 2004). Fermented foods are prepared from plants and animals by processes in which microorganisms play an important role by modifying the substrates physically, nutritionally and organoleptically (Steinkraus, 1997). During the process, the microorganisms could also produce a wide range of metabolic end-products that function as preservatives, texturizers, stabilizers, flavoring and coloring agents and these make fermented foods more acceptable (Harlander, 1992; Basillico *et al*., 2008). Although the physico-chemical properties of the food may be altered during fermentation, their nutritive values are usually retained or enhanced (Oyewole, 1992).

Since fermentation could increase protein content of food over the unfermented ones, fermented food is therefore desirable in developing countries like Nigeria where protein deficiency is a problem. Fermented foods are largely consumed in Africa, where they constitute a bulk of the food. They include 'ogi' and 'gari' in Nigeria, 'uji' in Kenya, 'mahewu' in South Africa, 'kenkey' in Ghana and 'saalga' in Burkina Faso (Olukoya et al., 1993; Olsen *et al*., 1995; Valenzuela *et al*., 2006). In Nigeria, many food substrates have been fermented to produce different fermented foods which are consumed either as beverages, main course meals or food condiments. According to Odunfa (1988) sources of Nigerian fermented foods may be classified as tubers, cassava (e.g. 'gari', 'fufu', 'akpu' and 'lafun'); cereals (e.g. 'eko', 'kati', and 'ogi'); legumes (e.g. 'iru', dawadawa 'ogiri' and 'ugba'); animal protein (e.g. 'wara and 'nono') and alcoholic beverages (e.g. 'oguro', 'burukutu', 'agadagidi' and 'pito'). The methods used for the preparation of these foods have been described earlier (Odunfa, 1988; Olukoya *et al*., 1993; Aderiye and Adebayo, 1999). Aderiye and Laleye (2003) in their study on the relevance of fermented foods in the South- Western Nigeria reported that at least 69.2% of adults consumed 'gari', 'ogi', 'iru', 'elubo' and 'fufu'. They concluded that the ease of food preparation, price of food and the length of storage of these products contribute to the frequency of consumption of these foods. Many Nigerian fermented food products are through lactic acid fermentation (Oyewole, 1992). This is desirable as it improves consumer's safety of the fermented foods (Nout, 1990). Lactic acid fermentation is generally inexpensive and has been employed in the production and preservation of wholesome fermented foods (Steinkraus, 1997). Naturally occurring lactic acid bacteria are the organisms mainly responsible for such fermentations. Lactic acid bacteria have been associated with the fermentation of foods and feeds for many centuries (Magnusson and Schnurer, 2001). They have the advantage of health and nutritional benefits and contribute to the flavor, aroma and increased shelf life of fermented products (Aderiye and Adebayo, 1999).

Lactic acid bacteria are commonly found in fermented foods and feeds and consequently are regularly consumed. Their role is to promote sugar fermentation and other modifications of the raw materials which results in changes in their rheological and organoleptic properties and in the increase of the period of consumption. They equally play a fundamental role in microbial ecology, synthesizing a variety of antimicrobial compounds such as organic acids, hydrogen peroxide, diacetyl and bacteriocins (Lindgren and Dobroqosz, 1990). The antimicrobial compounds produced by the bacteria play an essential role in ensuring safety and extending the shelf life of food. Lactic acid bacteria produce many antimicrobial metabolites like hydrogen peroxide, diacetyl, organic acids and bacteriocins during fermentation. Their ability to produce antimicrobial substances against other competing microflora ensures their predominance and food safety. The use of antimicrobials produced by bacteria traditionally used in the manufacture of food has been studied extensively as a means of improving microbial barriers in formulated or minimally processed foods (Hugas, 1998).

1.4.6 GARI

Cassava, the enlarged root of *Manihot esculenta Crantz*, is a staple food for over 500 million people in the developing world, including about 80 million in Nigeria alone. Cassava has important agronomic advantages, but it has two important deficiencies (Melanie *et al.*, 2005).

Firstly, the bitter varieties contain the toxic cyanogenic glucosides linamarin and (to a lesser extent) lotaustralin, which have fatal consequences when consumed in unprocessed foods. Secondly, Gari is very poor in protein, containing only about 1% (Melanie *et el.*, 2005).

Cassava may be processed by boiling, roasting, drying or by fermentation, depending on the variety. The most popular processing method, however, especially for the varieties high in cyanogenic glucosides, is by fermentation (Melanie *et el.*, 2005).

One of the most popular foods derived from fermented cassava is Gari, which is consumed by nearly 200 million people across West Africa. Gari is prepared by grating the cassava root, followed by dewatering, fermentation for 2 days at ambient temperature and roasting ('garification') of the fermented mash. During grating, the endogenous linamarase in the root is released and degrades the linamarin. However, the endogenous linamarase is not sufficient to break down all the cyanogenic glucosides in the root, and traces are usually carried into the Gari (Melanie *et el.*, 2005).

It is now well accepted that the flavor of Gari results from the fermentative activities of lactic acid bacteria (LAB) and yeasts, many of which also produce linamarase. The microbiology of cassava fermentation for Gari production was originally considered a two-stage process, in which *Corynebacterium sp*. and *Geotrichum candidum* strains were reported to be responsible for acid and flavor production. Later studies have shown that among the microorganisms isolated from fermenting cassava, *Lactobacillus plantarum* produced the most typical Gari flavor.

However, the involvement of five different genera of microorganisms in the fermentation was reported. These include, *Leuconostoc sp*, *Alcaligenes sp*, *Corynebacterium sp*, *Lactobacillus sp* and *Candida sp*, and it was concluded that strains of the genus *Leuconostoc* were the most frequently occurring. Microorganisms (Melanie *et el.*, 2005).

To date, various investigations on the microbiology of cassava fermentation and Gari production have been done. Most investigations done so far identified microorganisms associated with the fermentation by phenotypic means. In the 2005 Benin study however, LAB isolated throughout the fermentation of grated cassava for Gari preparation were characterized and identified at both the phenotypic and genotypic levels (Melanie *et al.*, 2005).

Traditional fermentation of cassava is dominated by a lactic acid bacteria (LAB) population. Fermentation is important for improving product flavor and aroma as well as safety, especially by reduction of its toxic cyanogenic glucosides. The production of Gari from cassava in Benin typically occurs on a household or small industrial scale, and consequently suffers from inconsistent product quality and may not always be safe for consumption (Melanie *et el.*, 2005).

Therefore, the diversity of LAB from a typical cassava fermentation for the preparation of Gari, and their technologically relevant characteristics were investigated in the 2005 Benin study, with a view towards selection of appropriate starter cultures (Melanie *et el.*, 2005).

A total of 139 predominant strains isolated from fermenting cassava were identified using phenotypic tests and genotypic methods such as DNA hybridization and sequencing of the 16S rRNA genes were done for selected strains. *Lactobacillus plantarum* was the most abundantly isolated species (54.6% of isolates), followed by *Leuconostoc fallax* (22.3%) and *Lactobacillus fermentum* (18.0%). *Lactobacillus brevis*, *Leuconostoc pseudomesenteroides* and *Weissella paramesenteroides* were sporadically isolated. The *L. plantarum* strains were shown to be better acid producers and capable of faster acid production than the L. *fallax or L. fermentum* strains. The incidence of b-glucosidase (linamarase) activity was also highest among strains of this species. Production of antagonistic substances such as H_2O_2 and bacteriocins, however, was more common among *L. fallax* and *L. fermentum* strains (Melanie *et el.*, 2005).

Strains of all three species could utilize the indigestible sugars raffinose and stachyose. Therefore, a starter culture containing a mixture of strains from all three species was recommended (Melanie *et el.*, 2005).

1.4.7 PALMWINE

Palm wine is an alcoholic drink obtained by the natural fermentation of the sap of various types of palm trees; this beverage is produced and consumed in several tropical regions of the world. It plays an important role in traditional practices as an alcoholic beverage, so it is important to determine the physicochemical characteristics and microbiological aspects of its fermentation. During the tapping process of the palm wine production, lactic-alcoholic-acetic fermentation is conducted by the lactic acid bacteria (LAB), yeast and acetic acid bacteria (AAB), respectively. *Saccharomyces cerevisiae* is the main microorganism that has been identified as being responsible for the alcoholic fermentation and odorants production (Santiago *et al.*, 2014).

On the other hand, *Lactobacillus plantarum* and *Leuconostoc mesenteroides* have been reported as the predominant LAB. While *Gluconobacter* and *Acetobacter* genera are the predominant, the palm wine composition depends on the stage of tapping period in which it is consumed. Thus, ethanol concentration varies in the range of 1 to 6%, lactic acid concentration varies in the range of 0.1 to 0.5%, and acetic acid percentage varies between 0.02 and 0.4%. The principal components responsible for the odorants of the palm wine are higher alcohols, esters, acids, aldehydes and ketones (Santiago *et al.*, 2014).

The chemical composition of the palm sap is very similar among different species of palm trees. Sucrose is the main sugar presents in the sap and is the substrate in the natural fermentation conducted by lactic acid bacteria, yeasts and acetic acid bacteria. The palm wine involves three types of fermentations: lactic, alcoholic and acetic, making this traditional beverage an interesting environment where microorganism or genes with potential biotechnological applications can be isolated. Palm wine contains ethanol, lactic acid, acetic acid, as well as higher alcohols, esters, aldehydes and ketones. The composition of palm wine depends on several factors such as the source of the sap and the length of the fermentation (Santiago *et al.*, 2014).

Microorganism identification in this beverage has been performed using traditional techniques of identification which involves isolation of the microorganism, and some molecular techniques that do not need microorganism culture. Therefore, the structure of the microbial community probably is not completely identified. The microbiology and the biochemistry of palm wine must be fully understood which includes determining changes in the structure and the metabolic activity of the microbial community (Santiago *et al.*, 2014).

Microbial community metabolism can be evaluated by monitoring the relative expression of messenger RNA (mRNAs). Ratio of microorganism in the fermentation during tapping of palm tree can be performed by quantitative monitoring of microorganisms using real-time PCR technology. Other important point is, knowing the origin of the microorganism and microbial vectors (Santiago *et al.*, 2014).

On the other hand, many authors have reported that yeasts are responsible for the ethanol production mainly *S. cerevisiae*, however, other authors have attributed this to *Zymomonas mobilis* (Santiago *et al.*, 2014).

2. MATERIALS

2.1 MATERIALS

The materials used include Petri dishes, Incubator, Wire loop, Bursen burner, anaerobic jar, peptone water, oxidase reagent, slides, violet stain, iodine stain. These materials were sourced and sterilized as needed.

2.2 MEDIA

MRS (Demane Rogosa and Sharp) agar was used

2.3 COLLECTION OF SAMPLES

2.3.1 Collection of Gari

The liquid extract from traditionally fermented Gari was collected into sterile bottles and was aseptically transported to the laboratory. The collection of the samples was carried out on the day 2 and day 3 from the beginning of the process of the traditional fermentation of the Gari, allowing for 24 hours and 48 hours duration of fermentation respectively.

2.3.2 Collection of Palmwine

Freshly tapped palm wine samples were obtained from local farmers. These were aseptically transported to the laboratory in sterile bottles where the palm wine was left to ferment for 24 hours.

2.4 ISOLATION OF LAB

From the collected samples, a loopful of each of the sample was taken and inoculated onto the MRS agar. Each of the Petri dish was appropriately labeled and incubated for 48hours at 37°C in both aerobic and anaerobic conditions.

2.5 CHARACTERISATION AND IDENTIFICATION OF LAB

The isolated organisms after sufficient culturing to yield adequate amount were then subjected to various microbiological analysis in order to establish, they are LAB and eliminate other potential non- LAB microorganisms.

2.5.1 Gram Staining

A loopful of inocula was taken from each of the culture plate and each was subjected to Gram staining:

1. Slide smear was prepared

2. The smear was stained with crystal violet, followed by Iodine

3. This was then decolorized, and counter stained with Safranine

4. It was finally viewed under the microscope

2.5.2 Catalase Test

A loopful of inoculum was taken from each of the culture plate and subjected to Catalase test:

1. Smeared slide was prepared

2. A drop of 3% hydrogen peroxide was added while watching it for rapid oxygen evolution.

2.5.3 Oxidase Test

A loopful of inoculum was taken from each of the culture plate and subjected to oxidase test:

1. Filter paper was soaked with oxidase reagent

2. Filter paper moistened with distilled water

3. The filter paper was smeared with the inoculum, and observed for color change

2.6 EVALUATION OF LAB FOR PROBIOTIC POTENTIAL

Each of the colony confirmed to be LAB was subjected to several analytical processes to access if they conform to the different properties of a probiotic

2.6.1 Ability to Inhibit Known Pathogens

The LAB were cultured with *E. coli* and *Staphylococcus aureus* and evaluated for their ability to inhibit the growth of the pathogens.

1. The LAB were inoculated into peptone water

2. Both pathogens were inoculated in separate dishes

3. Using a pour plate technique, the LAB were also inoculated into the plates containing the pathogens. There was also a control plate into which no LAB was inoculated

4. The plates were incubated at 37°C and checked for growth at 24 and 48 hours.

2.6.2 Acid Survival and Growth

The inoculum were also tested for the ability to grow in an acidic environment:

1. The LAB were inoculated into culture plates

2. Diluted hydrochloric acid was added to the plate and incubated at 37°C for 48 hours.

3. Concentrated hydrochloric acid was added to another plate and incubated at 37 °C for 48 hours.

4. The plates were then monitored for growth

3. RESULTS

3.1 RESULTS

3.1.1 ISOLATION OF SAMPLES

After incubation of the collected samples on MRS Agar at 37°C in both aerobic and anaerobic conditions, the following results were obtained:

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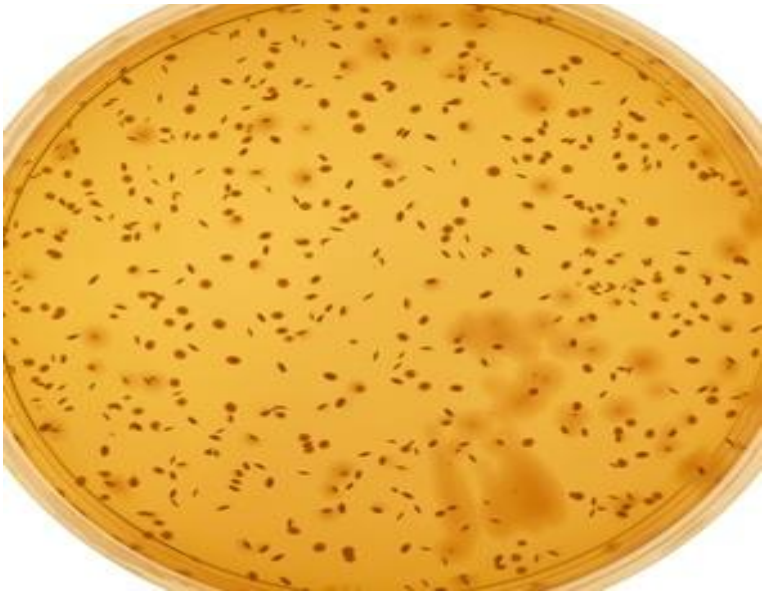

Figure 1: Diffused growth of bacteria present in Gari on MRS Agar

In the figure 1, pour plate method was used in inoculating the samples collected from Gari. After 48 hours, growths were observed in both the samples incubated in aerobic and anaerobic conditions, displaying diffused growth of the inoculated samples in varying sizes (small to medium) and shapes (round and rod-shaped) on a background of golden yellow MRS plate.

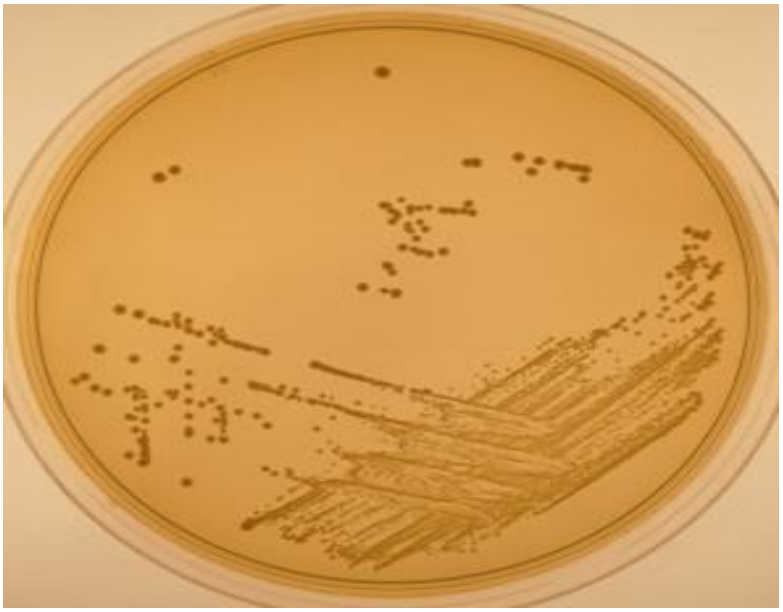

Figure 2: Distinct growth of bacteria present in Palmwine on MRS Agar

In the figure 2, after 24 hours of fermentation, the Palm wine samples were streaked onto MRS plates and incubated aerobically and anaerobically. After 48 hours of incubation, distinct growths were observed in the MRS Agar, displaying growths patterned along the streaked inocula, in varying sizes (small to medium) and shapes (round and rod-shaped) on a background of golden yellow MRS plate.

Table 1: Pattern of growth of bacteria present in Gari on MRS agar

	24 hours of incubation	48 hours of incubation
Aerobic culture	Growth	Growth
Anaerobic culture	Growth	Growth

From table 1, growths were observed in all the MRS plates inoculated with the Gari sample in both the aerobic and anaerobic cultures as early as 24 hours and after 48 hours.

Table 2: Pattern of growth of bacteria present in Palmwine on MRS agar

	24 hours of incubation	48 hours of incubation
Aerobic culture	Growth	Growth
Anaerobic culture	Growth	Growth

From table 2, growths were observed both after 24hours and 48 hours of incubation of the Palmwine samples on MRS agar.

3.1.2 CHARACTERISATION OF LAB

After series of sub-culturing, a pure colony was obtained from both Gari and Palmwine samples and subjected to various tests with the following results obtained.

Table 3: Cultural, morphological and biochemical characteristics of bacteria isolated from Gari and Palmwine

Cultural isolates	Cultural characteristics/colonial appearance	Gram reaction	Catalase reaction	Oxidase reaction	Inference
Aerobically-cutured Gari sample	Round and rod/shaped bluish colonies on MRS agar	+	-	-	LAB
Anaerobically-cultured Gari sample	Round and rod/shaped bluish colonies on MRS agar	+	-	-	LAB
Aerobically-cultured Palmwine sample	Round and rod/shaped bluish colonies on MRS agar	+	-	-	LAB
Anaerobically-cultured Palmwine sample	Round and rod/shaped bluish colonies on MRS agar	+	-	-	LAB

From table 3, the bacteria in both Gari and Palmwine samples were Gram positive (with mixture of both cocci and bacilli). They were however both catalase negative as well as oxidase negative.

3.1.3 EVALUATION OF LAB FOR PROBIOTIC POTENTIAL

3.1.3.1 ABILITY TO INHIBIT KNOWN PATHOGEN-

Table 4: Pattern of microbial inhibition by LAB isolated from Gari and Palmwine

	Staphylococcus aureus	*Escherichia coli*
LAB isolated from Gari	No growth	No growth
LAB isolated from Palmwine	No growth	No growth
Control (free from LAB)	Growth seen	Growth seen

As shown in table 4, the results obtained after culturing the LAB isolated from both Gari and Palm wine with known pathogens were represented above, with no growth seen in the presence of LAB in either the Gari or Palmwine, but growth seen in those cultured with the control (free from LAB).

3.1.3.2 ACID SURVIVAL AND GROWTH

Table 5: Effect of acid on the growth of LAB isolated from Gari and Palmwine

	Concentrated HCL	Diluted HCL
LAB isolated from Gari	No growth	Growth
LAB isolated from Palmwine	No growth	Growth

Table 5 shows that after subjecting the LAB in both Gari and Palmwine to acid test, growth was observed in the presence of diluted acid, but not in concentrated acid.

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4. DISCUSSION

4.1 DISCUSSION:

After inoculating the MRS plates with both samples which is the Gari sample and Palmwine sample, in an aerobic and anaerobic condition, growth was observed as early as 24hours and after 48hours. This result was also the same with most of the literatures reviewed (Arjun *et al*., 2012; Amoa-Awwa *et al*., 2007 and Nwachukwu *et al*., 2016). There was a little difference in the 2014 Indian study, where LAB were cultured on Lactobacillus MRS broth for 16h at 37°C with 10 percent CO_2 under anaerobic conditions (Ramdass *et al*., 2014). This finding was also different in the 2013 Ibadan study (Olaoluwa *et al*., 2013), where growth was only observed after 72h.

The longer incubation period recorded in the 2013 Ibadan study (Olaoluwa *et al*., 2013) was probably due to the serial dilution done on the samples (which could have reduced the actual amount of LAB) before plating them on the MRS agar. On the other hand, the shorter duration observed in the 2014 Indian study (Ramdass *et al*., 2014) may be due to their choice of sample, whereby the study was done on curds which were fermented by the LAB that was streaked on them.

The LAB isolated from the MRS Agar was found to be a mixture of both gram-positive cocci and bacilli. This finding is same with most literatures (Arjun *et al*., 2012; Amoa-Awa *et al*., 2007 and Nwachukwu *et al*., 2016). After isolating LAB from an MRS agar, sub-culturing was carried out to obtain a pure culture. The pure culture of LAB was subjected to some biochemical test, which are oxidase and catalase test, and they were all negative. This finding aligns with the 2012 India study (Arjun *et al*., 2012), 2007 Ghana study (Amoa-Awa *et al*., 2007) and the 2016 Nigeria study (Nwachukwu *et al*., 2016).

The properties of gram positivity, catalase negativity and oxidase negativity exhibited by the LAB in this study are similar with those described in the 2012 India study (Arjun *et al*., 2012), 2007 Ghana study (Amoa-Awa *et al*., 2007) and the 2016 Nigeria study (Nwachukwu *et al*., 2016).

The ubiquitous presence of these LAB, lend support to the theory already widely postulated (Arjun *et al*., 2012; Amoa-Awwa *et al*., 2007 and Nwachukwu *et al*., 2016), that the presence of these LAB enhances the traditional fermentation process. The findings here therefore do not deviate from the general agreement but further buttress the consensus.

By this, the consumption of these traditionally fermented foods also implies consumption of the LAB involved in their fermentation. This means that, most of these LAB are being consumed unknowingly by the majority of people who feed on this common food.

In this work, LAB exhibited antimicrobial property against *Staphylococcus aureus* and *Escherichia coli* by inhibiting the growth of these organisms when cultured simultaneously with the LAB. This finding corresponds with the findings in the work done by Guo *et al*. (2009) and Dunne *et al*. (2001) where the LAB inhibited the growth of other common gastrointestinal pathogens. The findings in this study compared to others are quite similar but only differ in terms of the pathogens they each inhibit. The ability of the LAB in both samples to survive acidic environment, it was observed that LAB survive in diluted hydrochloric acid but not in concentrated hydrochloric acid. The findings here also agree with the findings in the work done by Guo *et al*. (2009) and Dunne *et al*. (2001) where survival of the LAB was found to decrease with the decrease in pH.

4.2 CONCLUSION

The traditionally fermented Gari and Palmwine is a haven for many microorganisms among which are LAB, which are gram positive cocci and bacilli, catalase and oxidase negative organisms. These LAB are probiotic, exhibiting both acid survival and microbial inhibition.

4.3 RECOMMENDATIONS

1. Encouragement of extensive research into the probiotic potential of the LAB present in Gari and Palmwine.
2. Promotion of public enlightenment on the probiotic potential of the LAB present in Gari and Palmwine.
3. Establishment of agencies for the regulation and control of the use of LAB present in traditionally fermented foods and drinks.
4. Ensuring the teaching of the probiotic potential of the LAB present in traditionally fermented food and drink is instilled into the school curricula at all levels of our educational system.

5. Encouragement and financing of mass production of LAB present in Gari and Palmwine for their health benefits.
6. Provision of safe and conducive environment for the production, use and distribution of LAB present in Gari and Palmwine
7. Active involvement in research into the probiotic potential of LAB present in Gari and Palmwine.
8. Encouraging the involvement of academics, scientists and students into the development of fields that focus on LAB through grants and scholarships.
9. Ensuring that larger proportion of probiotics marketed for their health benefits are naturally obtained from traditionally fermented foods and drinks.
10. Improvement of methods and techniques used in harvesting LAB from traditionally fermented foods and drinks.
11. Ensuring that all safety protocols and standards are followed during the production of probiotic LAB.
12. Cautious use of LAB from Gari and Palmwine for their probiotic potential

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