



Research Article

Characterization and Microbiological Evaluation of Probiotic Isolated from Bambara Groundnut

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Keywords: Lactic acid bacteria; Bambara groundnut; fermentation; probiotic; isolates; nutraceuticals.

Abstract

The study was carried out to isolate, characterize, and study antimicrobial sensitivity of lactic acid bacteria (LAB) from Bambara groundnut. Dried Bambara groundnut was fermented by spontaneous method for seven days and its pH, TTA (Total Titratable acidity) and microbial load monitored for each of the fermentation days. Seven acid-producing cultures were isolated from the sample, and isolates were further classified first by phenotype. Phenotypic and biochemical characteristics led to identification of three bacterial groups. These were followed by in vitro assessment of antimicrobial activity against enteropathogenic bacteria. The most abundant type of LAB distributed in the isolates of fermented Bambara groundnut was *Lactobacillus delbrueckii*, followed by *Lactobacillus casei* in two of the isolates. *Lactobacillus brevis* was found in the remaining two isolates. The growth pattern at different salt concentrations revealed that the isolates were salt tolerant at 2% and 4% while at 6.5% there was no growth. At pH 4.5 and 6.0, there were also growth. The strain evaluated showed in vitro antibacterial activity against five pathogenic microorganisms namely *Escherichia coli*, *Salmonella sp*, *Shigella sp*, *Pseudomonas sp* and *Staphylococcus sp* using agar well diffusion method. These results suggested that various LAB were present in Bambara groundnut. The microorganisms isolated were then freeze dried using a freeze drier and then kept at a low temperature in the refrigerator so as to preserve/store the organisms for further processes. This report thereby showed that Bambara groundnut, being an underutilized legume can serve as potential candidate for probiotic nutraceuticals.

Introduction

Current nutritional research is aiming at health promotion, disease prevention and performance improvement. The provision of human being with required nutritional ingredients depends on both how well the host is supplied with balanced foods and what state of intestinal

microecology host has. Recent studies have shown that the application of natural fermentation processes to different food sources increases their nutritional quality and decreases the anti-nutritional compounds through the action of the microbial flora responsible for the fermentation

process (Farnworth, 2003; Granito *et al.*, 2007). Among the notable bacterial involved in improving the nutritional values, sensory properties and functional qualities of food is lactic acid bacteria (LAB). LAB produce several antimicrobial, including organic acids, lactic acid, acetic acid and formic acid. Additionally, among other functions, LAB improve the natural texture of products like yoghurts, ice cream and sour cream due to exopolysaccharidase production (De Vuyst *et al.*, 2001). With these various functions performed by LAB, they may be enhanced by implantation into various forms for specific purpose. The formulation of the dietary supplements, functional foods or herbal products into marketed medicinal products is known as “nutraceuticals”; a term which combines “nutrition” and “pharmaceuticals” (Noha, 2013). Among various nutraceuticals of major importance in diseases prevention is probiotics (Smith and Charter, 2010).

The human body, on the other hand, although created with a proper ratio of good to bad bacteria, frequently alters the ratio of bacteria as a result of today’s modern lifestyle. These lifestyle factors can affect the population of sensitive healthy bacteria. The use of antibiotics inhibits not only bad bacteria but also good bacteria, thus permitting bad bacteria, to invade the gastrointestinal tract (GIT) and multiply in high numbers that disturb the delicate balance between the good and bad bacteria. In order to have increased access to beneficial bacteria, there is need for artificial techniques to absorb the negative effect imposed by modern life-style. Biotechnology applications in the food processing sector, therefore, target isolation, characterization, identification and manipulation of beneficial microorganisms involved in food fermentation. Isolation is done to obtain pure bacterial cultures. Pure culture is essential in the study of the morphology, physiology, biochemical characteristics, and susceptibility to antimicrobial agents of a particular bacterial strain. These life microorganisms when administered in adequate amount confer a beneficial health benefit on the host and they are termed probiotic bacteria (Kalui *et al.*, 2010). Bacterial strains most commonly used as probiotics belong to the genera *Lactobacillus* and *Bifidobacterium*, but other organisms are also applied such as *Lactococcus* and *Enterococcus* (Enujiugha and Badejo, 2017). *Lactobacillus* species from which probiotic strains have been isolated include *L. acidophilus*, *Lactobacillus johnsonii*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus gasseri* and *Lactobacillus reuteri*. *Bifidobacterium* strains include *Bifidobacterium bifidum*, *Bifidobacterium longum* and *Bifidobacterium infantis* (Heller, 2001).

Probiotics can be delivered commercially either as nutritional supplements, pharmaceuticals or foods. A large number of probiotic products are available in the market in the form of milk, yoghurts, cheeses, ice creams, dairy spreads and fermented soya products. Also, special freeze-

dried pharmaceutical dietary preparations are available in the form of tablets (FAO, 2002). With an increase in the consumer vegetarianism in the developing countries coupled with the low purchasing power of low-income people especially in sub-Sahara Africa to consume much dairy product needed for probiotics, there is also a demand for the vegetarian probiotic products. Non-dairy probiotic products have also shown a big interest among vegetarians and lactose intolerance customers. Potential sources of vegetarian probiotics are legumes due to their large distribution and important nutritive value.

Bambara groundnut (*Vigna subterranean*), an indigenous African legume known to have been domesticated in West Africa from its presumed wild ancestor (Adeoye *et al.*, 2018). Bambara groundnut (BG) contains about 61.3% carbohydrate, 20.7% protein and 6.0% oil (Yusuf *et al.*, 2008) and is used as main food, snacks, relish and medicine, and has high ceremonial value (Oluwole *et al.*, 2007). As an essential step to harness the probiotics nutritional benefit of Bambara groundnut and to have increased access to beneficial probiotic bacteria locked in it due to its low satiety value, it was subjected to fermentation process to enhance the quality parameters and functional properties of the raw material. Fermentation with well-characterized starter cultures, yeast or lactic acid bacteria (LAB), is a potential means to improve the palatability and process ability of the whole-meal flours (Salmenkallio-Marttila *et al.*, 2001). This study therefore, targets isolation, characterization, identification and antimicrobial sensitivity of microorganisms involved in fermentation of Bambara groundnut (BGN).

Materials and Methods

Dried Bambara groundnut (*Vigna subterranea* (L) verde) was obtained from Institute of Tropical Agriculture, Ibadan, Nigeria. All chemicals used were of analytical grades and they include: Sodium hydroxide (NaOH), sodium chloride (NaCl), potassium dihydrogen phosphate (KH₂PO₄), hydrochloric acid (HCl), sodium citrate, trichloroacetic acid (TCA), ethanol (C₂H₅OH), iodine crystals, potassium dichromate (VII) (K₂Cr₂O₇), d-biotin, safrarin distilled water, peptone water, glucose phosphate etc. Media used are de Man, Rogosa Sharpe (MRS) broth (BIOMARK, India), nutrient agar, agar agar, muller hinton agar, bile salt agar, phenolphthalein indicator.

Bambara Groundnut Fermentation

Two hundred grams (200 g) of the sorted seeds was weighed using an analytical balance (AS 60/220.X2, India) into a sterile container and was subjected to spontaneous fermentation in which seeds were soaked in 400 mL of distilled water in the ratio of 1:2 w/v in a clean plastic bowl with cover and allowed to ferment for seven days at 28 ± 2 °C. Fresh samples were taken from the fermented BGN for microbiological analyses. In addition, pH and total titratable

acidity (TTA) of the samples were also measured. Fermentation was done in duplicate.

Determination of pH

The pH was measured using a standardized/ calibrated water test meter (Hangzhou Qi Wei, instrument Co, Ltd.). The pH meter was first calibrated using buffer solution of pH 4, pH 6 and pH 9 in order to determine the accuracy of the pH meter to be used. 10 ml of the fermented substrate was measured into a beaker and the calibrated pH meter was dipped into the measured substrate and allowed to be stabilized before the result displayed on the meter was taken and recorded. The pH was checked at 0, 1, 2, 3, 4, 5, 6 and 7th day. (Prescott *et al.*, 2008).

Temperature (°C)

The temperature of the fermentation medium was determined using a well calibrated Standard clinical thermometer. The thermometer was dipped inside the soaked Bambara groundnut and the temperature was checked at 0, 1, 2, 3,4,5,6 and 7th day. (Prescott *et al.*, 2008).

Determination of Total Titrable Acidity (TTA)

Fermented substrate (10 mL) was measured into a conical flask and 90 ml of distilled water was added to it. Sodium hydroxide (0.1M) was poured into the burette to fill the burette and 2 drops of phenolphthalein indicator were carefully dropped into the substrate in the beaker and shaken. The initial volume of the alkaline in the burette was noted. The alkali was run briskly into the beaker containing the substrate and indicator which was consistently shaken until there was a sharp change of the substrate to pink. The volume of the alkali in the burette at this point was noted to enable the determination of the volume of base (titre value of alkali) used. The TTA was checked at 0, 1, 2, 3, 4, 5, 6 and 7th day and was expressed as the amount of NaOH used (ml). The Titrable acidity was then calculated using the formula in Equation 1 (Prescott *et al.*, 2008).

%TTA =

$$\frac{\text{Average base titre (ml)} \times \text{molarity of base (mol)} \times 100}{\text{Volume of sample (ml)}}$$

Preparation of Culture Media and Diluent

The media used for both isolation and identification of microorganisms were nutrient agar (Oxoid), De Man Rogosa Sharpe (MRS) agar, bile salt agar and Muller Hinton agar. They were prepared according to manufacturer's instruction and sterilized by autoclaving at 121 °C for 15 mins at 760 psi and cooled to 45 °C before dispensing into sterile Petri dishes and left to gel. The diluent was prepared by adding 0.85 g NaCl to 100 ml of distilled water and 9 ml each was measured into dilution bottles which were covered tightly and sterilized in an autoclave at 121 °C and 15 mmHg for 15 minutes and cooled to 45 °C.

Isolation of Lactic Acid Bacteria and Determination of Total Viable Count

Isolation of microorganisms was done by the method described by Harrigan and McCance, 1990. After sterilization of agars and diluents, the workbenches were sterilized using ethanol and cotton wool and a spirit lamp was lighted, placed on the work bench to create an aseptic environment. The sterile Petri dishes were arranged and labelled on the sterilized work bench, the cooled dilution bottles were also placed on the work bench and serial dilution was done at 3-fold dilutions (100, 10-1, 10-2 and 10-3). 1ml of dilution factor 10-2 was inoculated via pour plate method on MRS agar by adding 0.3 mL of lactic acid. The Petri dishes containing the substrate and medium were incubated in an anaerobic jar which was put into an incubator at 370 °C for 48 h. Discrete colonies from each plate were sub cultured on fresh bacteriological media until pure cultures were obtained. The pure isolates obtained were preserved in a prepared broth bottle containing nutrient agar slants and stored at 4 °C in a refrigerator before biochemical tests were further carried out.

Identification and Characterization of Isolates

Colonies were selected randomly and were characterized using morphological and biochemical tests such as Gram stain, Sugar fermentation, motility, catalase, e.tc. Bacterial isolates were identified with reference to Cowan and Steel's Manual for the Identification of Medical bacteria (Cowan, 2004) and Bergey's manual of determinative Bacteriology (Holt, 1994). The bacterial isolates identification was based on colony morphology, cultural characteristics and biochemical tests using the methods described by Cheesebrough (2006).

Morphological Characterization

The morphological characteristics such as colour, shape, elevation, edge, consistency, colony surface and pigmentation of the distinct colonies were observed physically and noted with the aids of Bergeys Manual of Systematic Bacteriology (Holzapfel and Wood, 1995). The isolates cultures were subsequently screened by streaking on the appropriate sterilized agar plate till a pure colony appears. Pure cultures of presumed lactic acid bacteria were aseptically picked and maintained on appropriate double strength sterilized media (deMann Rogosa Sharpe and extract agar slants) and broths with 10% glycerol, which were then stored in refrigerator at 4°C.

Biochemical Characterization

Distinct pure colonies observed to be dominant were checked for gram reactions using microscopic examination for cell morphology. Some of the key tests for identification include the following:

Gram Stain

The method used was that described by Harrigan and McCance (1990). A smear of the test organism was made on a grease free glass slide and heat fixed. The smear was stained with crystal violet for 1 minute, rinsed with water and excess water was drained off. The slide was flooded

with lugol's iodine for 1 minute and it was rinsed with running water. The smear was decolorized with absolute alcohol until blue colouration ceased to leave the smear. It was rinsed immediately with water and drained off excess water for 5–10 s. The slide was counterstained with 0.5 % safranin for 1 min, the slide was rinsed with water and allowed to dry. Immersion oil was added and was observed under the microscope using the oil immersion (x100) objective lens. Gram positive bacteria retained the colour of the primary stain which is purple. Gram negative bacteria take the colour of the counter stain (safranin, neutral red, dilute carbol-fuchsin) which is red or pink.

Spore Stain

The malachite green staining method was used. The staining was carried out as described by Harrigan and McCance, 1990. Smears of the pure isolates were made on grease-free glass slide and heat fixed. The slides were flooded with 5% v/v malachite green solution. The slides were flamed in such a way that the stain steamed but did not boil. The slides were then allowed to stand for 5 min. The stain was then washed out in running tap water. The smears were counter stained with safranin for 30 s. This was stained with safranin for were blotted, dried and examined under the oil immersion objective. The spores stained green while vegetative cells-stained red.

Motility Test

For this test, the hanging drop technique was employed and the technique was carried out as described by Harrigan and McCance, 1990. Vaseline jelly was rubbed around the cavity of a hanging drop slide. A drop of peptone water containing the pure culture was placed on a cover slip. The hanging drops slide was then placed over the drop of peptone water in such a way that the centre of the depression lies over the drop. The slide was quickly inverted and viewed under the microscope, using oil immersion objective.

Sugar Fermentation Tests

Each of the isolates was tested for its ability to ferment a given sugar with the production of acid and gas or acid only. Since most bacteria especially Gram-negative bacteria utilize different sugars as source of carbon and energy with the production of both acid and gas, or acid only, the test is used as an aid in their differentiation. The growth medium used was peptone water and the method used was that described by De Man *et al.*, 2016. Peptone water was prepared in a conical flask and the indicators phenol red was added. The mixture was dispensed into test tubes containing Durham tubes. The tubes with their content were sterilized by autoclaving at 121 °C for 15 min. One percent (1%) solution of the sugar was prepared and sterilized separately at 115 °C for 10 min. This was then aseptically dispensed in 5 ml aliquot volume into the tubes containing the peptone water and indicator. The tubes were inoculated with young culture of the isolates and incubated at 37 °C. Acid and gas

production only were observed after about 24 hours of incubation. Acid production was indicated by the change of the medium from pale red to yellow colour, while gas production was indicated by the presence of gas in the Durham's tubes. The control tubes were not incubated (Prescott *et al.*, 2008).

Catalase Test

The method employed here was that described by Jagbir, 2011. A loop of the pure colony was transferred into a plain, clean glass slide. The sample was then mixed with a drop of 3% v/v hydrogen peroxide. It was observed for effervescence; immediate bubbling indicates that the organism is catalase positive.

Methyl Red Test

The methyl-red test was employed to detect the production of acid during fermentation of glucose such that the pH of a culture was sustained below a value of 4.5 as shown by the change in colour of the methyl-red indicator which was added at the end of the period of incubation (Prescott, 2008)

Voges Proskauer (VP) Test

This test was carried out to detect acetoin in a bacterial broth culture. It is used to determine if an organism produces acetymethyl cabinol from glucose fermentation. This test was carried out by the method described by Olutiola *et al.* (2000).

Indole Test

The test was carried out using the method described by Olutiola *et al.* (2000). Tubes of sterilized peptone water was prepared according to the manufacturer specification and it was then inoculated with a young culture of the presumed LAB isolates. It was later incubated at 37 °C for 48 h alongside a control tube (containing sterilized peptone water without inoculum). Four drops of Kovac reagent were added into each culture tubes. Positive result was indicated by a red colour that appeared immediately at upper part of the test tubes while yellow colouration indicates a negative result.

Oxidase Test

This was carried out using the method of Cheesbrough, 2006

Starch Hydrolysis Test

It was carried using the method described by Cheesbrough, 2006.

Endospore Test

It was carried using the method described by Cheesbrough, 2006.

Coagulase Test

This test was carried with the method described by Olutiola *et al.*, 2000.

Citrate Test

Simon citrate agar of 2.4 g was dissolved in 100 mL of distilled water. About ten millilitres (10 mL) of citrate medium was dispensed into each tube and covered, then sterilized and allowed to cool in a slanted position. The tubes were inoculated by streaking the organisms once across the surface. A change from green to blue indicates utilization of the citrate.

Casein Hydrolysis

Skim milk of 0.25 g was weighed and added to 25 mL of already prepared nutrient agar broth (24 g/ 1000 L) in a conical flask. The flask was shaken to ensure even distribution of the milk in the agar broth. 5 ml of the mixture was taken with a sterile pipette into three sterile and well labelled test tubes respectively. The test tubes were sealed with cotton wool wrapped with aluminium foil. The test tubes were then sterilized by autoclaving at 121 °C for 15 min under 15mmHg pressure. They were then cooled to about 40° C and inoculated with the isolates leaving one of the test tubes un-inoculated (serving as control). They were then incubated at 37 °C for 48 h.

Growth at Different Temperatures

This was done by a modified method of Buchanan and Gibbons (1974). Overnight isolated cultures were inoculated at 10% (v/v) in MRS broth and incubated at 15 °C, 37 °C and 45 °C for 24 hours. Total populations were determined by the streaking method, incubating the plates at 37 °C for 48 hours.

Tolerance to Bile Salt

Prepared bile salt agar was poured on sterile, well-labelled petri-dishes. The plates were gently swirled for proper distribution. The agar content of the plates was allowed to solidify. It was then inoculated with the isolates by streaking method and incubated in an anaerobic condition at 37 °C for 24-48 h.

Antibacterial Activity of Probiotic Isolates

The antagonistic activity of the probiotic isolates against some pathogenic bacteria (*Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus sp.*, *L. monocytogenes*, *Klebsiella sp.*, *B. subtilis*, *P. aeruginosa*, *E. faecalis*, and *Staphylococcus aureus*) was investigated using agar well diffusion. The test pathogens were seeded on a sterile molten nutrient agar. After solidification, wells were bore on seeded agar plates, and the probiotic-isolates were introduced into the wells. The plates were first incubated at 34 °C for 60 min to allow the test material to diffuse in the agar, and were then incubated at 37 °C for 18 h. After incubation, the diameters of the resulting clear zones (zones of inhibition) were measured from the center of the well in centimeters (cm) and later converted to milliliters (mL).

The flow diagram for fermentation process and eventual harvesting is shown in Fig. 1.

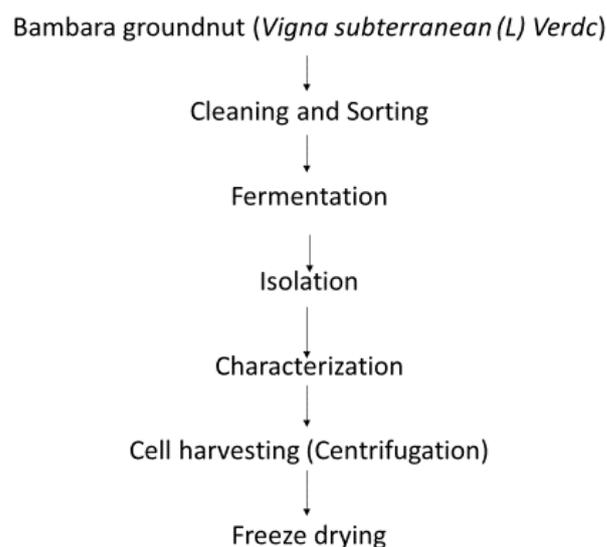


Fig. 1: Flowchart for the harvesting process of isolates from Bambara groundnut

Results and Discussion

Determination of pH, Total Titratable Acidity (TTA) and Microbial Load

The pH, titratable acidity (TTA) and temperature values of fermented Bambara groundnut in relation to fermentation days are shown in Figures 2 - 4. During the fermentation days, it was observed that the pH which has the initial value of 7.2 (neutral pH) decreases with the fermentation days but on the seventh day, an increase in the pH was observed. In the studies involving other legumes, Achinewhu (1987) as well as Ogbadu and Okagbue (1988) observed a steady increase in pH with fermentation. This difference may be due to the unique chemical composition of Bambara groundnut which is higher in starch and lower in protein than the other legumes. The fermenting microorganisms might have started fermentation by hydrolyzing available carbohydrate to acid before embarking on extensive proteolysis. Amino acids produced on proteolysis might have degraded to ammonia which may be responsible for the rise in pH. The gradual development of ammonia odor which became prominent on seventh day of fermentation was in agreement with the observed increase in pH. For this reason, the fermentation of Bambara groundnut was stopped on the 6th day.

The titratable acidity of the fermentation increases with the increase in fermentation days, but on the seventh day a slight decrease was noticed. The results for both pH and titratable acidity show that acidity of the fermentation medium increases which may be related to the production of lactic acid by the fermenting microorganisms. However, the change that occur in the pH and TTA at seventh day can be linked with the microorganism succession in an environment where the lactic acid producing microorganisms begin to die of as a result of the unfavourable condition of their metabolite (lactic acid).

Temperature of fermentation initially decreases from 31.5 °C of day one to 28 °C on the second day but remains constant (ie. at 28 °C) throughout the remaining days of fermentation. The pH, TTA and temperature are shown to be correlated at different level of significance as shown in Table 1. The correlation between pH and temperature (R value of 0.882), % TTA and days of fermentation (R value of 0.948) are significant at 0.01 level. This implies that there is strong direct proportionate relationship between the pairs ie. increase in value of one lead to increase in value of its pair. However, the correlation between % TTA and pH

showed 0.05 level of significance with negative R value that implies the pair is inversely proportional.

The bacterial load of fermenting Bambara groundnut revealed that the raw sample of Bambara groundnut on the De Man Rogosa Sharpe (MRS) agar used for the isolation of lactic acid bacteria showed no growth. After 24 hours of spontaneous fermentation, the highest bacterial count, 1.26×10^5 CFU g⁻¹ was observed on MRS agar which later decreased significantly ($p < 0.05$) to 4.70×10^4 CFU g⁻¹ after 72 hours of fermentation.

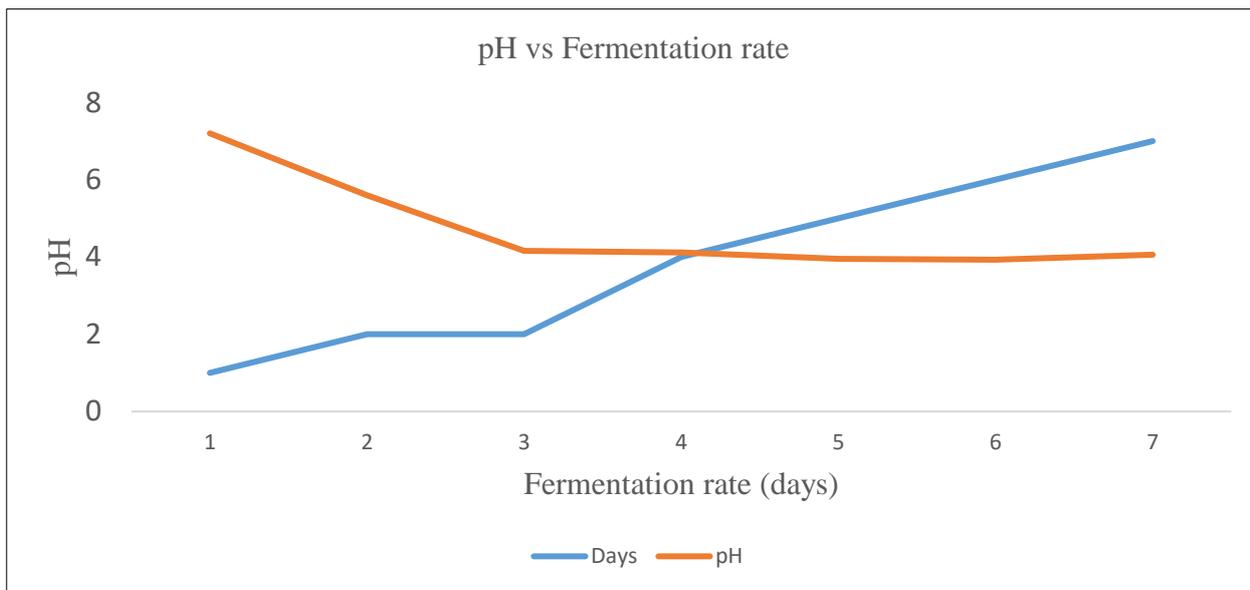


Fig. 2: Relationship between pH and fermentation rate

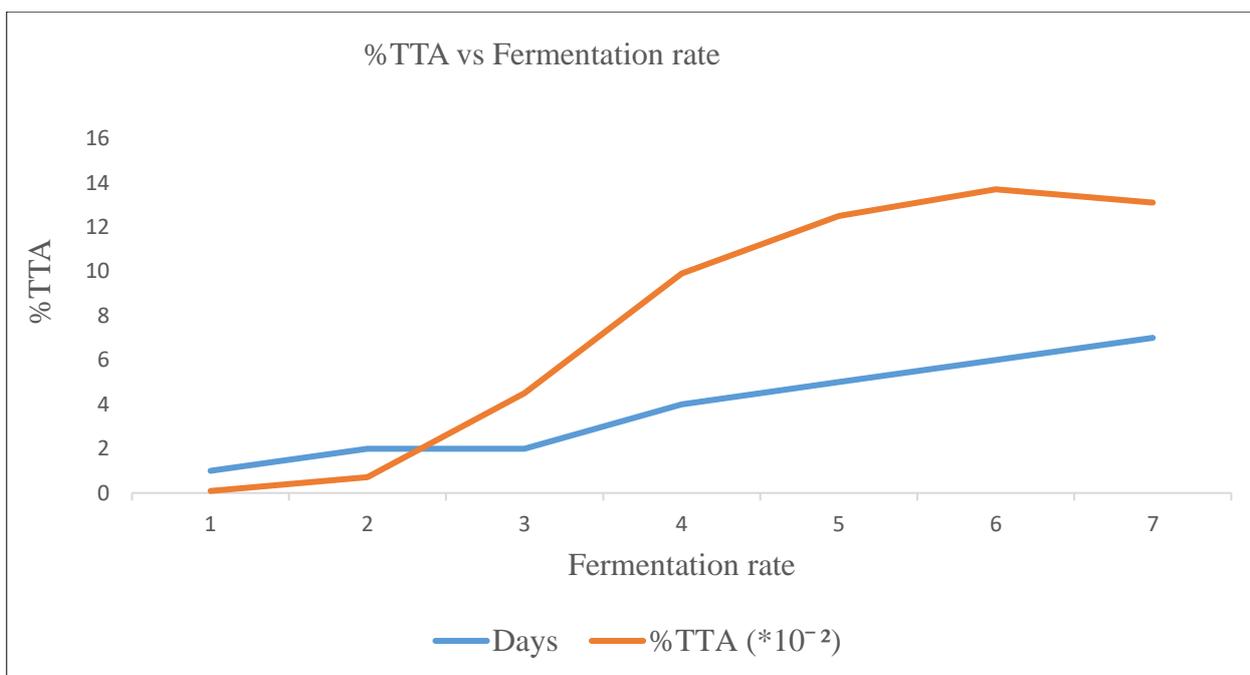


Fig. 3: Relationship between % TTA and fermentation

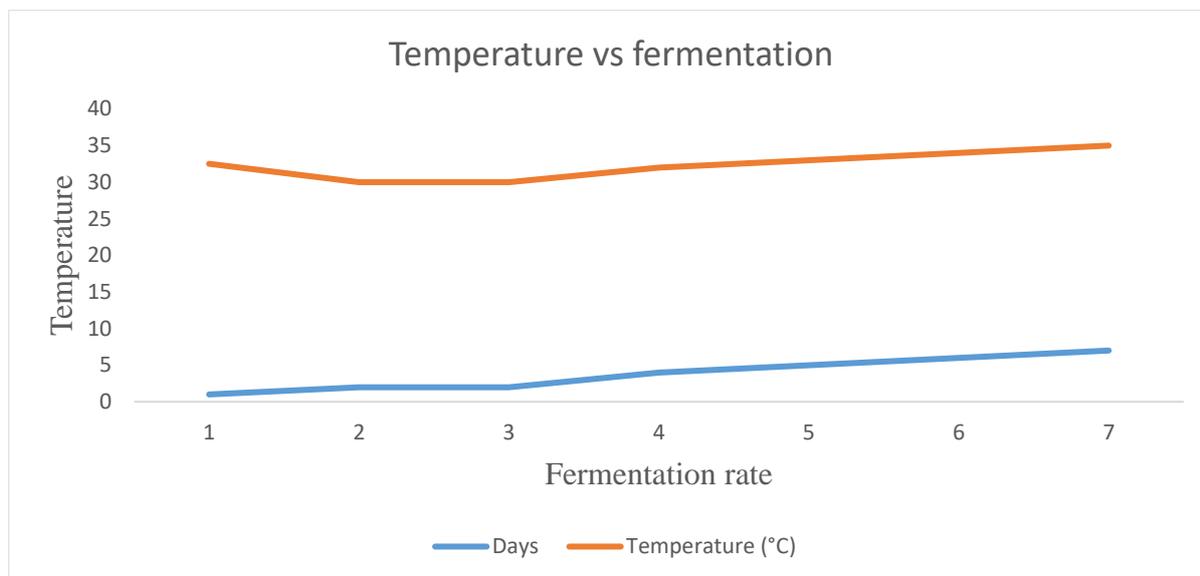


Fig. 4: Relationship between temperature and fermentation rate

Table 1: Correlation between pH, %TTA and temperature

	Days	Temperature °C	pH	%TTA(*10 ⁻²)
Days	1			
pH	-0.556	1		
Temperature °C	-0.732	0.882**	1	
%TTA (*10 ⁻²)	0.948**	-0.574	-0.829*	1

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

Increase in microbial growth was observed after 48 h of fermentation which might be as a result of the metabolic activities of the organisms that produce carbon dioxide and water (Chutmanop *et al.*, 2008). The gradual decrease observed in the lactic acid bacteria load as the fermentation progress may be due to the changes observed in the acidity of the fermenting medium. Abiola and Oyetayo (2016) reported a decrease in microbial load after 48 h of fermentation of cocoyam.

The decrease observed in bacterial load after 48 h of spontaneous fermentation may also be as a result of some bioactive substances which might have produced an inhibitory effect on other organisms involved in the fermentation. This is in line with the report of Chen and Hover (2003), Ouoba *et al.*, (2003, 2007) and Kalui *et al.* (2010). Increase in microbial growth was observed after the 6th day of fermentation which may be as a result of the metabolic activities of undesirable organisms.

Identification of Isolates

Each of the isolates was subjected to Gram stain test and were examined under light microscope. Each of the isolates gave blue-purple colour with staining; hence they were all Gram-positive bacteria. The isolates from MRS plates were rod-like bacilli with short or rounded ends. The

morphological characteristic is shown in Table 2. Spore staining and motility test were negative for all isolates on the MRS plates. This implies that none of the isolates formed spores and are non-motile. All of the isolates on MRS plates were tested for catalase. None of them showed catalase activity. This showed that they are catalase negative. The three isolates were observed to be negative to both methyl red and Voges Proskauer indicating that they cannot produce acetoin as the end product of glucose metabolism. The three isolates were observed to be negative to coagulase test as shown in Table 3. By implication, they cannot coagulate blood and this characteristic shows that they are non-pathogen. The yellow colouration observed during indole test indicates a negative result. This shows that the three isolates cannot produce the compound indole. Furthermore, Casein hydrolyses test for the three isolates were observed to be negative which indicates that they cannot produce the enzyme casease that breakdown casein. The result of starch hydrolysis as shown in Table 3 indicates that the three isolates were not able to hydrolyze the tested starch sample as indicated by the formation of a blue colour in the presence of iodine. The biochemical identification result for isolates on MRS plates is shown in Table 3.

The triple sugar iron (TSI) test revealed that isolate 1 was able to ferment all the sugars as shown to be positive in

Table 4, with no production of gas and acid. Isolate 2 was not able to ferment high molecular weight lactose and sucrose but fermented low molecular weight glucose with no gas and acid production. Isolate 3 was able to ferment all

the three sugars without gas and acid production. In addition to TSI test result, all the three isolates were able to ferment monitol and were all negative for xylose and citrate.

Table 2: Morphological characteristics of isolated organism

Morphological characteristics	Probable organism
Purple, short rod, irregular shape	<i>Lactobacillus</i> sub sp
Purple, short rod, regular shape	<i>Lactobacillus</i> sub sp
Purple, short rod but scanting	<i>Lactobacillus</i> sub sp

Table 3: Biochemical identification result for isolates on MRS plates

Biochemical Identification	Isolate 1	Isolate 2	Isolate 3
Morphology	Bacilli	Bacilli	Bacilli
Gram stain	Positive	Positive	Positive
Spore stain	Negative	Negative	Negative
Motility	Negative	Negative	Negative
Catalase	Negative	Negative	Negative
Methyl red and Voges Proskauer	Negative	Negative	Negative
Coagulase	Negative	Negative	Negative
Indole	Negative	Negative	Negative
Casein hydrolyses	Negative	Negative	Negative

Table 4: Result of TSI and other sugar fermentation

Test organism	Lactose	Sucrose	Glucose	Gas	Acid (H ₂ S)	Mannitol	Xylose	Citrate	Most probable Organism
Isolate 1	Positive	Positive	Positive	Negative	Negative	Positive	Negative	Negative	<i>Lactobacillus casei</i>
Isolate 2	Negative	Negative	Positive	Negative	Negative	Positive	Negative	Negative	<i>Lactobacillus delbruekii</i>
Isolate 3	Positive	Positive	Positive	Negative	Negative	Positive	Negative	Negative	<i>Lactobacillus brevis</i>

Tolerance to Bile Salt

The isolates were observed to grow at different concentration (2%, 4% and 6.5%) of bile salt agar after 24-48 hours of observation. Isolate 1 and 2 showed growth at a 2% and 4% concentration but no growth at 6.5%. Isolate 3 showed growth at 2% but no growth at 4% and 6.5% concentration. This indicates that they have tolerance to bile salt. The implication of this is that each of the isolates can survive the harsh condition of the gastrointestinal tract of both human and animal.

Growth at Different Temperatures

The isolates growth pattern at different temperatures was distinguishing within the groups as no growth was observed at 10 °C and 15 °C. However, all the isolates grew at 30 °C. This can imply that all the isolates are mesophiles. All isolated strains presented better growth at 37- 45 °C and small growth was observed at 15 °C. Based on the research carried out by Kandler and Weiss, (1989) and Cherubin,

(2003), *Lactobacilli* grow at temperatures between 25-53 °C, where the optimal temperature generally is between 30 °C and 40 °C. According to Bergey's Manual (Buchanan and Gibbons, 1974), the hetero-fermentative species grows at 45 °C and does not present any growth at 15 °C.

Antimicrobial Sensitivity

Observation reveals that the two isolates tested (1 and 2) have inhibitory capacity to destroy some pathogenic organism over the antibiotic control (ciprofloxacin) used as shown in Fig. 5. The first isolate has the highest inhibitory capacity on *Salmonella* sp and *Staphylococcus aureus* over control and isolate 2 but has no observable inhibition on *Shigella* sp. On the other hand, the second isolate has the widest clear zone on *E. coli* and *Shigella* sp than control and isolate indicating that it has the highest inhibitory capacity over the pathogen. However, there was no observable clear zone on *Staphylococcus aureus*.

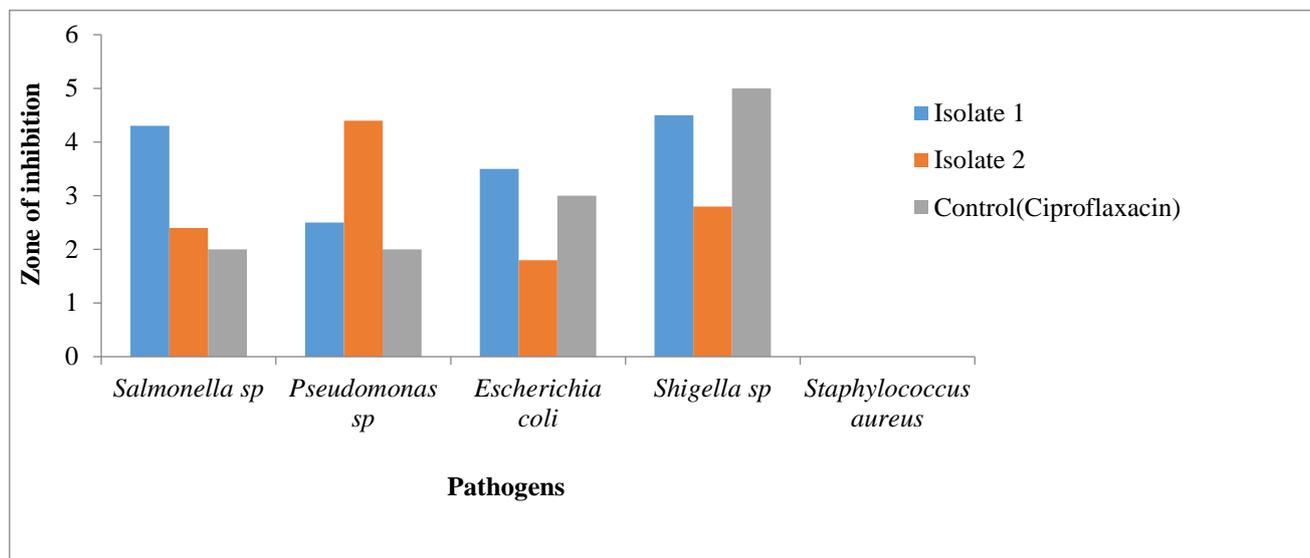


Fig. 5: Antimicrobial Susceptibility.

Conclusion

In this study, Lactic Acid Bacteria (LAB) was isolated, characterized, identified and stored from spontaneously fermented bambara groundnut. The results showed that isolates had probiotic properties like acid-bile tolerance and antimicrobial activity against food spoilage organisms and gastro intestinal tract (GIT) pathogens. These make them potential candidates for probiotic product, which can be beneficial to human and can then be used for nutraceuticals in the production of encapsulated products for the enhancement of immune system and proper functioning of the human GIT.

Authors' Contribution

Adeoye Babatunde Kazeem & Aransiola Elizabeth Funmilayo designed the research plan; Adeoye Babatunde Kazeem, Felix Oluwasola, & Adepoju Oluwarinu Aduragbemi performed experimental works & collected the required data. Alebiowu Gbenga & Bisi-Johnson Mary Adejumo analysed the data; Adeoye Babatunde Kazeem & Adepoju Oluwarinu Aduragbemi prepared the manuscript. All authors critically revised, finalized the manuscript & approved the final form of the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest with present publication.

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