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Characterization of the indigenous fermenters for the production of fermented condiments from soybean seeds

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Abstract

Several studies have shown that most condiments consumed in Nigeria today are produced from allochtonous microorganisms, and these organisms are usually associated with diseases or create negative impacts into the condiments. Several attempts have been made in order to control this ugly situation but there is still controversial thought in the choice of microorganism to be used in order to produce a non-toxic and palatable condiment. The aim of this study was to isolate and characterize the indigenous fermenters for the production of fermented condiments from soybean seeds. Soybean (*Glycine max)* sample were gotten from the market and were transferred aseptically to the laboratory for further analysis, the samples were prepared and fermented in a wrapped *Thaumatococcus danielli* leaves (called *Uma* in Igbo and *Ewe eran* in Yoruba). The specific microbial genera were enumerated on appropriate selected media and identified using standard identification parameters with the aid of identification and taxonomic manuals. Soybean sample was fermented with indigenous microorganisms isolated from 7 days old fermented soybean sample. This was oven-dried, pulverized and packaged in a cleaned sterile screw capped container. *Lactobacillus plantarum* strain ZS 2058 (L), *Bacillus subtilis strain* 168 (B) and *Saccharomyces cerevisiae* strain YJM555 (Y) were the indigenous microbes isolated from the 7 days locally fermented soybean and these were used for the production of light to dark brown condiments with water activity ranging from 0.27 – 0.37 for the fermented soybean in the plate and 0.22 - 0.36 for the fermented soybean wrapped with *Thaumatococcus danielli* leaves (called "uma" in Igbo and "ewe eran" in Yoruba). It was observed that the condiments produced using indigenous consortium of BLY was significantly ($p<0.05$) more preferable to the ones produced from single strain. Therefore this study has shown indigenous B, L, Y, BL and BLY produced good and palatable condiments from fermented soybean.

Keywords: Indigenous fermenters; Soybean; *Lactobacillus*; *Bacillus subtilis*; *Glycine max*.

1. Introduction

Soybean (*Glycine max* L.) is one of the nutritionally richest natural vegetable foods known to human kind, records of its usage dates back to 2838 BC in China. Due to its high satiety value caused by high oil content, poor digestibility, green beany taste, long cooking time and persistent bitterness, it has little direct use although it has high protein content, minerals, vitamins and bioactives. Soybeans like other bean species impact blood glucaose and lipid indices [1, 2]. Soybean as a food is used as soymilk, soyflour, soy oil, feed for livestock and poultry, soy concentrate, protein isolates, soy yoghurt, tofu and fermented foods such as Tempeh, soy sauce, Miso, Natto ans sufu [3, 4].

Fermented foods play an essential role in tackling issues of poverty, malnutrition, and hunger among African consumers [5]. Fermented foods are rich source of nutrients, with fermentation bringing about a "pre-digestion" of food substrates to make the associated nutrients more bioavailable and, in some instances, removing allergens (including 2S albumin

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proteins, profilins, cupin, prolamine), antinutritional compounds (such as phytate, tannins, lectins, protease inhibitors, saponins, alkaloids, and oxalate) and toxins (such as cyanogenic glycosides, bacterial toxins, mycotoxins, biogenic amines) [5-7]. The microbes associated have health-promoting properties either directly, with some probiotic strains from most commonly consumed and extensively investigated fermented foods in Africa or indirectly, through the production of health-promoting metabolites. Several fermented foods also contain prebiotic components [3, 8]. Indigenous fermentation processes represent traditional, reliable, and affordable methods of preserving nutritional and sensory qualities of associated substrates while extending shelf life and enhancing safety. The preservative effects of fermented foods are particularly important in Africa considering the tropical climatic conditions across large regions of the continents. Food fermentation is the only option for preserving foods, resulting in products with enhanced acceptability, digestibility, functionality, and nutritional quality. Such enhancements are more controllable where there is a corresponding understanding of the microbiota of the fermented food. Since ancient times, fermented foods have been produced by a process of natural (wild, spontaneous) fermentation, carried out by indigenous microorganisms naturally present in the raw material or processing environment [8]. The dominance of fermenting microorganisms, their metabolites and the changing pH of the raw material inhibit the growth of pathogenic microorganisms. Natural fermentation occurs also when a component containing a large number of microorganisms that initiate the fermentation process is added to the raw material. In both cases, the microorganisms involved in fermentation and the microclimate impact a product quality. The backsloping method, involving the use of a previously fermented product to inoculate a new batch, has also been used. This approach increases the chances of the desired microorganisms domination and competition with microorganisms that responsible for the product spoilage or disease. These traditional fermentation methods are still used today, primarily in home-based, local food production, or small-scale production. However, in the twentieth century, the development of microbiology, including food microbiology, has led to starter cultures introduction, which initiate the fermentation process and at the same time ensure greater product standardization. Such method results in products with constant organoleptic properties. Fermentation with well-defined cultures has found application, especially in the case of products obtained on an industrial scale. The process conducted under controlled conditions, it allows increasing the pace of the process and its throughput. The predominance of native microbiota allows limiting the growth of undesirable strains or species of microorganisms, as well as to reducing the toxic compounds they produce, ensuring the food safety [8]. Thus, while, to date, there is a corresponding understanding of many of the functional pathways and metabolic potential of several fermenting genera including Leuconostoc, Lactobacillus, Pediococcus, Debaryomyces, Kluyveromyces, Saccharomyces [5]. *Bacillus spp*. is the most dominant naturally fermenting agents in soybean, these hydrolytic bacteria are associated with utilization and reduction of indegistible oligosaccharides and polysaccharides. The organism has also shown to reduce the anti-nutrients that hinders availability of proteins and phytochemicals in soybeans [3], it also completely removes the beany odour of raw soybeans and increase sensory quality of the product [3]. Although several studies have been done on use of microorganism in production of condiments from fermented soybean, little is known about use of combination of microorganism, this present paper is aimed at characterizing indigenous fermenters for the production of fermented condiments from soybean seeds.

2. Material and methods

2.1. Sample Collection

This was carried out using the modified method of Suleiman and Omafe [9]. Soybean seeds were collected randomly from different shops and open markets in Eke Awka, Awka South LGA. Anambra State. Sampling was performed manually from different bags and basins, such that soybean seeds were collected from different parts of the bags and basins. The samples were aseptically pooled and mixed properly to form a bowl and placed in sterile nylon bag, the soybean seeds were properly labeled and taken to the laboratory for analysis.

2.2. Transportation

A sterile polythene bag containing ice blocks placed inside a cooler was used for the transportation of the sample. The temperature of the cooler was carefully checked and adjusted to 28^{o}C -30^{o}C in order to prevent or reduce microbial shock by reducing the quality of the ice inside the cooler. The samples were aseptically arranged inside the cooler without direct contact with the ice bag. The cooler was covered properly with packing tape to prevent accidental opening of the cooler. The cooler was taken to the laboratory safely for the analysis.

2.3. Preparation and Local Fermentation of the Soybean

Two hundred and fifty grams of cleaned soybean seeds were weighed using an analytical weighing balance and steeped in 500ml bucket of water overnight, after which the seedcoat were removed by rubbing between the palms and then the chaff were removed using sieve. The soybean seed were then thoroughly washed and placed inside cleaned

Thaumatococcus danielli leaves (called "uma" in Igbo and "ewe eran" in Yoruba) and wrapped properly and then kept inside 500ml bucket that was well covered with the lid for fermentation to take place for 7 days at room temperature.

2.4. Processing of the Fermented Soybean

After the fermentation the fermented soybean were prepared for culturing and the diluents used was peptone (BIOTECH) water whi ch was prepared according to the manufacturers instruction, then was sterilized by autoclaving at 121°C for 15min at 15 psi. Ten grams of the fermented soybean was aseptically weighed using analytical weighing balance into a 200 ml beaker (G.G) and little amount of the diluent was added and homogenized and then make upto 100 ml, part of these preparations was transferred into 100 ml beaker (G.G) and boiled for 10-15 min using a pressure pot.

2.5. Isolation of the Test Sample

The media used for this isolation includes Sabourand dextrose agar (SDA), de Man Rogosa and Sharpe broth (MRS) and Nutrient agar (BIOTECH). A 0.1 ml of the preparation/inoculum collected using a sterile pipette and aseptically plated onto solidified sabourand dextrose agar plate (90 mm x 15 mm) which was prepared according to the manufacturers instruction and the procedures described in Cheesbrough [10] supplemented with chloramphenicol (0.05 %) and spread using a spreading rod, 0.1 ml of the boiled preparation/inoculums was collected and plated unto solidified nutrient agar plate also 1 ml of the inoculums was collected using sterile pipette and aseptically inoculated into sterile 100 ml conical flask (Glassco) containing MRS broth (Oxoid) which was prepared according to the manufacturers instruction and the conical flask were incubated in a microaerophic environment (containing candle used to evacuate all traces of oxygen thereby creating an environment having only carbon iv oxide). The incubation was done for 24 – 72 h at (30±2°C). The SDA and NA were incubated in an inverted position for 24 h at 35±2°C (for NA) and 30±2°C (for SDA) in an incubator (STXB128)

2.6. Purification of the Isolates

The plate that showed discrete colonies were selected after 24 h and each colony was aseptically streaked using a sterile wireloop on a sterile poured plate (90 mm x 15 mm) containing nutrient agar (BIOTECH) prepared according to the manufacturers description. Similar procedure was repeated on SDA plate (90 mm x 15 mm) for the yeast and also on DeMan Rogosa Sharpe (MRS) agar plate (90 mm x 15 mm) that was prepared according to the manufacturers instruction after which it was incubated at their required growth conditions.

2.7. Characterization of the Bacteria Pure Isolates

The pure isolates were characterized using the morphological, biochemical and molecular characteristics as described by Iheukwumere *et al.* [11].

2.8. Morphological characteristics of the Bacteria isolates

The cultural descriptions (size, appearance, edge, elevation, colour) of the isolates were carried out as described in Goldman and Green [12]. The Gram staining technique which revealed the Gram reaction, cell morphology and cell arrangement were also carried out using the procedure described by Cheesbrough [10], Goldman and Green [12] and Frank and Robert [13]. The presence or absence of capsule was also carried out as described by Goldman and Green [12]. The presence or absence of flagellum was determined by carrying out motility test as described by Cheesbrough [10].

2.9. Gram staining technique

A thin smear was made in a cleaned grease free microscopic slide (75mm×25mm), air dried heat fixed. The smear was flooded with crystal violet solution (0.2%) for 60 seconds and rinsed with cleaned water. Gram iodine solution (0.01%) was then applied and allowed for 60 seconds. This was rinsed with cleaned water. This was followed by decolourizing the slide content with 95%w/v ethyl alcohol for 10seconds and then rinsed with cleaned water. The smear was then counter stained with safranin solution (0.025%) for 60 seconds, rinsed with cleaned water, blot drained and air dried. The stained smear was covered with a drop of immersion oil and observed under a binocular compound light microscope using × 100 objective lens.

2.10. Capsule staining technique

A thin smear was also made in a cleaned grease free microscopic slide (75 mm×25 mm), air dried and heat fixed. Crystal violet (1 %) was applied and allowed for 2 min. The crystal violet was gently washed off with 20 % copper sulphate solution. This slide was blotted and dried, and observed under compound binocular light microscope using oil immersion lens.

2.11. Motility test

A semi-solid medium prepared by mixing 5.0 g of bacteriological agar (BIOTECH) with 2.0 g of nutrient broth (BIOTECH) in 1 Litre of distilled water was used. The solution was dissolved and sterilized using autoclaving technique after dispensing 10 ml portion in different test tubes. The test tubes were allowed to set in vertical positions and then inoculate the test organisms by performing a single stab down the centre of the test tube to half the depth of the medium using sterile stabbing needle. The test tubes were kept in an incubator in vertical position at 35 ± 2^{o}C for 24h.

2.12. Biochemical characteristics of the isolates

The capability of the isolates to produce catalase, indole, oxidase, acetoin, grow in 6.55 % NaCl and to utilize sugars, sugar alcohols and other substances (ribose, sorbitol, arabinose, sacharose, glucose trehalose, lactose, starch, inulin, salicin, hiparate) and also the haemolytic activity of the isolates were done using the methods described by Cheesbrough [10], Goldman and Green [12] and Frank and Robert [13].

2.13. Indole test

Indole is a nitrogen containing compound formed when the amino acid tryptophan is hydrolyzed by bacteria that have the enzyme tryptophanase. This is detected by using KOVAC's reagent. For this test, isolates were cultured in peptone water in 500.0 ml of deionized water. Ten millilitres of peptone water was dispensed into the test tubes and sterilized. The medium was then inoculated with the isolates and kept in an incubator at 37˚C for 48 hr. Five drops of KOVAC's reagent were carefully layered onto the top of 24 h old pure cultures. The presence of indole was revealed by the development of red layer colouration on the top of the broth cultures.

2.14. Sugar fermentation test

The capability of the isolates to metabolize some sugars (glucose, xylose, ducitol, maltose, arabinose, inositol, mucate and lactose) with the resulting formation of acid and gas or either were carried out using sugar fermentation test. One litre of 1 % (w /v) peptone water was added to 3 mL of 0.2 % (w/v) bromocresol purple and 9 ml was dispensed in the test tube that contained inverted Durham tubes. The medium was then sterilized by autoclaving. The sugar solution were prepared at 10% (w/v) and sterilized. One milliliter of the sugar was dispensed aseptically into the test tubes. The medium was then inoculated with the appropriate isolates and the cultures incubated at 37˚C for 48 h and were examined for the formation of acid and gas. Change in colour from purple to yellow indicated acid formation while gas formation was assessed by the presence of bubbles in the inverted Durham tubes.

2.15. Hydrogen sulphide production

This was performed using triple sugar iron (TSI) agar. The TSI agar was made in accordance to the manufacturer's instruction. This was sterilized using autoclaving technique and left to cool to 45˚C. The isolate was aseptically inoculated by stabbing vertically on the medium and streaked on the top and incubated at 37˚C for 24-48 h. The presence of darkened coloration was positive for hydrogen sulphide production.

2.16. Ornithine decarboxylase test

This test determines the capability of the organism to produce the enzyme decarboxylase which removes the carboxyl group from an amino acid. The medium ornithine decarboxylase broth is used. The medium was prepared according to the manufacturer's instruction and dispensed into test tubes, sterilized using autoclaving technique and left to cool to 45˚C. The medium was inoculated with the appropriate culture and incubated at 37˚C for 24-48 h and were examined for colour change. A change in color from purple to yellow indicates a positive result.

2.17. Methyl red test

The glucose phosphate broth was prepared according to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 hr. After incubation, five drops of 0.4 % solution of alcoholic methyl red solution was added and mixed thoroughly, and the result was read immediately. Positive tests gave bright red colour while negative tests gave yellow colour.

2.18. Voges-Proskauer test

The glucose phosphate broth was prepared in accordance to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48hr. After incubation, 1.0 mL of 40% potassium hydroxide (KOH) containing 0.3 % Creatine and 3 ml of 5 % solution of α-naphthol was added in the absolute alcohol. Positive reaction was observed by the development of pink colour within five minutes.

2.19. Citrate utilization test

The Simmon's Citrate Agar was prepare according to the manufacturer's direction and the isolates were inoculated by stabbing directly at the center of the medium in the test tubes and incubated at 37˚C for 48 hr. Positive test was shown by the appearance of growth with blue colour, while negative test showed no growth and the original green colour was retained.

2.20. Catalase test

The test was carried out as described by Cheesbrough [10]. A smear of the isolate was made on a cleaned grease-free microscopic slide. Then, a drop of 30% hydrogen peroxide (H_2O_2) was added on the smear. Prompt effervescence indicated catalase production.

2.21. Oxidase test

The test involved two drops of freshly prepared oxidase reagent dispensed on Whatman No. 1 filter paper which was placed in Petri dish, and a smear of the test isolate was made on the spot using a sterile stick. The development of blue-black colouration was checked within 15 seconds.

2.22. Urease test

This was carried out as described by Cheesbrough [10]. The urea agar slant was prepared in accordance to the manufacturer's direction and the isolates were aseptically inoculated into sterilized medium. This was incubated at 37^0C for 48 h. After incubation, observation was made for the presence of purple-pink colouration.

2.23. Gelatin hydrolysis test

Gelatin agar (BIOTECH) was used for this test. The medium was prepared according to the manufacturer's direction and poured into Petri dishes and allowed to solidify. The test isolates were aseptically streaked on the surface of the medium and then incubated for 24-48 h at 35±2^{0}C. The hydrolysis of the medium was checked after 24-48 h incubation.

2.24. Starch hydrolysis

This is used to determine the ability of the bacteria to hydrolyze starch. This was carried out as described by Cheesbrough [10]. The starch agar was prepared in accordance to the manufacturer's direction and the isolates were aseptically streaked into sterilized medium and incubated for 48 h at 37^{0}C, the surface of the plate was flooded with iodine solution with a dropper for 30 sec., excess iodine was poured off, presence of clear zone around the line of bacterial growth indicates positive results while a blue, purple or black coloration of the medium indicate negative result.

2.25. Coagulase test

The test was carried out as described by Cheesbrough [10]. A smear of the isolate was made on a cleaned grease-free microscopic slide that contained a drop of physiological saline, a drop of human plasma was added and gently mixed, after 10 sec. presence of clump indicate positive result.

2.26. Esculine test

Bile esculine agar (BIOTECH) was used for this test. The medium was prepared in accordance to the manufacturer's direction, and poured into test tubes (Pyrex) in slant positions. The test isolates were asceptically streaked on the surfaces of the slant medium and then incubated for 24-48 h at 35±2^{0}C. The blackening of more than half of the medium was checked after 24-48 h incubation.

2.27. Identification of Fungal Isolates

The fungal isolates were identified to the genus/species level based on macroscopic, microscopic and molecular characteristics of the isolates obtained from pure cultures as described in the study published by Iheukwumere *et al*. [14].

2.28. Macroscopy

The colonies were carefully examined for fungal characteristics. The rate of growth, color, shape, texture, consistency of the growth and other peculiar features of the colonies were observed as described in the study published by Iheukwumere *et al*. [14].

2.29. Microscopy

This was carried out using Needle mount technique. A drop of lactophenol cotton blue (LCB) solution was placed on the center of a clean grease-free slide. A fragment of the colony was placed in the drop of the LCB using sterile wire loop and covered with a cover-slip to avoid air bubbles. Excess fluid from the outside of the cover slip was wiped with cotton wool and slide was passed through the flame to warm the staining so as to remove the remaining air bubbles and facilitate staining of the fungal element. The slide was then examined under the microscope, using low-power objective of ×10 magnifications, and followed by high-power objective of ×40 magnifications to reveal the nature of the hyphae, shape, size, texture and arrangement of the conidia. The pictorial nature of the fungal organisms was confirmed using the fungal atlas as described in the study published by Iheukwumere *et al*. [14].

2.30. Molecular characterization of the isolates

2.30.1. Extraction and purification of DNA

All strains were plated on Nutrient Agar (Biotech) and incubated at 37°C for 24 hr. By means of the procedures of Zymo Research (ZR) DNA miniprepTM kit, bacterial genomic DNA was then extracted and purified (Category No. D6005; Irvine, California, USA) as described by Iheukwumere *et al.* [11].

2.30.2. Determination of the quality of extracted DNA

Using mass spectrophotometer (Nanodrop), One micro litre (1µL) was aseptically dropped into a fresh space in the chamber and the chamber was lightly closed which was then linked to a computer system which showed the window that discovered the value of the sample at 260/280nm as described by Iheukwumere *et al.* [11].

2.30.3. Amplification of DNA and gel electrophoresis of PCR product

This was analysed using Master cycler Nexus Gradient (Eppendorf). A mixture of primer (20 µL), template DNA (20µL), water (72 µL) and master mix (108 µL), which comprises taq polymerase, dimethylsulfoxide (DMSO), magnesium chloride ($MgCl_2$) and nucleotides triphosphates (NdTPs), was made in 1.5 mL tube and homogenized using vortex mixer (Eppendorf). This was then positioned in the block chamber of the master cycler and then programmed. The PCR program for conditions were as follows: initial incubation at 94°C for 5 mins, followed by 35 cycles of denaturation at 94°C for 15 secs, annealing at 55°C for 15 secs, elongation at 72°C for 21 secs and final extension period for 10 mins at 72°C. The amplified products were electrophorezed in 1.0% agarose gel and a1kb DNA ladder was used as a size reference. After staining with 3µL of nucleic acid stain (GR green), the gel was documented with gel documentation apparatus [11].

2.30.4. DNA sequencing of 16s rRNA fragment

The 16S rRNA amplified PCR products generated from universal primer (16S), was used for the sequencing using ABI DNA sequencer (Applied Biosystem Inc) at International Institute of Tropical Agriculture (IITA), Ibadan using the method of Iheukwumere *et al.* [11].

2.30.5. Computational Analysis

This was analysed making use of the modified method of Iheukwumere *et al.* [11]. The chromatograms generated from the sequences were cleaned to obtain regions with normal sequences. The cleaned nucleotides were aligned using pair wise alignment tool. The consensus sequences formed by the alignment of the forward and reverse sequences were used to perform the Basic Local Alignment Search Tool (BLAST) using National Centre for Biotechnology Information BLAST over the internet. The sequences of the isolates with 95% and above similarities were accepted. Also the

maximum scores, total scores and accession numbers of the isolates were assessed. The relatedness of the isolates was determined by tracing their phylogenetic tree using DNA distance neighbour phylogenetic tree tool.

2.31. Preparation of Soybean Condiments

2.31.1. Processing of soybean for fermentation

This was carried out using the modified method of Farinde *et al.* [15]. One kilogram of soybean were carefully picked and weighed using analytical weighing balance and steeped in 200 ml bucket of water overnight for fermentation to take place, after the soybean were dehaulled by rubbing between the hands to remove seed coat, after the chaff/seed coat were properly removed using a clean sieve, the soybean was then properly washed and placed inside a beaker and then autoclaved at 121^{o}C for 15 min at 15 psi.

2.31.2. Fermentation Process

This was carried out using the modified method of Hu *et al.* [16] and Chukeatirote *et al*. [17]. After autoclaving the soybean, a 100g of soybean was weighed using analytical weighing balance and placed inside 6 different *Thaumatococcus danielli* leaves (called "uma" in Igbo and "ewe eran" in Yoruba) which was properly sterilized using electric oven at 180^{0}C for 2 h, each of the leaves containing the soybean were inoculated with the fermenters prepared and diluted to a turbidity that matched 0.5 MacFarland standard that was prepared by mixing 0.6 mL of 1% $BaCl_2$. $2H_2O$ and 99.4 mL of 1% Conc. H_2SO_4, 10 ml of suspension *Bacillus* was added and labeled as "B" , 10 ml of suspension of *Lactobacillus* was added and labeled as "L", 10ml of suspension of yeast was added and labeled as "Y" , consortium of suspensions 5ml of Bacillus and 5 ml of Lactobacillus was added and labeled as "BL", consortium of suspensions of 3ml of *Bacillus*, 3 ml of *Lactobacillus* and 4ml of yeast were added and labeled as "BLY" consecutively and one of the leaves containing only soybean was set aside as the control. These leaves were carefully wrapped. This same method was repeated using sterile plates. The wrapped leaves and the plates containing the soybean were kept at room temperature for fermentation to take place for 7days.

2.31.3. Storage and packaging

After fermentation, the fermented samples were aseptically dried using an electric oven at 80^{0}C for 7days**.** After drying water activity of the fermented samples was determined, after which it was grinded into powder and stored in a sterile screw capped container for subsequent analysis.

2.32. Data Analysis

The data obtained in this study were presented in tables and figures. Their percentages were also calculated. The sample means and standard deviations of some of the analytical data were also calculated. The significance of the prevalence of the isolates in the studied samples was determined at 95% using one way analysis of variance (ANOVA). Pairwise comparison was carried out using student "t" test.

3. Results

The isolate were culturally and morphologically characterized using their appearance on agar plate, edge, surface of the colony, optical nature, gram reaction, cell morphology, spore, position of spore, capsule, motility and formation of bud. Isolate A, isolate M and isolate X showed similar appearance on the agar plate, gram positive and did not possess capsule, isolate M and isolate X showed similarity on their edge, surface of the colony, optical nature, lacks spores, lacks capsule, are non-motile while they differ in their elevation, cell morphology and formation of bud. They were tested for the following catalase, coagulase, citrate, Gelatin, MR, Vp, indole, urease, oxidase, H_2S, starch hydrolysis, ornithine decarboxylase and Esculin hydrolysis. The isolates were all negative for coagulase, indole, and urease and ornithine decarboxylase. Isolate A and X are both positive to catase and hydrogen sulphude production while isolate A and M are positive to starch hydrolysis and Esculin hydrolysis. The study revealed the ability of the isolate to utilize some sugars. The sugars tested include D-mannitol, Arabinose, glucose, galactose, fructose, sucrose, lactose, maltose, xylose, arabitol, dricitol, inositol, sorbitol, starch and trehalose. The isolates were all able to utilize D-mannitol, Arabinose, glucose, fructose, sucrose, and maltose but were not able to utilize arabitol, ducitol. Isolate M and isolate Y are not able to utilize inositol, xylose, sorbitol and starch. The qualities of nucleic acids (DNA) extracted form isolate L, B and Y, were within the stipulated range (1.80-1.90). Purity of nucleic acids (DNA) was determined by calculating the ratio of the absorbance $A260/A280$ as shown in Table 4. The amplicous generated from nucleic acid (DNA) extracted from the isolates were cleaned and re-electropheresed and the sequencing of the amplified regions of isolate L, B and Y showed 100% identities

of each of the isolates. The study revealed the presence of *lactobacillus plantarum* strain 252058 (isolate L), *Bacillus subtilis* strain 168 (isolate B) and *saccharomyces cerevisae* strain YJM 555 (isolate Y) as shown in Table 5.

The soybean was initially yellow in colour, solid and dried, after overnight steeping and peeling the soybean became moist and slippery. After fermenting the soybean there were changes in the colour, the colour of ones in 'uma' leaves (*Thaumatococcus danielli*) became light brown, has a characteristic odour, slippery and moist while the ones in plate are dark brown coloured, slippery and moist with a characteristic odour. After drying the soybean some of the ones in leaves became light brown coloured while some are cream coloured, had a characteristic odour, dried with a water activity ranging from 0.27 to 0.37, while the ones in plates, some are dark brown while some are light cream coloured with water activity ranging from 0.22 to 0.26. The prepared condiments were grounded into powder some of the ones in leaves were light cream coloured with a characteristic odour with water activity that ranged from 0.27 to 0.37, some of the ones in plates were dark creamed and some light brown with water activity that ranged from 0.22 to 0.26. The prepared condiments were packaged with a sterile transparent screw capped container and properly labeled for futher analysis.

Table 1 Cultural and morphological characteristics of the isolates

Parameter	**Isolate A**	**Isolate M**	**Isolate X**
Appearance on agar plate	Cream/white	Cream/white	Cream
Elevation	Flay	Low-convex	Raised
Edge	Irregular	Smooth	Smooth
Surface of the colony	Rough	Smooth	Smooth
Optical nature	Opaque	Transparent	Transparent
Gram reaction	+ve	+ve	+ve
Cell morphology	Rod	Rod with round ends	Oval shape
Spore	+	-	-
Position of spore	Central spore	-	-
Capsule	+ve	-	-
Motility	+	-	-
Formation of bud	-	-	+

Key: + = positive; - = negative

Table 2 Biochemical characteristics of the isolates

Parameter	**Isolate A**	**Isolate M**	**Isolate X**
Catalase	+	-	+
Coagulase	-	-	-
Citrate	+	-	-
Gelatin	+	-	-
MR	-	-	+
Vp	+	-	-
Indole	-	-	-
Urease	-	-	-
Oxidase	+	-	-
H_2S	+	+	-
Starch hydrolysis	+	+	-
Ornithine decarboxylase	-	-	-
Esculinhydrolysis	+	+	-

Key: + = positive - = negative

Table 3 The sugar utilization potential of the isolate

Parameter	Isolate A	Isolate M	Isolate X
D-mannitol	+	+	+
Arabinose	+	+	+
Glucose	+	+	+
Galactose	+/-	-	+
Fructose	+	+	+
Sucrose	+	+	+
Lactose	+/-	+	+
Maltose	+	+	+
Xylose	+	-	-
Arabitol	-	-	-
Ducitol	-	-	-
Inositol	+	-	-
Sorbitol	+	-	-
Starch	+	-	-
Trehalsoe	+	+/-	+

Key: + = positive; - = negative; +/- = slightly positive (50%)

Table 4 DNA quantification of microbial nucleic acid samples with nanodrop-2000 spectrophotometer

Sample ID	Nucleic Acid (ng/Nl)	260nm	280nm	260/280
L	122.70	3.5148	1.9312	1.82
B	166.40	3.7208	2.0010	1.86
Y	99.20	2.0473	1.258	1.82

Table 5 Molecular identities of the microorganisms used for this study

Parameter	Isolate L	Isolate B	Isolate Y
Max Score	4783	6835	5171
Total score	15366	6835	9914
Query cover (%)	100	100	100
E-value	0.0	0.0	0.0
Identity (%)	100	100	100
Accession length	3198337	4316079	84195
Accession Number	CPO 12343.1	CPO53102.1	CPO006491.2
Description	*Lactobacillus*	Bacillus	*Saccharomyces*
	Plantarum	*Subtils*	*Cerevisiae*
	Strain ZS2058	Strain 168	Strain YJM555
	Complete genome (L)	Complete genome (B)	Complete genome (Y)

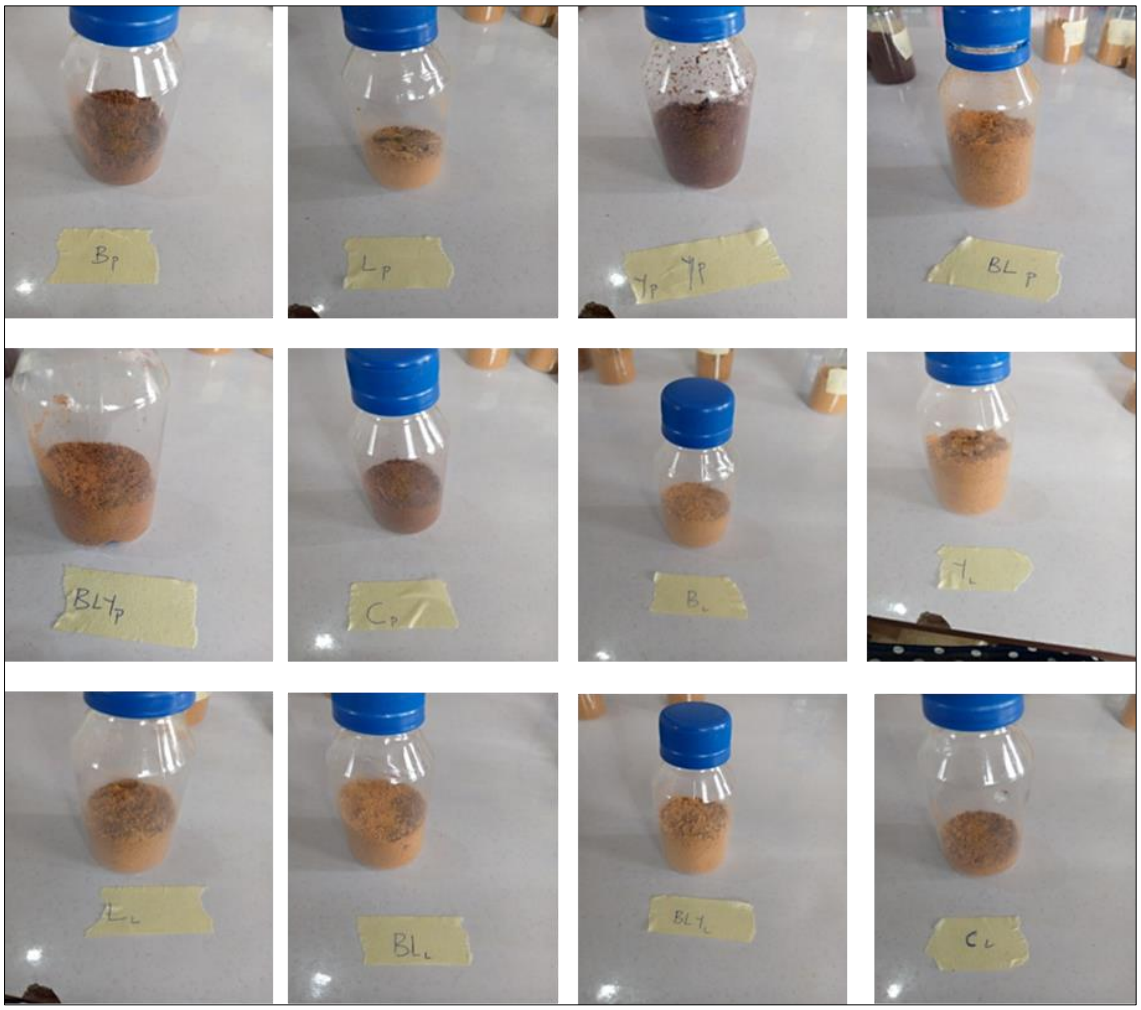

Figure 1 Prepared condiments

4. Discussion

The occurrences of *Bacillus substilis* strain 168 (B), *Lactobacillus plantarum* strain ZS2058 (L) and *Saccharomyces cerevisiae* strain YJM 555 (Y) as indigenous organisms supported the findings of many researchers [18-21]. The occurrences of these organisms could be attributed to favourable and optimum environmental conditions which is suitable for the survival and multiplication of the organisms. The above report corroborated with the reports of other researchers [20].

The prepared condiments from the indigenous microorganisms had some characteristic features which differ from that produced by many researchers [22, 23]. The condiments used in the present study were prepared using individual isolates (B, L and Y) and consortium of the isolates, and these differ with other condiments that were prepared by other researchers 22, 23]. The water activity and packaging of the present condiments differ from the condiment prepared by Olasupo and Okorie [22] and Adamu *et al*. [23]. Also the dark brown or dark appearances associated with the condiments prepared from fermented soybean wrapped in *Thaumatococus daniellii* ("Uma" leaves) leaves could be attributed to the secretion from some bioactive components of the leaves. The water activity of the prepared condiments was within the stipulation of the regulatory bodies, World Health Organization (WHO) and Nigeria Industrial Standard (NIS) for regulation of microorganisms and preservation of dried food samples. The regulatory bodies stated that water activity (aw) of 0.85 or less is suitable for controlling the growth of microorganisms in food samples.

5. Conclusion

This study has shown that indigenous *Bacillus subtilis* strain 168 (B), *Lactobacillus plantarum* strain ZS2058 (L) and *Saccharomyces cerevisiae* strain YJM555 (Y) can produced good and palatable condiments, of which consortium of BLY is most preferable.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declare no conflict of interest.

References

[1] Chang JH, Kim MS, Kim TW, Lee SS. Effects of soybean supplementation on blood glucose, plasma lipid levels, and erythrocyte antioxidant enzyme activity in type 2 diabetes mellitus patients. Nutr Res Pract. 2008;2(3):152-157. doi:10.4162/nrp.2008.2.3.152.

[2] Egbuna C, Ifemeje JC, Nwaka AC, Olisah MC, Lukong CB, Patrick-Iwuanyanwu KC, Ifemeje MO, Iheukwumere IH, Iheukwumere CM. Blood glucose level and serum lipid profile of wistar albino rats fed four species of local beans consumed in South-east, Nigeria. *European Journal of Biomedical and Pharmaceutical Sciences*, 2020; 7(5): 30-39.

[3] Shrestha AK, Dahal NR, Ndungutse V. Bacillus fermentation of soybean:A review. *Journal of Food Science and Technology.* 2010; 15: 30 – 365.

[4] Das, S., Sharangi, A.B., Egbuna, C., Jeevanandam, J., Ezzat, S.M., Adetunji, C.O. et al. (2020). Health Benefits of Isoflavones Found Exclusively of Plants of the Fabaceae Family. In: Egbuna, C., Dable Tupas, G. (eds) Functional Foods and Nutraceuticals. Springer, Cham. https://doi.org/10.1007/978-3-030-42319-3_22.

[5] Obafemi YD, Oranusi SU, Ajanaku KO, Akinduti PA, Leech J, Cotter PD. African fermented foods: overview, emerging benefits and novel approaches to microbiome profiling. *Nature Partner Journal Science of Food.* 2022; 6: 15 – 25.

[6] Ifemeje, JC, Egbuna C, Eziokwudiaso JO, Ezebuo FC. Determination of the Anti-nutrient Composition of *Ocimum gratissimum, Corchorus olitorius, Murraya koenigii Spreng* and *Cucurbita maxima*. *International Journal of Innovation and Scientific Research*, 2014; 3(2): 127-133.

[7] Egbuna C, Ifemeje J.C. Biological Functions and Anti-nutritional Effects of Phytochemicals in Living System. IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS), 2015; 10(2): 10-19.

[8] Palai, S., Derecho, C.M.P., Kesh, S.S., Egbuna, C., Onyeike, P.C. (2020). Prebiotics, Probiotics, Synbiotics and Its Importance in the Management of Diseases. In: Egbuna, C., Dable Tupas, G. (eds) Functional Foods and Nutraceuticals. Springer, Cham. https://doi.org/10.1007/978-3-030-42319-3_10.

[9] Suleiman, Omafe KA. Identification and characterization of hyphomycetous fungi in bean seeds. *American Journal of pathology.* 2013; 114: 23–27.

[10] Cheesbrough M. District Laboratory Practice in Tropical Countries (2nded.). Cambridge University Press: Cambridge, England. 2010; 45–70.

[11] Iheukwumere IH, Olusola TO, Chude C. Molecular characterization and diversity of enteric bacteria isolated from chicken feeds. *Journal of Natura Sciences Research.* 2018; 8: 21–33.

[12] Goldman E, Green LH. Practical Handbook of Microbiology. (2nded.). CRC Press: London. 2009; 31–53.

[13] Frank EB, Robert CJ. Practical Medical Microbiology for Clinicians. (1sted.).Wiley & Sons Inc: Hoboken, New Jersey, USA. 2015; 13–40.

[14] Ihekwumere CM, Umedum CU, Iheukwumere IH. Identities and prevalence of Aspergillus species on *Phaseolus vulgaris* (Bean) seeds sold in Ihiala, Anambra State, Nigeria. *Greener Journal of Microbiology and Antimicrobials.* 2020; 5(1): 16 – 25.

[15] Farinde A, Owolarafe O, Ogungbemi I. An overview of production, processing, marketing and utilization of Okra in egbedore local government area of Osun State, Nigeria. *Agriculture and Engineering.* 2007; 4: 1 – 17.

[16] Hu Y, Ge C, Yuan W, Zhu R, Zhang W, Due L, Xue J. Characterization of fermented black soybeans Natto inoculated with *Bacillus* Natto during fermentation. *Journal of Science food and Agriculture*. 2010; 90: 1194 – 1202.

[17] Chukeatitrote E, Dajanta K, Apichartssrangkoa A. Thua Nao indigenous Thai fermented soybeans. *Journal of Biological Science*. 2010; 10: 581 – 583.

[18] Oz E, Kaban G, Baris O, Kaya M. Isolation and identification of lactic acid bacteria from pastirma. *Food Control*. 2017; 77: 158 –162.

[19] Salam NA, Elly R, Evy R, Endang P, Husmaini S. Isolation and identification of lactic acid bacteria from okara and evaluation of their potential as candidate probiotics. Pakistan Journal of Nutrition. 2017; 16: 618 – 628.

[20] Zhu J, Geo M, Zhang R, Sun Z, Wang C, Yang F, Huang T, QU S, Zhao L, Li Y, Hao Z. Effects of soybean meal fermented by *Lactobacillus plantarum, B. subtilis and S. cerevisiae* on growth, immune function and intestinal morphology in weaned piglets*. Microbial Cell Factories.* 2017; 16: 191 – 201.

[21] Anggraeni SD, Husmaini H, Sabrina S, Zulkarnain Z, Rossi E. Viability of *Lactobacillus planetarium and Lactobacillus pentosus* isolated from solid waste of soy milk as candidate probiotic for poultry. *International Conference of Bio-Based Economy and Agricultural Utilization.* 2019; 497: 1 – 7.

[22] Olasupo NA, Okorie PC. African Fermented Food Condiments: Microbiology Impacts on Their Nutritional Values. In: Solís-Oviedo, RL, de la Cruz Pech-Canul, Á, editors. Frontiers and New Trends in the Science of Fermented Food and Beverages [Internet]. London: IntechOpen; 2019 [cited 2022 May 23]. Available from: https://www.intechopen.com/chapters/65270 doi: 10.5772/intechopen.83466.

[23] Adamu AS, Farouq AA, Magashi MA, Ibrahim AD. Taste profile and consumer preference of "dawadawa" produced from the seeds of Parkia biglobosa, Glycine max and Hibiscus sabdariffa. Int. J. Biol. Chem. Sci. 2019; 13(1): 178-185.