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Research Article

Isolation and Biochemical Characterization of Microorganisms Associated with the Fermentation of Kersting's Groundnut (*Macrotyloma geocarpum*)

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Abstract

The present research is centered on the microorganisms associated with liquid and solid fermentation of Kersting's groundnut (*Macrotyloma geocarpum*) using standard techniques. The characterized microbial isolates were: *Bacillus licheniformis*, *B. subtilis*, *B. megaterium*, *B. polymyxa*, *B. coagulans*, *Lactobacillus plantarum*, *Staphylococccus aureus*, *Aspergillus niger*, *Rhodotorula* spp., *Aspergillus parasiticus*, *Rhizopus stolonifer* and *Saccharomyces cerevisiae*. Total Bacterial Count (TBC) for liquid fermentation ranged from 3.67×10^3 CFU g^{-1} to 9.67×10^3 CFU g^{-1}, while, TBC for solid fermentation were between 3.67×10^3 CFU g^{-1} to 8.00×10^3 CFU g^{-1}. There was no fungi growth recorded in the raw sample before fermentation. However, significant growth of 2.67×10^3 SFU g^{-1} and 2.33×10^3 SFU g^{-1} was recorded after 24 h of fermentation in solid and liquid fermentation, respectively. The growth increased to 6.67×10^3 SFU g^{-1} and 4.67×10^3 SFU g^{-1} in solid and liquid fermentation, respectively, after 72 h of fermentation. A total colony of 1.23×10^4 CFU g^{-1} was observed for lactic acid bacteria after 24 h of liquid fermentation, the growth decreased significantly ($p<0.05$) to 4.33×10^3 CFU g^{-1} after 72 h of fermentation. No lactic acid bacteria was observed in solid fermentation. All the five species of Bacillus isolated were present in both liquid and solid fermentation, with *Bacillus subtilis* as the predominant organism throughout the fermentation period.

Key words: Fermentation, Kersting's groundnut, biochemical characterization, percentage occurrence

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Kersting's groundnut also known as Geocarpa groundnut or Groundbean (*Macrotyloma geocarpum*) is the third subterranean legume after groundnut and bambara groundnut (Marechal and Baudet, 1977; Dako and Vodouhe, 2006; Adu-Gyamfi *et al*., 2012). It is an annual leguminous crop that belongs to the family leguminosae and the subdivision Papilionoideae (Bayorbor *et al*., 2010). It is closely related to Bambara groundnut (*Vigna subterranea*) in having a similar geocarpic character. However, unlike Bambara groundnut and common groundnut (*Arachis hypogaea* L.), it is Cleistogamous. It is an indigenous crop cultivated in parts of West Africa for food (Buah and Huudu, 2007; Aremu *et al*., 2006). It is a promising alternative source of high quality protein and feed for the tropics (Obasi and Agbatse, 1994; Adu-Gyamfi *et al*., 2012). It is reported to be an under-exploited legume (Amujoyegbe *et al*., 2007; Adu-Gyamfi *et al*., 2012; IPGRI., 2001).

Fermentation is a process which involves the conversion of large molecules to small molecules or molecular oxidation/reduction mechanisms mediated by selected microorganisms (Yadav *et al*., 2011). The mechanism of food fermentation is essentially the conversion of carbohydrates to alcohols and carbon dioxide or organic acids by yeasts, bacteria or a combination thereof, under anaerobic conditions (Willams and Dennis, 2011). In fermentation, the substrate is only partially oxidized, ATP is formed exclusively by substrate-level phosphorylation and oxygen is not needed. Traditional fermentation processes can also be called uncontrolled/spontaneous fermentation which is dependent on microorganisms from the environment. Traditional fermentation process is the most available and affordable food preservation method, which is of great economic importance to developing countries (FAO., 2008; Adesulu and Awojobi, 2014). The microorganisms responsible for the fermentation may be the micro-flora indigenously present on the substrate, or they may be added as starter cultures (Adesulu and Awojobi, 2014).

Microorganisms are living creatures that are microscopic in size and are heterogeneous organisms such as algae, fungi (mould and yeasts) and bacteria (Oyewole and Isah, 2012). The multiplication of microorganisms in food is greatly influenced by the inherent (intrinsic factors) and environmental characteristics of the food (Onyenekwe *et al*., 2012). Indigenous natural fermentation takes place in a mixed colony of microorganisms such as moulds, bacteria and yeasts (Willams and Dennis, 2011). Nearly all food fermentations are the result of more than one microorganism, either working together or in sequence, but growth is generally initiated by bacteria, followed by yeasts and then moulds (FAO., 1998; Adesulu and Awojobi, 2014). Compounds formed during fermentation processes includes; organic acids (palmitic, pyruvic, lactic, acetic, propionic, malic, succinic, formic and butyric acids), alcohols (mainly ethanol) aldehydes and ketones (acetaldehyde, acetoin, 2-methyl butanol) (Ari *et al*., 2012). The aim of this research is to isolate, characterize and compare the percentage occurrence of microorganisms associated with liquid and solid fermentation of Kersting's groundnut (*Macrotyloma geocarpum*).

MATERIALS AND METHODS

Source of kersting's groundnut: Kersting's groundnut (*Macrotyloma geocarpum*) seeds were purchased from a seller at Isolo market, Akure South Local Government area of Ondo State, Nigeria. The seeds were identified and authenticated at the Department of Crop, Soil and Pest Management of Federal University of Technology, Akure, Ondo state. The seeds were de-husked, cleaned and sorted for further study.

Processing of kersting's groundnut: The sorted seeds were divided into three portions, coded A, B and C of 500 g. Portion A was subjected to solid fermentation, where the de-husked seeds were wrapped in blanched banana (*Musa acuminata*) leaves and allowed to ferment for three days at $28\pm2^\circ$C in a clean plastic bowl with cover. Portion B was subjected to liquid fermentation in which seeds were soaked in water in the ratio of 1:3 w/v in a clean plastic bowl with cover and allowed to ferment for three days at $28\pm2^\circ$C. Portion C which serves as control was analyzed raw.

Isolation of microorganisms and determination of total viable count: One gram of macerated Kersting's groundnut (raw and fermented) was put into 9 mL of sterile water and diluted serially to obtain a dilution of 10^{10} dilution factor. One milliliter was pipetted from dilution tube (10^{-3}) into sterile petri-dishes. Thereafter, 20 mL of Nutrient agar, De Man Rogosa Sharpe and Potato dextrose agar cooled to 45°C then poured separately onto each of the plates in triplicates and the plates were gently swirled and allow to solidify. The nutrient agar plates were incubated at 37°C for 24 h while Potato Dextrose Agar plates were incubated at 25°C for 3-5 days. The agar plates of De Man Rogosa Sharpe which is used for isolation lactic acid bacteria were incubated anaerobically in an anaerobic jar. After incubation, the bacterial colonies were observed and counted using a colony counter (Gallenkamp).

Representative colonies of bacteria were selected and sub-cultured on fresh bacteriological media until pure cultures were obtained (Fig. 1).

Identification of bacteria: The identification of bacteria was based on morphological characteristics and biochemical tests carried out on the isolates. Morphological characteristics observed for each bacteria colony after 24 h of growth included colony appearance; shape, elevation, edge, optical characteristics, consistency, colony surface and pigmentation. Biochemical characterizations were done according to the method of Fawole and Oso (2004). Some of the key tests for identification include the following:

Gram staining techniques: A thin smear of each of the pure 24 h old culture was prepared on clean grease-free slides, fixed by passing over gentle flame. Each heat-fixed smear was stained by addition of 2 drops of crystal violet solution for 60 sec and rinsed with water. The smear were again flooded with Lugol's iodine for 30 sec and rinsed with water, decolourized with 70% alcohol for 15 sec and were rinsed with distilled water. They were then counter stained with 2 drops of Safranin for 60 sec and finally rinsed with water, then allowed to air dry. The smears were mounted on a microscope and observed under oil immersion objective lens. Gram negative cells appeared pink or red while gram positive organisms appeared purple (Fawole and Oso, 2004).

Spore staining technique: This test is to detect the presence of bacteria endospores. Heat-fixed smears of the organisms were prepared on separate slides and flooded with 5% Malachite green solution and steamed for a minute. The stain was washed off with water and counter stained with 2 drops of Safranin solutions for 20 sec. The slides were allowed to air dry and examined under oil immersion objective (100) lens. Endospores stained green while vegetative cells stained pink (Cheesbrough, 2006).

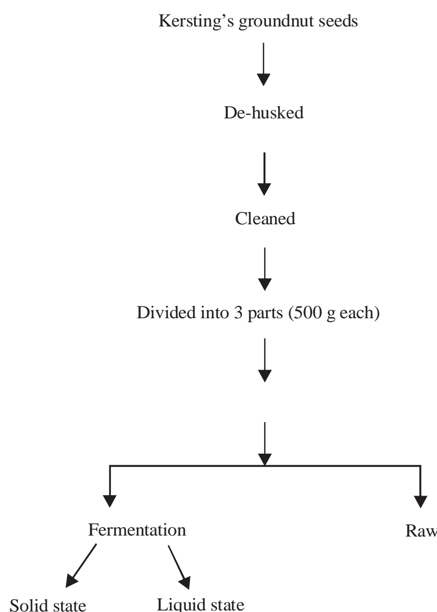

Fig. 1: Flow chart of the processing of Kersting's groundnut

Motility test: A sterile needle was used to pick a loop of a 24 h old culture and was stabbed onto nutrient agar in glass vials. The vials were incubated at 37°C for 24-48 h. Non-motile bacteria had growth confined to the stab line with definite margins without spreading to surroundings area while motile bacteria gave diffused growth extending from the surface (Olutiola *et al*., 2000).

Catalase test: A small quantity of 24 h old culture was transferred into a drop of 3% Hydrogen peroxide solution on a clean slide with the aid of sterile inoculating loop. Gas seen as white froth indicates the presence of catalase enzyme (Cheesbrough, 2006).

Coagulase test: Coagulase is an enzyme capable of coagulating certain blood plasma, notably human and rabbit plasma. This test differentiates pathogenic from non-pathogenic *Staphylococcus* spp., the test was carried out using 18-24 h old culture. A loopful of isolated bacterium was emulsified with normal saline solution on a microscope slide. A drop of undiluted plasma was added to the suspension and stirred for five seconds. A coagulase-positive result was indicated by clumping of colonies together (Olutiola *et al*., 2000).

Methyl red test: Five millimeters of glucose phosphate broth (1 g glucose, 0.5% KH_2PO_4, 0.5% peptone and 100 mL distilled water) were dispensed in clean test tubes and sterilized. The tubes were then inoculated with the test organisms and incubated at 37°C for 48 h. At the end of incubation, few drops of methyl red solution were added to each test and colour change was observed. A red colour indicates a positive reaction (Olutiola *et al*., 2000).

Voges-proskaeur test: Five millimeter of glucose phosphate broth (1 g glucose, 0.5% KH_2PO_4, 0.5% peptone and 100 mL distilled water) were dispensed in clean test tubes and sterilized. The tubes were then inoculated with the test organisms and incubated at 37°C for 48 h. After incubation, 6% α-naphhtol and 6% Sodium hydroxide were added

to about 1 mL of the broth culture. A strong red colouration formed within 30 min indicates positive reaction (Olutiola *et al*., 2000).

Indole test: Tryptone broth (5 mL) was placed into different test tubes after which a loopful of the bacterial isolates was inoculated into the test tubes, leaving one of the test tubes uninoculated to serve as control. The test tubes were then incubated at 37°C for 48 h. After incubation, 0.5 mL of Kovac's reagent was added and shaken gently; it was allowed to stand for 20 min to permit the reagent to rise. A red or red-violet colour at the top surface of the tube indicates a positive result while yellow colouration indicates a negative result (Cheesbrough, 2006).

Starch hydrolysis: This is used to assay the ability of microorganisms that can produce enzymes that degrade substrate with carbon compounds. Nutrient agar was prepared with 1% soluble starch and was sterilized. The medium was poured into sterile plates and were inoculated by streaking the organisms once across the plates after solidifying. The plates were incubated at 37°C for 24 h after which they were flooded with Gram's iodine. Unhydrolysed starch forms a blue colour with the iodine. Hydrolysed starch appears as a clear zone due to alpha amylase activity while reddish brown zones around the colony indicates partial hydrolysis of starch (to dextrans).

Citrate test: This test detects the ability of an organism to use citrate as a sole source of carbon and energy. About 2.4 g of citrate agar was dissolve in 100 mL of distilled water. About ten milliliter (10 mL) of citrate medium was dispensed into each tubes 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.

Oxidase test: A piece of filter paper was soaked with few drops of oxidase reagent. Sterile inoculating loop was used to pick a colony of the test organism and smeared on the filter paper. If the organism is oxidase producing, the phenylenediamine in the reagent will be oxidized to a deep purple colour (Cheesbrough, 2006).

Sugar fermentation: Sugar fermentation test was carried out to determine the ability of organisms to ferment sugars with production of acid and gas. Sugar indicator broth was prepared using peptone water medium containing 1% fermentable sugar and 0.01% phenol red. About ten milliliters of sugar broth was dispensed into each of the test tubes, durham tube which would trap the gas if produced was inverted carefully. The test tubes were autoclaved and inoculated with a loopful of 24 h old culture of the test organisms after then incubated for 2-7 days at 36±1°C and observed daily for acid and gas production. Yellow colouration indicates acid production while gas production was indicated by displacement of the medium in the durham tube (Fawole and Oso, 2004).

Identification of fungi: The fungal colonies were sub-cultured on Potato Dextrose Agar (PDA). The isolates were identified based on their morphological and microscopic features. Two drops of cotton-blue-in-lactophenol were placed on clean glass slide and small piece of mycelium free of medium was removed with sterile inoculating needle and transferred on to the stain. The mycelium was teased (picked) out with the needles and covered with clean cover slip carefully avoiding air bubbles and observed under the microscope for vegetative and reproductive structures (Hunter and Bamett, 2000).

Statistical analysis: All experiments were carried out in triplicates. Data obtained were analyzed by one-way analysis of variance (ANOVA) and means were compared by Duncan's New Multiple Range test (SPSS 21.0 version). Differences were considered significant at $p<0.05$.

RESULTS

Total viable count obtained from fermenting Kersting's groundnut: The bacterial load of fermenting Kersting's groundnut as shown in Fig. 2, revealed that the raw sample of Kersting's groundnut has a total count of 3.67×10^3 CFU g^{-1} on nutrient agar while the De Man Rogosa Sharpe (MRS) agar used for the isolation of lactic acid bacteria showed no growth for the raw sample. After 24 h of liquid fermentation, the highest bacterial count, 1.23×10^4 CFU g^{-1} was observed on MRS agar which later decreased significantly ($p<0.05$) to 4.33×10^3 CFU g^{-1} after 72 h of fermentation. Total viable count on nutrient agar after 24 h of liquid fermentation was 9.00×10^3 CFU g^{-1}, but later reduced to 5.33×10^3 CFU g^{-1} after 48 h. The viable count on nutrient agar later increased significantly ($p<0.05$) to 9.67×10^3 CFU g^{-1} after 72 h of liquid fermentation. Total colonies on nutrient agar after 24 h of

solid fermentation was 8.00×10^3 CFU g^{-1}, but later reduced significantly to 5.33×10^3 CFU g^{-1} after 48 h of fermentation. After 72 h of solid fermentation, viable colonies was observed to have increased significantly ($p<0.05$) to 7.67×10^3 CFU g^{-1}.

Fungal load of the fermenting Kersting's groundnut as shown in Fig. 3 revealed that there was no fungi growth recorded in the raw sample before fermentation. After 24 h of solid state fermentation the viable colonies was 2.67×10^3 SFU g^{-1}, the load increased significantly ($p<0.05$) to 6.67×10^3 SFU g^{-1} after 72 h of fermentation. While in liquid fermentation, a total colony of 2.33×10^3 was recorded after

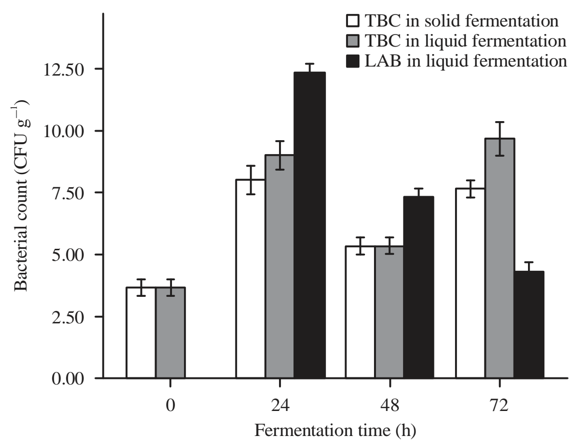

Fig. 2: Bacterial load (CFU g^{-1}) of Kersting's groundnut during fermentation bars are presented as Mean±S.E of replicates (n = 3), TBC: Total bacterial count, LAB: Lactic acid bacteria

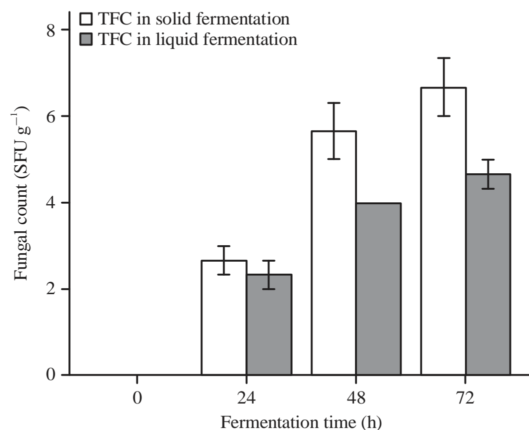

Fig. 3: Fungal load (SFU g^{-1}) of Kersting's groundnut during fermentation bars are presented as Mean±S.E of replicates (n = 3), TFC: Total fungal load Correct the figure (zero value)

24h of fermentation, which significantly ($p<0.05$) increased to 4.67×10^3 SFU g^{-1} after 72 h of fermentation. Fungal load was observed to be higher in the solid state fermentation than the liquid state.

Colonial, morphological and biochemical characteristics of bacteria and fungi isolated from raw and fermented Kersting's groundnut: The colonial, morphological and biochemical characteristics of the microorganisms isolated from the raw and fermenting Kersting's groundnut were shown in Table 1, 2 and 3, respectively. Microorganisms tentatively identified are: *Bacillus licheniformis*, *B. subtilis*, *B. megaterium*, *B. polymyxa*, *B. coagulans*, *Lactobacillus plantarum*, *Staphylococccus aureus*, *Aspergillus niger*, *A. parasiticus*, *Rhizopus stolonifer, Rhodotorula* spp. and *Saccharomyces cerevisiae*.

Occurrence of microorganisms during the fermentation of Kersting's groundnut: The occurrence of microorganisms in the raw and fermenting Kersting's groundnut was shown in Table 4. *Bacillus licheniformis*, *Bacillus subtilis* and *Staphylococccus aureus* was present in the raw sample. *Bacillus subtilis*, was present throughout the fermentation period. *Lactobacillus plantarum* was present throughout the liquid fermentation. *Bacillus megaterium* and *Bacillus polymyxa* were isolated after 24 and 72 h of both liquid and solid fermentation. *Saccharomyces cerevisiae* was not isolated in the raw sample, but was isolated through the liquid fermentation process. *Aspergillus niger* and *Rhizopus stolonifer* were isolated both in liquid and solid fermentation. *Aspergillus parasiticus* was isolated after 72 h of liquid fermentation while *Rhodotorula* spp., was isolated after 72 h of solid fermentation.

Percentage occurrence of microorganisms during the fermentation of Kersting's groundnut: The percentage occurrence of microorganisms during the liquid and solid fermentation as shown in Fig. 4 revealed that all the five species of Bacillus were present in liquid and solid fermentation of the Kersting's groundnut. *Lactobacillus plantarum* and *Saccharomyces cerevisiae* were only isolated from the liquid fermentation. *Staphylococcus aureus* was isolated in both liquid and solid fermentation. *Aspergillus niger* and *Rhizopus stolonifer* were present in both fermentation techniques. *Aspergillus parasiticus* was isolated only in the liquid fermentation, while, *Rhodotorula* spp. was isolated only in solid fermentation.

Table 1: Colonial and morphological characteristics of microorganisms isolated from Kersting's groundnut

Isolate code	Colony shape	Cell shape	Gram's reaction	Endospore formation	Motility test
FKG 01	Large flat creamy, wide spreading and glistening surfaced colonies	Short rods	+	+	+
FKG 02	Colonies are large, margin is undulate, with circular form and flat elevation	Shot rods	+	+	+
FKG 03	Creamy yellow irregular glistening surfaced colonies	Short rods	+	+	+
FKG 04	Colonies are punctiform, convex with an entire margin. The smallness of the colonies is due to the inefficient metabolism of these microbes	Long rods	+	-	-
FKG 05	Colonies are large, irregular and flat with an undulate margin	Short rods	+	+	+
FKG 06	Fluffy cream colonies	Purple oval shape	+	-	-
FKG 07	Rhizoid flat, whitish, glistening, convex, smooth entire edges	Short rods	+	+	+
FKG 08	Small, non-spreading glistening yellowish colonies with entire smooth edges	Cocci in clusters	+	-	-

+: Positive, -: Negative, FKG: Fermented Kersting's groundnut

Table 2: Biochemical characterization of Kersting's groundnut isolates

Tests	FKG 01	FKG 02	FKG 03	FKG 04	FKG 05	FKG 06	FKG07	FKG08
Catalase	+	+	+	-	-	+	+	+
Coagulase	-	-	-	-	-	-	-	+
Starch hydrolysis	+	+	+	-	+	-	+	-
Indole	-	-	-	-	-	-	-	-
Citrate	+	+	+	-	-	-	+	-
MR/VP	+/+	-/+	-/-	+/-	+/+	+/-	+/+	-/-
Oxidase test	-	-	-	-	-	-	-	-
Arabinose	AG	A	A	AG	AG	AG	AG	AG
D-mannitol	AG	AG	A	AG	AG	AG	A	AG
Galactose	AG	AG	A	AG	AG	AG	AG	AG
Sucrose	AG	A	A	-	AG	AG	A	AG
Glucose	A	-	A	AG	AG	AG	A	AG
Maltose	AG	AG	A	A	AG	AG	A	AG
Lactose	AG	-	A	AG	AG	AG	-	AG
Frutose	AG	AG	A	A	AG	AG	AG	AG
Suspected organism	*B. licheniformis*	*B. subtilis*	*B. megaterium*	*L. plantarum*	*B. polymyxa*	*S. cerevisae*	*B. coagulans*	*S. aureus*

+: Positive, -: Negative, AG: Acid and gas, A: Acid

Table 3: Cultural and morphological characteristics of fungi and their tentative identification

Cultural characteristics	Morphological characteristics	Identification
Dark- brown mycelium	Dark-brown conidia, conidiophores are long, globose vesicles that are completely covered with biseriate phialides, phialides are borne on brown metulae	*Aspergillus niger*
Red-pink colonies	Spherical to enlongated budding yeast-like cells or blastoconidia	*Rhodotorula* spp.
Yellowish oil green to cedar green mycelium	Conidia globose to subglobose, biseriate phialides, metulae phialides, vesiciles globose to pyriform	*Aspergillus parasiticus*
White cotton-like fluffy mass mycelium	Non-septate hyphae and coenocytic twin sporagiophores, well developed collumela which is in umbrella-like in shape	*Rhizopus stolonifer*

Table 4: Occurrence of microorganisms during fermentation of Kersting's groundnut

	Liquid fermentation time (h)				Solid fermentation time (h)			
Organisms	0	24	48	72	0	24	48	72
Bacillus lichenifomis	+	+	-	+	+	+	-	+
Bacillus subtilis	+	+	+	+	+	+	+	+
Bacillus megaterium	-	+	-	+	-	+	-	+
Lactobacillus plantarum	-	+	+	+	-	-	-	-
Bacillus polymyxa	-	+	-	+	-	+	-	+
Bacillus coagulans	-	+	+	-	-	+	+	-
Staphylococcus aureus	+	+	-	-	+	+	-	-
Aspergillus niger	-	+	+	+	-	+	+	+
Rhodotorula spp.	-	-	-	-	-	-	-	+
Aspergillus parasiticus	-	-	-	+	-	-	-	-
Rhizopus stolonifer	-	+	+	+	-	+	+	+
Saccharomyces cerevisiae	-	+	+	+	-	-	-	-

+: Positive, -: Negative

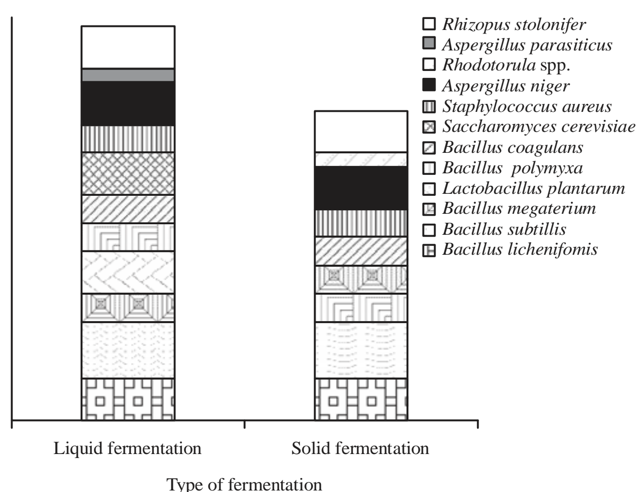

Fig. 4: Microbial composition and abundance during two type of fermentation techniques. Each colored band in a column represent the genus of a microorganism and the band width shows its relative abundance

More microorganisms were isolated in the liquid fermentation compared to the solid fermentation.

DISCUSSION

The decrease observed in bacterial load after 48 h liquid fermentation may 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). Low microbial growth observed after 48 h of solid fermentation may also be as a result of reduction in the moisture content, which is known to be the most important factor in microbial growth (Baysal *et al.*, 2003; Nester *et al.*, 2006). Increase observed in microbial growth after 72 h solid fermentation may be as a result of the metabolic activities of the organisms that produce carbondioxide and water (Chutmanop *et al.*, 2008). 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. Foluso *et al.* (2014) has reported a decrease in microbial load after 48 h of fermentation of cocoyam.

The significant increase observed in fungal load during solid fermentation may be due to the low water activity requirement of fungi which make them to thrive more than bacterial even at a very low moisture content (Nester *et al.*, 2006). More microorganisms are associated with liquid fermentation compare to solid fermentation. This may be as a result of the free access of the microorganisms to the nutrient in the fermenting medium (Subramaniyam and Vimah, 2012).

Biochemical characterization of the isolates revealed the presence of *Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus polymyxa*, *Bacillus coagulans*, *Lactobacillus plantarum*, *Staphylococccus aureus*, *Aspergillus niger*, *A. parasiticus*, *Rhizopus stolonifer*, *Rhodotorula* spp. and *Saccharomyces cerevisiae* as microorganisms associated with the fermentation of Kersting's groundnut. These organisms have been found to be responsible for the fermentation of most legumes and cereals (Wood and Holzapfel, 1995; Lei and Jacobsen, 2004; Adesulu and Awojobi, 2014). The presence of these organisms during the fermentation periods confirms that they grow in close association with the food substrate and produce extracellular enzymes (Steinkraus, 2002). The isolation of *Bacillus* species as the most predominant bacterial flora in the fermentation of Kersting's groundnut may be as a result of their ability to survive both in slightly acidic and alkaline environment (Kuta *et al.,* 2009). This is in accordance with the previous works of Babalola and Giwa (2012) during fermentation of soybeans. *Bacillus* spp., have better competitive ability compared to other bacteria species present in the same environment (Kuta *et al.*, 2009).

Percentage occurrence of the isolates in liquid and solid fermentation techniques revealed the presence of *Bacillus* spp., as dominant organism may be due to their xerophytic nature (Kuta *et al.*, 2009). *Lactobacillus plantarum* and *Saccharomyces cerevisiae* were only isolated from the liquid fermentation. *Staphylococcus aureus* isolated after 24 h of both fermentation techniques may be as a result of contact with the skin (Baird-Parker, 2000; Lawrynowicz-Paciorek *et al.*, 2007). *Aspergillus niger* and *Rhizopus stolonifer* isolated in both solid and liquid fermentation may be as a result of the association of the organisms with plants, soil and decaying vegetation (Schuster *et al.*, 2002). Isolation of *Rhodotorula* spp. signified cross-contamination in the laboratory during fermentation because *Rhodotorula* spp have been reported as a saprophyte from skin, vaginal and respiratory specimens of human (Gomez-Lopez *et al.*, 2005; Jimoh *et al.*, 2012). In conclusion, no lactic acid bacteria was observed in solid fermentation. All the five species of Bacillus isolated were present in both liquid and solid fermentation, with predominance *Bacillus subtilis* throughout the fermentation period.

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