Molecular Characterization of Bacterial Isolates from Conventionally Stored African Oil Bean Seed in Ilorin Metropolis

Ihum T. A.[1*] John W. C.[2] and Oledibe C. F.[3]

1. Durable Crops Research Department, Nigeria Stored Product Research Institute (NSPRI), Ilorin, Nigeria
2. Department of Pest Management Technology, Federal College of Forestry Jos, Nigeria
3. Research Operations Department, NSPRI, Ilorin, Nigeria

* Corresponding author: temiokusami@gmail.com

Abstract: The African oil bean seed (*Pentaclethra macrophylla*) is widely used as a traditional food condiment and is valued for its high protein content. This study focused on assessing the microbiological quality and molecular characterization of microorganisms associated with African oil bean seeds stored under conventional methods. Samples were obtained from three different markets in the Ilorin metropolis of Nigeria: Mandate, Ojo Oba, and Ganmo. Isolates were identified using 16S RNA GENE sequencing and the BLAST algorithm. Microbial counts ranged from $3.7 \pm 1.4 \times 10^6$ to $6.3 \pm 0.5 \times 10^6$ cfu/g African oil bean seeds from the Mandate market had the highest bacterial load of $6.3 \pm 0.5 \times 10^6$ cfu/g followed by those from Ganmo with a bacterial load of $5.3 \pm 0.5 \times 10^6$ cfu/g, while samples from the Oja Oba market had counts of $4.5 \pm 2.2 \times 10^4$ cfu/g. The bacteria isolates were identified as *Staphylococcus aureus* CIP 9973; and *Pectobacterium carotovorum* subsp. *carotovorum* Pec 1; *Enterobacter cloacae* AS10 *Klebsiella aerogenes* OFM28; *Escherichia coli* 2013C-3342; *Proteus mirabilis* UPMSD3; *Lactobacillus plantarum* NCU116; *Lactobacillus plantarum* NRIC 0383. This study emphasized the importance of molecular characterization in determining the genetic diversity and assessing the microbial quality of the African oil bean seed in ensuring food safety and public health.

Key word: Bacteria, characterization, oil seeds, quality

INTRODUCTION

The African oil bean seed (*Pentaclethra macrophylla*) commonly called Ukpaka/ Ugba in Igbo land, Apara in Yoruba land, and Ukana in Efik is an important and cheap source of protein (Afia, 2020). It is very popular among the Ibos and has also become a major delicacy and food check spelling agent in other ethnic groups in Nigeria. The seed is rich in oil content (53.98%) and oleic acid (29.0%) and its oil yield is greater than that of castor bean (42.2%) and locust bean (20.68%) (Aladekoyi, *et al* 2016, Oboh, 2019). Despite its importance, the oil seed has been neglected thus, limiting the potential of the crop. Furthermore, the production of this delicacy in Nigeria is a small-scale process and most times consideration for good manufacturing practices (GMP) and sanitation practices is minimal. This is mostly due to poor hygiene and the use of contaminated water in the fermentation and packaging process (Amaefula *et al*., 2024) consequently, introducing spoilage and pathogenic microorganisms. Studies have also shown that fermented African oil bean seeds are majorly contaminated by *Bacillus, Escherichia, Proteus, Micrococcus, Staphylococcus, Streptococcus, Alcaligenes, Pseudomonas, Corynebacterium,* and *Enterococcus* species (Onyekachi, *et al*., 2021). These pathogens can be introduced during production (Onyekachi *et al*., 2021, Amaefula *et al*., 2024). One major concern in the food industry is contamination by pathogens, which are frequent causes of food-borne diseases (Awojobi *et al*., 2016). According to Onyekachi *et al*. (2021), contamination during post-fermentation constitutes a major issue to the safety and microbial quality of this product. Packaging of the African oil bean seed for storage is done traditionally by wrapping the fermented product with different kinds of leaves believed to aid the fermentation process. Ogbulie *et al*. (1998) studied the possibility of using polyethylene bags and foil wraps with the aid of preservatives to package and extend the storage duration of African oil bean seeds. This method was able to keep the product for only eight days after which the product quality was found to

degrade. Onyekachi *et al*. (2021) opined that the use of leaves in wrapping and poor handling measures during packaging can contaminate the product. This could therefore allow microbial contamination from openings thereby resulting in deterioration hence, this study is aimed at assessing the microbial quality and molecular identities of associated microorganisms of African oil bean seed sold in major markets in the Ilorin metropolis.

MATERIALS AND METHODS

Sample collection: African oil bean seed (500 g) measured using a weighing scale (EMC LVD C-Tick Rohs, China) was purchased from five different vendors at random locations in Mandate, Ganmo, and Oja oba, Markets, in Ilorin, Kwara State, Nigeria. All samples were kept at a temperature of 4^{o}C in an ice pack and transported to the Microbiology Laboratory of the Nigeria Stored Products Research Institute, Ilorin, Nigeria for further analysis.

Processing of samples: African Locust bean sample (300 g) was weighed using a weighing scale, and reduced into smaller portions using a mortar and pestle pre-sterilized using 70% alcohol. The different homogenates were collected in labeled sterile tubes and stored in sterile containers at 4^{o}C in the refrigerator (Haier Thermocool, China) for further use.

Isolation of Lactobacillus species from African locust bean samples: Isolation of *Lactobacillus* species from the samples was done according to the method of (Hwanhlem *et al*., 2013). Processed samples were serially diluted, by transferring 1 g/1 ml of each sample separately into 9 ml of sterile water to make a stock mixture. A homogenized mixture (1 ml) was then taken into an appropriately labeled test tube to make 10^{-1} of the mixture. The serial dilution was continued until 10^{-8} was obtained. An aliquot (0.1 ml) of the respective dilutions was spread over MRS (de Man, Rogosa, Sharpe) agar plates. The MRS agar plates were then incubated anaerobically in an anaerobic jar (Microbiology AnaerotestX Merck, Darmstadt, Germany) at 37 ± 2^{o}C for 48 hours. Growth on MRS plates was then observed after 48 hours and counts were recorded.

Purification of bacterial isolates: Colonies cultured on MRS agar plates were sub-cultured twice on MRS agar and incubated in an incubator (Swiss *model* NU-5700, UK) at 37 ± 2^{o}C for 48 hours to get pure colonies. Purified isolates from the samples were maintained at 4^{o}C (Haier Thermocool, China) in MRS and nutrient broth medium respectively for further characterization and identification.

Bacterial DNA extraction: Fresh cultures of isolates from the samples were duplicated in sterile bottles containing nutrient broth for molecular identification. Broth culture (1000 µl) was centrifuged (Sorvall RC6 PLUS, Thermo-electron Corporation, Asheville, NC, USA) at 10, 0000 g for 5 min to get the pellets. The supernatant was decanted and 1 ml of DNA Extraction Buffer (DEB) containing proteinase K (0.05 mg/ml) was added and vortexed using a vortex mixer, after which 50 µl of 20% sodium dodecyl sulfate (SDS) was added and incubated in a water bath at 65^{o}C for 30 minutes. The tubes were then allowed to cool to room temperature, before adding 100 µl of 7.5 M potassium acetate and mixed briefly. The solution was then centrifuged (Sorvall RC6 PLUS, Thermo-electron Corporation, Asheville, NC, USA) at 13000 g for 10 minutes. The supernatant was transferred into fresh autoclaved tubes. Two-third volumes of isopropyl alcohol were then added to the supernatant in the test tubes and inverted gently before incubating in an incubator shaker (Thermo Scientific) at 20^{o}C for 1 hour. After incubation the solution was centrifuged at 13000 g for 10 minutes and the supernatant was discarded. Thereafter 500 µl of 70% ethanol was added and the supernatant was further centrifuged again for 5 minutes at 13000 rpm. The supernatant was carefully discarded with the DNA pellet intact and the DNA pellets dried at 37^{o}C for 10-15 minutes. The pellets were later

resuspended in 50 µl of Tris-EDTA (TE) buffer. Aliquots of DNA obtained were then stored at 20^{o}C in a refrigerator (Haier, thermocool China) for further laboratory analysis (Zhou *et a*l., 2014).

Polymerase chain reaction (PCR) analysis: Polymerase chain reaction was done according to the method of √Zhou *et a*l. (2014). Polymerase Chain Reaction sequencing preparation cocktail consisted of 10 µl of 5x GoTaqcolourless reaction, 3 µl of 25 mM $MgCL_2$ (Magnesium Chloride), 1 µl of 10 mM of dNTPs (deoxyribonucleotide triphosphate) mix, 1 µl of 10 pmol each of the 16S rDNA gene forward primer (50-AGAGTTTGATCCTGGCTCAG-30), reverse primer: 50 (GTGTGACGGGCGGTGTGTAC-30), 0.3 units of Taq DNA polymerase (Promega, USA) made up to 42 µl with distilled water and 8 µl DNA template. Polymerase chain reaction was carried out in a Gene Amp 9700 PCR System Thermal cycler (Applied Biosystem Inc., USA) with a PCR profile consisting of an initial denaturation at 94°C for 5 min; followed by 30 cycles consisting of 94°C for 30 seconds annealing of primer at 56^{o}C and 72°C for 1 minute 30 seconds; and a final termination at 72°C for 10 minutes. These were then allowed to chill at 4^{o}C in a refrigerator (Haier Thermocool, China).

Determination of the integrity of the DNA Gel: The integrity of the DNA and PCR amplification product were checked on 1% agarose gel. Tris Borate EDTA (1XTBE) buffer was used to prepare the agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60°C and stained with 3 µl of 0.5 g/ml ethidium. A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was further allowed to solidify for 20 minutes to form the wells. Tris/Borate/EDTA (1XTBE) buffer was then poured into the gel tank to barely submerge the gel. Two microlitres (2µ l) of 10 x blue gel loading dye (which gives color and density to the samples to make it easy to load into the wells and monitor the progress of the gel) were added to 4 µl of each PCR product and loaded into the wells after the 100 bp DNA ladder was loaded into well 1. The gel was electrophoresed at 120 V for 45 minutes visualized with ultraviolet trans-illumination and photographed (Zhou *et a*l., 2014). The sizes of the PCR products were estimated by comparison with the mobility of a 100 bp ladder that ran alongside experimental samples in the gel.

Purification of amplified 16SrRNA gene product: Amplified fragments were further purified using 95% ethanol to remove the PCR reagents. A 3 M, 7.6 µl of sodium acetate, and 240 µl of 95% ethanol were added to each 40 µl PCR amplified product in a sterile 1.5 µl Eppendorf tube and mixed thoroughly using a vortex mixer (Cole-ParmerIndia, Pvt limited) and kept at -20°C in the refrigerator (Haier, Thermocool, China) for 30 minutes. This was then centrifuged (Sorvall RC6 PLUS, Thermo-electron Corporation, Asheville, NC, USA) for 10 minutes at 13000 rpm at 4°C followed by removal of the supernatant after which the pellet was washed by adding 150 µl of 70% ethanol and centrifuged for 15 min at 7500 g at 4°C in a refrigerator (Haier, Thermocool, China). All supernatant was repeatedly removed and tubes were inverted on paper tissue and left to dry in the fume hood at room temperature (28 ± 2^{o}C) for 10 -15 minutes after which it was suspended with 20 µl of sterile distilled water and kept in the refrigerator (Haier, Thermocool, China) at -20^{o}C before sequencing. The purified gene product fragment was checked on a 1.5% agarose gel run on a voltage of 110 V for about 1 hour as previously described, to confirm the presence of the purified product and quantified using a Nanodrop Model 2000 UV-Vis spectrophotometer.

Sequencing of amplified fragments: Sequencing of amplified PCR fragments was carried out by InquabaBiotec Sequencing Service, South Africa using the method of (Yuehua *et al*., 2016). Amplified fragments were sequenced using a Genetic analyzer

3130xl sequencer (Applied Biosystems) using the manufacturers' manual, while the sequencing kit used was a Big Dye terminator cycle sequencing kit. Bio-Edit software and MEGA 6 were also used for all genetic analyses. The obtained sequences were compared with those available in the Gen Bank database, using the Basic Local Alignment Search Tool (BLAST) at the National Center of Biotechnology Information website (http://www.ncbi.nlm.nih.gov/).

RESULTS

A total of seven (7) bacteria were isolated from the African locust bean namely *Staphylococcus aureus, Pectobacterium carotovorum, Enterobacter cloacae, Klebsiella aerogenes, Escherichia coli, Proteus mirabilis* and *Lactobacillus plantarum.* The bacterial isolates from the different markets were labeled numerically for molecular identification (Table 1). The occurrence of bacterial isolates from locust beans is outlined in Table 1. African oil bean seeds from Ganmo market had the highest number of bacteria occurrence (5), while those from Mandate and Ojo oba markets had an occurrence of 4 bacterial isolates. The findings of bacterial load of the African oil bean seeds from the different markets showed that they varied with type and source (markets). However, based on the market where the samples were bought, the average bacterial load of African oil bean seeds, showed that seeds from the Mandate market (vendor C) had the highest bacterial load of $6.3 \pm 0.5 \times 10^6$ cfu/g (Table 2), while the highest counts from Oja Oba was $4.5 \pm 2.2 \times 10^4$ cfu/g vendor C, in the same vein Ganmo had the highest bacterial load of $5.3 \pm 0.5 \times 10^6$ cfu/g. Generally microbial counts ranged from $3.7 \pm 1.4 \times 10^6$ to $6.3 \pm 0.5 \times 10^6$ cfu/g (Table 2).

Genetic distance between all isolates is depicted in Figure 1. Similarity searches with sequences in the ribosomal data project database revealed 82.30% identity with *Staphylococcus aureus* CIP 9973, 94.10% with *Pectobacterium carotovorum* Pec1, 97.70% with *Enterobacter cloacae* AS10, 93.80% with *Klebsiella aerogenes* OFM28, 91.80% with *Proteus mirabilis* UPMSD3, 94.50% with *Escherichia coli* 2013C-3342, 95.90% identity with *L. plantarum* NRIC 0383 and 83.40% with *L. plantarum*NCU116 (Table 3). Sharp band amplicons corresponding to the 16S rRNA intergenic spacer region were observed for the test bacteria and bacteriocin-producing LAB. The negative control without any template did not give any band (Plate 1).

Table 1: Occurrence of *Lactobacillus* Isolates in Locust Beans and Cow Milk Samples

S/N	Markets	Microbial occurrence
1.	Mandate	*Enterobacter cloacae, Klebsiella aerogenes Escherichia coli Lactobacillus plantarum*
2.	Oja Oba	*Klebsiella aerogenes, Lactobacillus plantarum Staphylococcus aureus, Proteus mirabilis,*
3.	Ganmo	*Staphylococcus aureus, Enterobacter cloacae, Klebsiella aerogenes, Proteus mirabilis, Lactobacillus plantarum*

Table 2: Average Bacterial Load (Log CFU/ml ± SD) of Africa oil bean samples purchased from selected markets in Ilorin

Vendors	Mandate Bacterial counts cfu/g.	Oja-Oba	Ganmo
A	$4.8 \pm 1.5 \times 10^4$	$4.8 \pm 0.5 \times 10^4$	$5.3 \pm 0.5 \times 10^6$
B	$4 .1 \pm 0.8 \times 10^4$	$3.7 \pm 1.4 \times 10^6$	$4.2 \pm 1.8 \times 10^6$
C	$6.3 \pm 0.5 \times 10^6$	$4.5 \pm 2.2 \times 10^4$	$3.7 \pm 1.5 \times 10^6$

	8	7	1	5	6	2	4	3
8		0.15	0.93	0.91	0.85	0.87	0.89	0.86
7	0.15		1.00	0.98	0.92	0.93	0.96	0.92
1	0.93	1.00		0.35	0.29	0.31	0.33	0.30
5	0.91	0.98	0.35		0.13	0.16	0.21	0.18
6	0.85	0.92	0.29	0.13		0.10	0.15	0.12
2	0.87	0.93	0.31	0.16	0.10		0.17	0.14
4	0.89	0.96	0.33	0.21	0.15	0.17		0.15
3	0.86	0.92	0.30	0.18	0.12	0.14	0.15	

Figure 1: Genetic distance between molecularly characterized isolate

Key: 1: *Staphylococcus aureus CIP* 9973; 2: *Pectobacteriumcarotovorum* subsp. *carotovorum* Pec 1; 3: *Enterobacter cloacae* AS10 4: *Klebsiellaaerogenes* OFM28; 5: *Escherichia coli* 2013C-3342; 6: *Proteus mirabilis* UPMSD3; 7(A): *Lactobacillus plantarum* NCU116; 8(B): *Lactobacillus plantarum*NRIC 0383.

Table 3: Similarity searches of bacteria isolate with sequences in the ribosomal project database

Isolate Codes	Pairwise Identity (%)	NCBI Accession	Organism Description
1	82.30%	MG650162	*Staphylococcus aureus* partial 16S rRNA geneCIP 9973
2	94.10%	MH532568	*Pectobacterium carotovorum* subsp. *carotovorum* strain Pec1 16S ribosomal RNA gene, partial sequence
3	97.70%	MH605571	*Enterobacter cloacae* strain AS10 16S ribosomal RNA gene, partial sequence
4	93.80%	MH542333	*Klebsiella aerogenes* strain OFM28 16S ribosomal RNA gene, partial sequence
5	91.80%	MH393635	*Proteus mirabilis* strain UPMSD3 16S ribosomal RNA gene, partial sequence
6	94.50%	CP027766	*Escherichia coli* strain 2013C-3342 chromosome, complete genome
A	83.40%	CP016071	*Lactobacillus plantarum* strain NCU116, complete genome
B	95.90%	AB362652	*Lactobacillus plantarum* gene for 16S rRNA, partial sequence, strain NRIC 0383

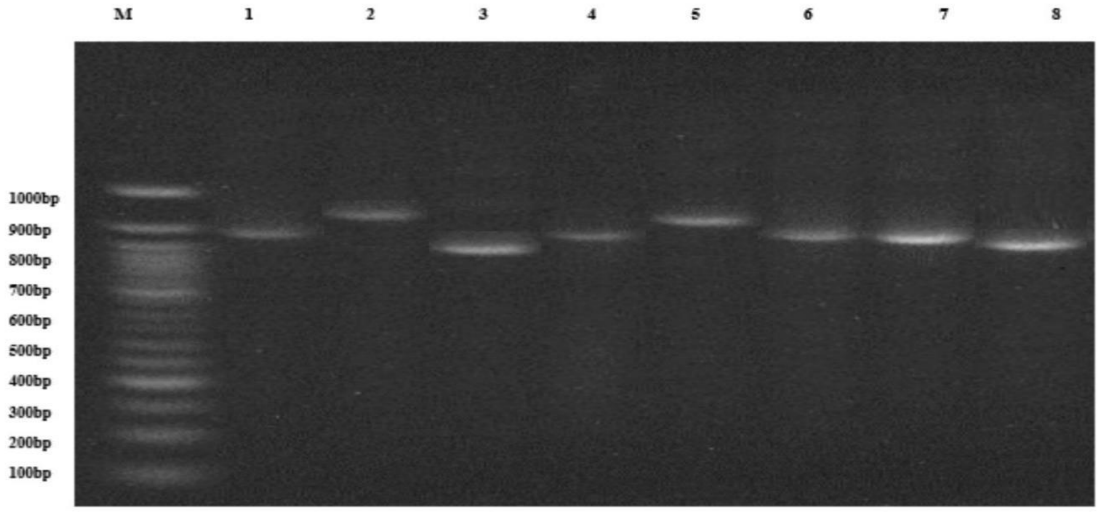

Plate 1: Polymerase Chain Reaction Amplification Product of Bacteria Isolates visualized by Agarose Gel Electrophoresis

Key: Lane M: 100bp marker; Lane 1: *Staphylococcus aureus* CIP 9973; Lane 2: *Pectobacterium carotovorum* subsp. *carotovorum* Pec1; Lane 3: *Enterobacter cloacae* AS10; Lane 4: *Klebsiella aerogenes* OFM28; Lane 5: *Escherichia coli* 2013C-3342; Lane 6: *Proteus mirabilis* UPMSD3; Lane 7 (A): *Lactobacillus plantarum* NCU116; Lane 8 (B) *Lactobacillus plantarum* NRIC 0383

DISCUSSION

Bacterial isolates from African oil bean seed belonged to seven genera identified as *Enterobacter aerogenes, Staphylococcus aureus, Escherichia coli, Klebsiella, Proteus, Pseudomonas,* and *Bacillus* species. Except for *Pectobacterium carotovorum* all the bacteria identified in this study were also isolated and identified by Isu and Njoku (1997), Ogbulie *et al*. (2014), and Mbah *et al*. (2018) from different parts of Nigeria. Some of the bacteria isolated in this study are part of the natural flora of the African oil seeds fermentation process or contaminants from the processing, production, and packaging process. The presence of lactic acid bacteria (LAB) in the oil seeds was evidence of LAB as a normal microflora of fermented foods (Opara *et al*., 2013). It also showed that traditionally fermented food products can potentially be good sources of probiotic organisms (Ogbulie *et al*., 2014). Isu and Njoku, (1997), Ogbulie *et al*. (1998), and Mbah *et al*. (2018) also isolated *Bacillus* spp., *Lactobacillus* spp., *Staphylococcus* spp., *Micrococcus* spp. and members of the family Enterobacteriaceae, from the African oil bean samples. The authors opined that only *Bacillus* spp. was found to ferment African oil bean seeds to Ukpaka/ Ugba, Apara, Ukana and were the predominant microorganisms present, in the total microbial population density (Njoku and Okemadu, 1998). Ansari *et al.* (2012), Opara *et al*. (2013), and Okoye (2016), also reported that these microorganisms play active roles in the process of fermentation. In this study, microbial load of the African oil bean seeds was within $4.1 \pm 0.8 \times 10^4$ - $6.3 \pm 0.5 \times 10^6$ cfu/g. Omeh *et al*. (2014) and Okoye (2016) also reported microbial counts of $1.3 \pm 0.5 \times 10^6$ cfu/g $6.0 \pm 0.5 \times 10^6$ cfu/g. Homology searches of 16s rDNA sequences of the bacteria isolates showed 82.30% identity with *Staphylococcus aureus* CIP 9973, 94.10% with *Pectobacterium carotovorum,* 97.70% with *Enterobacter cloacae* AS10, 93.80% with *Klebsiella aerogenes* OFM28, 91.80% with *Proteus mirabilis* UPMSD3, 94.50% with *Escherichia coli* 2013C-3342, 95.90% identity with *L. plantarum* NRIC 0383 and 83.40% with *L. plantarum* NCU116 using the BLAST algorithm indicated the extent to which two (nucleotide or amino acid) sequences have the same residues at the same positions in an alignment. These findings showed the importance of using molecular methods for identifying newly isolated microorganisms. According to Yuehua *et al*. (2016), genotypic methods are independent of variations in growth conditions, if species-specific primers or probes are available; which offer a very fast method of confirming the identity of the target organism. Furthermore, identification of LAB based on the morphological, physiological, and biochemical characteristics are often considered unreliable, since different species may have similar morphological and nutritional requirements. Phenotypic characterization based on sugar fermentation profile may be used as a presumptive identification but, does not provide reliable identification (Dubernet *et al.,* 2002). However, genotype-based methods such as 16S rDNA are robust to identify bacteria as a complement or alternative to phenotypic methods (Yuehua *et al*., 2016).

CONCLUSION

The study conducted on the molecular characterization of bacterial isolates from conventionally stored African oil bean seeds in Ilorin Metropolis, Nigeria sheds light on the microbial quality of fermented African oil bean seeds. The presence of various bacteria genera highlights the importance of understanding the microbiological profile of these seeds. Also, employing molecular methods for bacterial identification would reliably reveal the diversity and population structure of bacterial species associated with African oil bean seeds. This would no doubt provide insight into the microbial diversity of the processed seeds and offer a basis for assessing the effectiveness of storage techniques and the quality of the seeds

ultimately improving food safety and quality standards.

REFERENCES

Afia, K. (2020). A review of *Pentaclethra macrophylla* (African oil bean) seed. *International Digital Science Resource,* 2579-0781.

Aladekoyi, G., Orungbemi, O.O., Karimu, O.A., and Aladejimokun, A.O. (2017). Comparative studies of the nutritional and phytochemical constituents of African oil bean (*Pentaclethra macrophylla* Benth) and African bean (*Anthonotha macrophylla*) for human consumption. *Chemistry Research Journal*, 2(3):16-21.

Amaefula, J. O., Obiegbuna, J. E., Agu, H. O. & Amaefuna, O. C. (2024). Proximate Composition of Cowpea, Fermented African Oil Bean Seed Flour Blends, and Sensory Properties of Steamed Paste (moi moi) Prepared from the Blends. *Asian Journal of Food Research and Nutrition*, 3(1), 98-107.

Ansari, A., Aman, A., Siddiqui, N. N., Iqbal, S. and Qader, S. A. (2012). Bacteriocin (BAC-IB17): screening, isolation, and production from *Bacillus subtilis* KIBGEIB-17. *Pakistan Journal of Pharmaceutical Sciences*, 125: 195–201.

Dubernet, S., Desmasures, N. and Guéguen, M. (2002). A PCR-based method for identification of *Lactobacilli* at the genus level. *FEMS Microbiology Letters.* 214 (2):271-5.

Hwanhlem, N., Biscola, V., El-Ghaish, S., Jaffr-es, E., Dousset, X. and Haertl, V.S. (2013). Bacteriocin-producing lactic acid bacteria isolated from mangrove forests in southern Thailand as potential bio- control agents: Purification and characterization of a bacteriocin produced by *Lactococcuslactis* subsp. *Lactis* KT2W2L. *Probiotics and Antimicrobial Proteins*, 5(4): 264-278.

Isu, N. R. & Njoku, H. O. (1997). An evaluation of the microflora associated with fermented African oil bean (*Pentaclethra macrophylla* Bentham) seeds during ugba production. *Plant Foods for Human Nutrition*, 51: 145-157.

Mbah, G.O., Onyeabo, U.A., and Udeh, B.C. (2018). Effect of fermentation on the nutritional composition of African oil bean seed. *The Pacific Journal of Science and Technology*, 19(1), *pp*. 244-250.

Njoku, H.O. and Okemadu, C.P. (1998) Biochemical changes during the natural fermentation of the African oil bean for the production of *'ugba.'. Journal of Science and Food Agriculture*, 49: 457-465.

Oboh, G. (2019). *Pentaclethra macrophylla* Benth. PROTA (Plant Resources of TropicalAfrica/Ressources végétales de l'Afrique tropicale), Wageningen, Netherlands. Retrieved from http://www.prota4u.org/search.asp. (Accessed on October 8, 2019).

Ogbulie, J.N., Njoku, H.O., Uwaezuoke, J.C., and Onyicha, R.I. (1998). Evaluation of packaging materials for the preservation of African oil beans (*Pentaclethra macrophylla*) for the production of ugba. *Journal of Innovative Life Sciences*, 3: 110-118.

Ogbulie, T.E., Nsofor, C.A., and Nze, F.C. (2014). Bacteria species associated with ugba (*Pentaclethra macrophylla*) were produced traditionally and in the laboratory and the effect of fermentation on the product of oligosaccharide hydrolysis. *Nig. Food J*., 32(2):73-80.

Okoye, E.I. (2016). Extraction, characterization, and pharmaceutical screening of oil obtained from seeds of *Pentaclethra macrophylla* Benth (African oil bean seed). *The*

Pharmaceutical and Chemical Journal, 3(2):88-91.

Onyekachi, I. A., Ifeanyichukwu, O. N. V. and Simonyan, K. J. (2021). Postharvest processing, packaging, and storage of African oil bean seed. *Poljoprivrednatehnika*, 46(1):31-46.

Opara, C.C., Inyang, I., and Iheonye, K.A. (2013). Kinetics of fermentation of oil bean seed. *Food Science and Technology*, 14(1): 68-72.

Yuehua, J., Lanwei, Z., Fei, L., Huaxi, Y. and Xue, H. (2016). Complete genome sequence of *Enterococcus faecalis* LD33, a bacteriocin-producing strain. *Journal of Biotechnology*, 227**:** 79–80.

Zhou, F., Zhao, H., Bai, F., Piotr, D., Liu, Y. and Zhang, B. (2014): Purification and characterization of bacteriocin produced by *Lactobacillus plantarum*, isolated from Chinese pickle. *Czech Journal of Food Science*, 32: 430–436.