



ANTIMICROBIAL ACTIVITIES OF *LACTOBACILLUS* SPP ASSOCIATED WITH LAFUN AGAINST SOME PATHOGENIC AND SPOILAGE MICROORGANISMS

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Abstract

Lafun is among the foods produced from the fermentation of cassava tubers. *Lafun* flours were obtained from six different communities in Akoko area of Ondo State. Total bacterial counts and the lactic acid bacterial counts of the samples were determined. The pH, total titratable acidities TTA and temperature of the samples were also evaluated. The antibacterial activities of the predominant lactic acid bacteria from the samples were assayed against some pathogenic microorganisms using agar well diffusion methods. The highest bacterial counts and lactic acid bacteria counts of 4.61×10^6 cfu/g and 4.85×10^6 cfu/g were obtained from Iwaro Oka sample while the lowest counts of 1.2×10^6 cfu/g and 1.5×10^6 cfu/g were obtained from Akungba-Akoko and Ayegunle-Oka samples respectively. Bacteria that were isolated from the samples were *Lactobacillus plantarum*, *L. fermentum*, *Leuconostoc mesenteroides*, *Streptococcus faecalis*, *Staphylococcus aureus* *Escherichia coli* and *Bacillus cereus*. The highest and the lowest pH values of 5.4 and 3.4 were obtained from unfermented sample and the laboratory fermented sample respectively. The highest and the lowest total titratable acidities were obtained from the laboratory fermented sample (3.3%) and the unfermented sample (0.5%) respectively. The temperature of the *lafun* samples ranged from 28°C to 31°C. *Lactobacillus plantarum* and *L. fermentum* which were the predominant lactic acid bacteria from the samples inhibited *B. cereus*, *E. coli* and *S. aureus* and *Str. faecalis* isolated from the sample. This study suggested that the antimicrobial substances produced by *L. plantarum* and *L. fermentum* are potential bio-preservatives. The organisms should be investigated further for their health benefits.

Keywords: Cassava, fermented foods, *lafun*, lactic acid bacteria, fermentation

Introduction

Cassava (*Manihot esculenta*) is a staple food crop usually consumed in tropic and subtropical regions in the world. It is more resistant to drought, pest and disease attack. Global demand for cassava and cassava products mostly in Nigeria has resulted in the involvement of many individuals into farming activities. This has caused Nigeria to be rated as the largest producer of cassava in the world (Echebiri

and Edaba, 2008; Otekunrin and Sawicka, 2019). High rate of cassava production has been used in challenging food security in Africa countries and other countries around the world (Oyewole, 2002). Cassava also forms part of raw materials used in industries and in livestock feed formulation. Cassava tubers can also be used to produce instant noodles, beverages, sweeteners (glucose and fructose) and seasonings such as monosodium glutamate (Achi and

Akomas, 2006). Despite this, the consumption of cassava is limited because of its low or poor protein contents and presence of cyanogenic.

Fermentation technique has been employed in improving the nutritional quality and reduction in the antinutritional contents of most foods thereby making them available for human consumption (Umeh and Odibo, 2013). Processing of cassava tuber by fermentation for consumption helps to detoxify, preserve and extend the shelf life of the end products (Achi and Akomas, 2006). Popular foods obtained from fermented cassava tubers in Nigeria include *garri*, *fufu*, *chikwangue*, *pupuru*, *tapioca* and *lafun* (Achi and Akomas, 2006; Fakoya, and Adegbehingbe, 2007; Ayoade *et al.*, 2018 Adegbehingbe *et al.*, 2019).

Recent research has proven fermentation as the most economical methods of processing and preserving cassava tubers into desirable food products (Ayoade *et al.*, 2018). In developed countries, most fermented foods are produced under controlled conditions while in developing countries, they are processed using age old techniques. *Lafun* is a whitish colour dry powder produced from processed fermented cassava tubers which is prepared by stirring boiled water to form elastic dough which are eaten with soups or stews (Oyewole and Afolami, 2001; Omodamiro *et al.*, 2007;. Adebayo-Oyetero *et al.*, 2017). It is locally consumed by all classes of people, most especially the less affluent class (Achi and Akomas, 2006). It is prepared traditionally by peeling, washing, cutting, steeping and fermentation. Various biochemical changes had been reported during the fermentation of cassava tuber for *Lafun* production. They include hydrolysis of cyanogenic compounds and antinutritional factors, formation of flavour compounds and softening of the roots (Adebayo-Oyetero *et*

al., 2017).

Improper quality control measures in the production of most foods have resulted in the outbreak of food borne illness causing serious health problems in the society. Some microorganisms such as lactic acid bacteria under suitable conditions secrete antimicrobial substances which inhibit the growth of most food borne pathogenic organisms (Adebayo *et al.*, 2014). Lactic acid bacteria associated with most fermented foods contribute to their flavoring, souring, shelf life ability due to their ability to proliferate under high acidic conditions and secretion of organic acids such as lactic acid and butyric acid. The bacteriocin producing ability of lactic acid bacteria ensures preservation of foods, control of food borne pathogens capable of causing food spoilage or altering its structural form (Tebyanian *et al.*, 2017). Therefore, this study is aimed at antibacterial activities of lactic acid bacteria isolated from *fufu* samples against some pathogenic and spoilage microorganisms.

Materials and Methods

Collection of Samples

Fufu samples were obtained from the processors in six communities namely Akungba-Akoko, Ikare-Akoko, Iwaro-Akoko, Ayegunle-Akoko, Supare-Akoko and Oba-Akoko in all Akoko area, Ondo State, Nigeria. The samples were transported to Microbiology laboratory in iced packed bags for further analyses.

Laboratory Preparation of Lafun

The cassava root tubers were peeled and washed with potable water and allowed to drain. Five kg of the tubers were sliced into 7 cm thick and soaked in water in an open bowl in ratio 1:3 w/v in triplicates. The bowls were allowed to ferment for 72 hours at room temperatures. The soaking water was removed from the tubers, spread on trays, sun-dried for 4 days, milled into flours and sieved. They were packed in polythene bags

for further analyses.

Enumeration and Isolation of Microorganisms

Ten grams each of the samples were dissolved in 90 ml sterile peptone water solution and then homogenized to form the stock cultures. Isolation of microorganisms was done using serial dilution. Enumeration of viable bacteria and lactic acid bacteria was carried out on nutrient agar and de Man Rogosa and Sharpe (MRS) agar respectively. Bacterial cultures were incubated at 37°C for 24 hours while lactic acid bacteria were incubated anaerobically at 35°C for 48 hours. The colonies observed on the plates were counted and recorded. Pure cultures of the isolates were obtained by repeated streaking on their respective fresh bacteriological media. Pure bacterial isolates were subjected to various tests such as Gram's reaction and biochemical tests and identified as described by Olutiola *et al.* (2000).

Collection of test isolates

The test isolates *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Streptococcus Feacalis* were collected from the University Health Center of Adekunle Ajasin University, Akungba-Akoko, Nigeria. The organisms were maintained on Mueller-Hinton agar slants.

Standardization and Inoculation of the Organisms

All the organisms used were standardized to 0.5 McFarland standards. A 0.2 ml aliquot of 24 hour-old broth culture was dispensed in another sterilized 20ml Mueller-Hinton agar broth and incubated for 3-5 hours. One milliliter portion from the final broth is equal to 0.5 McFarland standard (6×10^8 cfu/ml) according to Oyeleke *et al.* (2008). A sterile swab stick was dipped into the standardized broth culture and excess liquid was drained from the swab stick by pressing it gently to the inner side of the test

tube containing the broth culture. The surfaces of the set media were streaked with the swab stick.

Antibiotic Sensitivity Testing of the Bacterial Isolates

The antibiotic sensitivity test of the microbial isolates was carried out using disc diffusion method (CLSI, 2009). The antibiotics sensitivity test was also investigated using GBMTS disc (Abtek Biological Limited) containing the following antibiotics ampiclox – APX (30 µg), gentamycin – GN (10 µg), erythromycin – E (10 µg), sparfloxacin – SP (10 µg), pefloxacin - PEF (10 µg), ciprofloxacin – CPX (10 µg), augmentin – AU (30 µg), streptomycin – S (30 µg), septrin – SXT (30 µg), chloramphenicol – CH (30 µg) and zinnacef – Z (20 µg). The commercial antibiotic discs were gently placed on Mueller-Hinton agar plates previously seeded with 24 hour old culture of each test microorganism using a glass spreader. The plates were incubated at 37°C for 24 hours. After the incubation, each plate was examined; the antibacterial susceptibility pattern of each of the isolates was indicated by clear zones of inhibition around each disc. The diameters of the zone of inhibition were measured and recorded. The isolates were categorized as either sensitive or resistant to antibiotics depending on their zones of inhibition.

Determination of Antimicrobial Activity of lactic Acid Bacteria from the Lafun

The antimicrobial activity of *Lactobacillus* sp. isolated against some pathogenic microorganisms such as *B. cereus*, *S. aureus* and *E. coli* was carried out using agar well diffusion method (Guessas and Kihal, 2004). Wells of 6.00 mm diameter were made in the solidified agar plates using sterile cork borer. The bacteriocin from the culture medium was obtained by cold centrifugation (4°C) at 3000 rpm for 30 minutes. Zero-point two milliliter (0.2 ml) cell-free filtrate was introduced into

the wells made on the grown plates while distilled water and standard antibiotics (ciprofloxacin 20mg/ml) were used as the negative and positive control respectively. The plates were incubated at 30°C for 48 hours and 37°C for 24 hours and the diameter of zones of inhibition was measured.

Determination of pH

The pH of the *lafun* was determined using a pH meter (Mettler Toledo, UK). This was carried out by weighing 10 g each of the sample into 90 ml sterile distilled water and then homogenized. The pH meter was calibrated with standard buffer (pH 7.0 and 4.0) and dipped into homogenized mixture and the results obtained were recorded (Olutiola *et al.*, 2000).

Determination of Total Titratable Acidity (TTA)

This was determined by diluting 10 g each of the *lafun* sample into 90 ml sterile distilled water. The mixture was homogenized and then allowed to settle. Twenty milliliters of the homogenate were titrated against sodium hydroxide with two drops of phenolphthalein as an indicator until the end point was reached and recorded (Olutiola *et al.*, 2000).

Data Analysis

The data generated were recorded and subjected to statistical analysis using standard procedure. Mean and standard deviations were obtained using ANOVA and the differences in the treatment mean were compared using Duncan's Multiple Range Test (DMRT).

Results

Figure 1 shows the total bacterial counts from the *lafun* samples. The highest bacterial count of 4.61×10^6 cfu/g and lactic acid bacteria count of 4.85×10^6 cfu/g were obtained from Iwaro-Oka sample while the lowest bacterial count of 1.20×10^6 cfu/g and lactic acid bacteria count of 1.5×10^6

cfu/g were obtained from fermented sample (control) respectively. *Staphylococcus aureus* and *Lactobacillus fermentum* were found in the ground unfermented sample while *Leuconostoc mesenteroides*, *L. fermentum* and *L. plantarum* were isolated from the laboratory fermented sample. *Lactobacillus fermentum* and *L. plantarum* and *Leuconostoc mesenteroides* lactic acid bacteria which were isolated from the *lafun* samples. *Lactobacillus plantarum* was isolated from all the samples while Supare-Akoko sample did not contain *L. fermentum* among the samples. Also, *Leuconostoc mesenteroides* were absent from Akungba-Akoko and Iwaro-oka samples. *Bacillus cereus* were isolated from Akungba and Iwaro-Oka. *S. aureus* from Akungba and Oba samples while *E. coli* were isolated from Akungba, Ikare, and Iwaro-Oka samples. *Streptococcus faecalis* was isolated from Ikare sample (Table 1).

Figure 2 shows the pH of the *lafun* samples. The highest pH of 5.4 was obtained from unfermented sample, followed by Akungba sample (5.0) while the least value of 3.4 was obtained fermented sample. However, laboratory fermented sample had the highest total titratable acidity (0.3%), followed by Oba sample (3.2%) while the unfermented sample recorded the least value (0.5%) (Figure 3). The temperature of the *lafun* samples ranged from 28°C to 31°C (Figure 4). The highest temperature of 31°C and the least value of 28°C were observed from Oba-Akoko and the laboratory fermented sample respectively. Fufu samples from Iwaro and Supare had temperature of 30°C while Ikare sample had temperature of 29°C.

Table 2 shows the antibiotic sensitivity pattern of the isolates from the fermented *lafun* samples. *Streptococcus faecalis* was most susceptible to Pefloxacin with zone of inhibition 27 mm. *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus faecalis* were susceptible to chloramphenicol

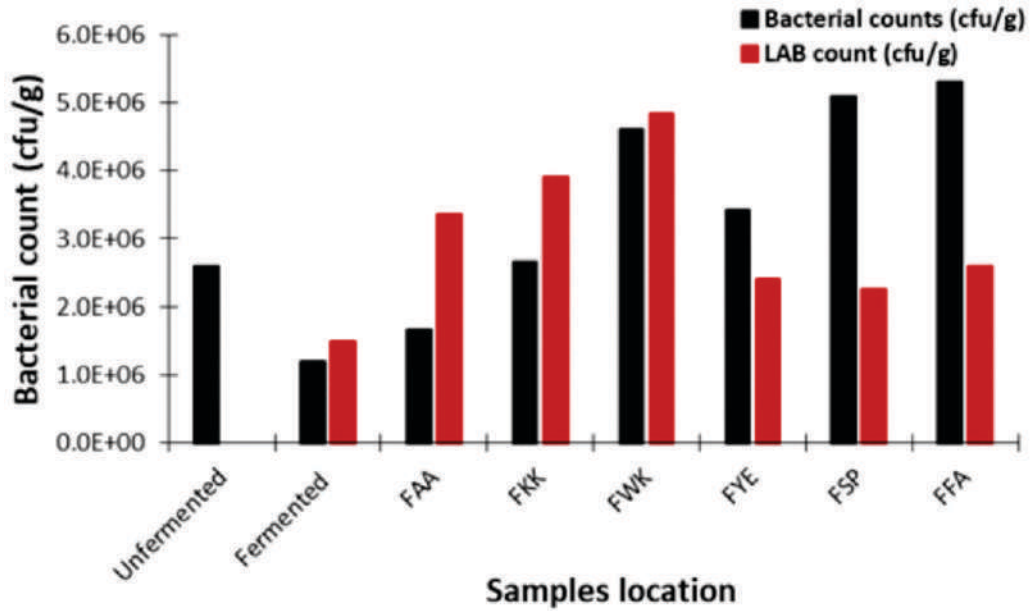


Figure 1: Total bacterial counts of the *lafun* samples.

FAA: Akungba-Akoko, FKK: Ikare-Akoko, FWK: Iwaro Oka, FYE: Ayegunle-Akoko, FSP: Supare-Akoko, FFA: Oba-Akoko.

Table 1: Occurrence of microorganisms from *lafun* samples

Sample code	<i>Bacillus cereus</i>	<i>Staphylococcus aureus</i>	<i>Leuconostoc mesenteroides</i>	<i>Streptococcus faecalis</i>	<i>Escherichia coli</i>	<i>Lactobacillus fermentum</i>	<i>Lb. plantarum</i>
UNF	-	+	+	-	-	-	-
FFF	-	-	+	-	-	+	+
FAA	+	+	-	-	+	+	+
FKK	+	-	+	+	+	+	+
FWK	-	-	-	-	+	+	+
FYE	-	-	+	-	-	+	+
FSP	-	-	+	-	-	-	+
FFA	-	+	+	-	-	+	+

-. Negative, +: Positive, UNF: Unfermented sample, FFF: Fermented samole, FAA: Akungba-Akoko, FKK: Ikare-Akoko, FWK: Iwaro-Oka, FYE: Ayegunle-Akoko, FSP: Supare-Akoko, FFA: Oba-Akoko.

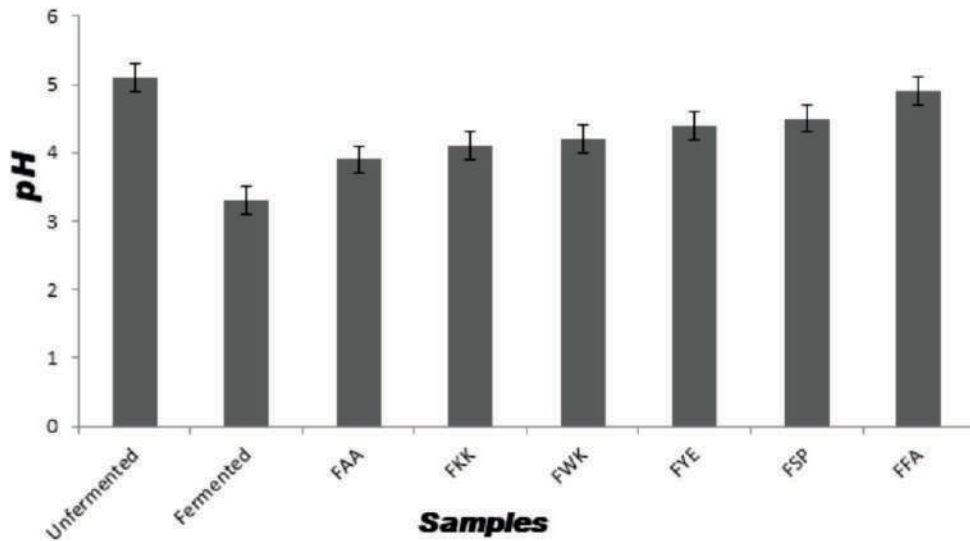


Figure 2: The pH of the *lafun* samples.

FAA: Akungba-Akoko, FKK: Ikare-Akoko, FWK: Iwaro Oka, FYE: Ayegunle-Akoko, FSP: Supare-Akoko, FFA: Oba-Akoko.

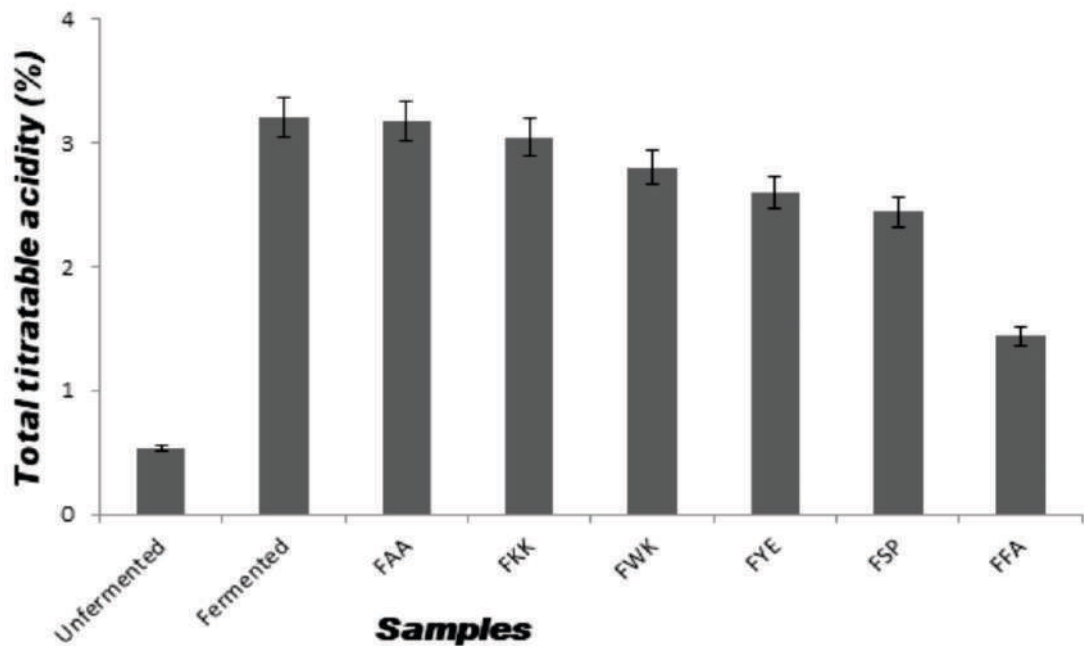


Figure 3: The total titratable acidity of the *lafun* samples.

FAA: Akungba-Akoko, FKK: Ikare-Akoko, FWK: Iwaro Oka, FYE: Ayegunle-Akoko, FSP: Supare-Akoko, FFA: Oba-Akoko.

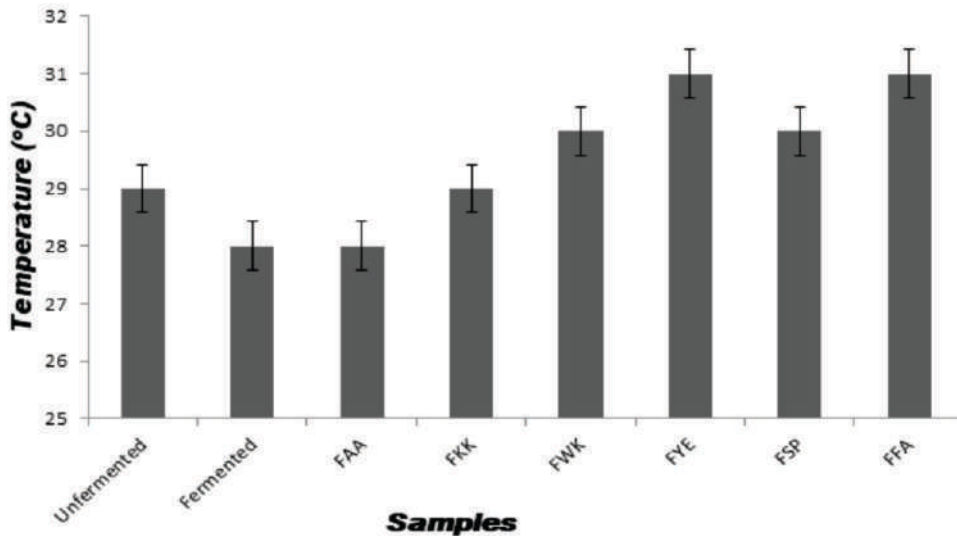


Figure 4: Temperature of the *lafun* samples

FAA: Akungba-Akoko, FKK: Ikare-Akoko, FWK: Iwaro Oka, FYE: Ayegunle-Akoko, FSP: Supare-Akoko, FFA: Oba-Akoko.

with zones of inhibition 13 mm, 14 mm and 15 mm respectively. The susceptibility of *B. cereus*, *E. coli* and *S. aureus* to Ciprofloxacin and Gentamycin were observed with zone of inhibition of 19 mm, 12 mm and 10 mm respectively. All the isolates were resistant to ampiclox, augmentin, sparfloxacin, septrin and zinnacef while *Streptococcus feacalis* was susceptible to Erythromycin with zone of inhibition 11 mm. *Staphylococcus aureus*, and *Streptococcus feacalis* were resistant to Ciprofloxacin.

Table 3 shows the antibacterial activity of the *Lactobacillus* spp. against the test isolates. All the pathogenic bacteria were susceptible to the LAB with various degrees of susceptibility. *Bacillus cereus* was the most susceptible to *L. plantarum* with the zone of inhibition of 14 mm followed by *E. coli* having 8 mm while the lowest susceptibility was observed in *Streptococcus feacalis* with 6 mm. *Lactobacillus fermentum* exhibited better inhibitory effect against *Staphylococcus aureus* (10 mm) followed by

B. cereus and *E. coli* (6 mm each) while *Streptococcus feacalis* was the least susceptible among them with 4 mm.

Discussion

Lactic acid bacteria play a major role in most fermentation processes, not only because of their ability to improve the flavor and aroma but also for their preservative effects on food. Predominance of diverse members of lactic acid bacteria in fermented foods had been reported during spontaneous fermentation of carbohydrate foods (Adewara and Ogunbanwo, 2013; Obi, 2015; Adegbehingbe *et al.*, 2019). Oyedeji *et al.* (2013) found *L. plantarum* and *Lc. mesenteroides* as the dominant lactic acid bacteria while producing *fufu*, and *L. cellobiosus*, *L. plantarum* and *Lc. lactis* during *ogi* fermentation. Ayoade *et al.* (2018) observed the predominance of *Lactobacillus brevis* and *L plantarum* during *gari* and *fufu* and production. The bacteria isolated from the *lafun* samples could be the microflora of the substrate (cassava tubers) of the samples.

Table 2: Antibiotic sensitivity pattern of the isolates from the fermented *lafun* samples

Antibiotics	Zone of inhibition (mm)			
	<i>Bacillus cereus</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus feacalis</i>
Ampiclox	R	R	R	R
Gentamycin	R	R	10	R
Erythromycin	R	R	R	11
Sparfloxacin	R	R	R	R
Pefloxacin	R	R	R	27
Ciprofloxacin	19	12	R	R
Augmentin	R	R	R	R
Amoxicillin	14	R	R	R
Streptomycin	12	R	R	R
Septin	R	R	R	R
Chloramphenicol	R	13	14	15
Zinnacef	R	R	R	R

R: Resistant

Table 3: Antimicrobial activity of *Lactobacillus* spp. Isolated from *lafun* against test isolates

Test isolates	Zone of inhibition (mm)	
	<i>Lactobacillus plantarum</i>	<i>Lactobacillus fermentum</i>
<i>Bacillus cereus</i>	14	6
<i>Staphylococcus aureus</i>	7	10
<i>Escherichia coli</i>	8	6
<i>Streptococcus feacalis</i>	6	4

There are other various ways in which microorganisms may come in contact with the substrate during spontaneous fermentation of foods. They could emerge from the raw materials, utensils and equipment used in its production. Other sources include insects or handlers which result in variation in the types of microorganisms from these substrates (Penido *et al.*, 2018). Higher bacterial counts from Iwaro-Oka sample could be due to lack of quality control check during processing, lack of proper hygiene measures by the processors, large surface area in the fermentation medium, availability and utilization of nutrients by the fermenting microorganisms.

The predominance of lactic acid bacteria in

the fermented food might be due to its tolerance to high acidity and subsequent low pH environment. Lactic acid fermentation contributes to the preservation, nutritional value, flavour and sensory properties of various fermented foods (Naik *et al.*, 2009). *Lactobacillus plantarum* has been reported as the dominant microorganisms implicated in various fermented foods such as *akamu*, *ogi*, *fufu*, *gappal*, *togwa*, *nono* and *burukutu* (Oyedeji *et al.*, 2013; Umaru *et al.*, 2014; Elyas, *et al.*, 2015; Tankoano *et al.*, 2019). The presence of *S aureus*, *E. coli* and *B. cereus* could be because of contamination from the environment during processing. Local equipment and primitive methods of food processing are major sources of microbial contamination in most fermented foods

(Sakhale *et al.*, 2012).

The fermentation of cassava tubers to produce *lafun* resulted to the decrease in pH and increase in total titratable acidity of the product. Reduction in pH values might be due to the increasing hydrogen ion content, fermentative transformation of carbohydrates to lactic acid and acetic acid by the microorganisms (Oyewole and Odunfa, 1992; Ogunbanwo, 2005; Oyedeji *et al.*, 2013).

The highest concentration of total titratable acidities from the laboratory fermented *lafun* (control) agreed with the findings of Ogunbanwo (2005). The acids produced such as lactic acid reduced the pH of the medium thereby making it acidic which is not conducive for the survival of some spoilage bacteria which might have found their way into the fermenting. The higher acids which is attributed to the proliferation of lactic acid bacteria is detrimental to the competing microorganisms. However, the presence of microorganisms such as *E. coli*, *S. feacalis* and *B. cereus* in some of the *lafun* samples could be from the handlers during or after fermentation (Oyewole and Odunfa, 1992; Adewumi *et al.*, 2009; Adegbehingbe *et al.*, 2019).

Temperature is one of the factors that affect the growth of microorganisms. Different microorganisms adapt to different temperature conditions. The mesophilic temperature could be useful in food preservation in traditional food fermentation where the temperature is within that which the isolates can tolerate. The variation observed in the temperature could be due to the prevailing storage conditions of the samples as well as the metabolic activities of microorganisms which were present in the samples (Adegbehingbe *et al.*, 2019).

The result of antibiotic effect against the isolated microorganisms agree with the

findings of Adewumi *et al.* (2009) who reported the resistance of *Bacillus* sp. to gentamycin, ampiclox and zinnacef but susceptible to ciprofloxacin. *Lactobacillus plantarum* and *L. fermentum* isolated from the samples had significant inhibitory activities against all the pathogenic bacteria isolated from the samples. The antimicrobial activity of lactic acid bacteria has been extensively reviewed (Ogunbanwo *et al.*, 2004; Adebayo *et al.*, 2014). The effect is due to the non-dissociated form of the acids, which can penetrate the membrane and liberate hydrogen ions in the neutral cytoplasm, thus leading to inhibition of vital cell functions (Adebayo *et al.*, 2014; Adegbehingbe and Bello, 2014; Afolabi *et al.*, 2016). Inhibitory activity of lactic acid bacteria has been reported to be due to a combination of many factors such as production of lactic acid which brings about reduction of pH of the fermentation medium and production of inhibitory bioactive compounds such as hydrogen peroxide and bacteriocins which are responsible for most antimicrobial activity (Ogunbanwo, 2005; Ishola and Adebayo-Tayo, 2012). The proliferation of spoilage microorganisms and food-borne pathogens can be prevented by low pH and high concentration of organic acids. The antimicrobial activity of *Lactobacillus fermentum* against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhimorium* has been reported (Atter *et al.*, 2014).

Conclusion

Production of antimicrobial substances from lactic acid bacteria as bio-preservative in foods could significantly be of importance in industries due to its less toxic effect. The inhibitory substances produced by lactic acid bacteria are known to contribute to the safety of fermented foods as natural inhibitors against spoilage microorganisms.

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