Bacteriocin Production using Lactic Acid Bacteria Isolated from Ugba (*Pentaclethra macrophylla* Benth)

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Abstract— *Lactic acid bacteria (LAB) play vital roles in our everyday life such as in fermentation, preservation and production of wholesome foods. This work is aimed at studying the bacteriocin production of lactic acid bacteria from Ugba (a local condiment) sold in Anambra state. A total of 5 samples of Ugba each were purchased randomly from three locations in Anambra state. Lactic acid bacteria were isolated and characterized using standard microbiological procedures. The isolates were subjected to antimicrobial activities by disc diffusion method while bacteriocin production was achieved by culture on appropriate medium and partially purified by centrifugation method and then characterized. Antimicrobial activities using cell free supernatant and partially purified bacteriocin of the isolates against Escherichia coli, Staph. aureus and Candida albicans found in Ugba were equally assessed. The isolate that gave the highest zone of inhibition was identified by 16s rDNA sequencing and was selected for further assay. Biopreservative study of the choice isolate against Escherichia coli, Staph. aureus and Candida albicans which were extraneously introduced into the Ugba was conducted. A total of Six LAB isolates designated with codes were obtained based on their catalase spot test reaction. zones of inhibition ranging from 13-20mm, 12- 18 mm and 10-19mm were obtained against Escherichia coli, Staphylococcus aureus and Candida albicians respectively. The LAB isolate code A1 with the Best Antimicrobial Activity was identified as Lactobacillus plantarum. The bacteriocin produces by Lactobacillus plantarum (A) was slightly stable at 40 and 60°C with a continuous decrease at 80 and 100°C for 30 mins but totally lost the activity at 121 °C for 15 min. The effect of pH on partially purified bacteriocin showed maximum activity against Escherichia coli, Staphylococcus auerus and Candida albicians. at pH 5.0 (18 mm ,17mm and 15mm respectively) and a continuous decrease as the pH increased from 6 to 9. The antimicrobial activity of bacteriocin was lost after treatment with proteolytic enzyme: trypsin and pepsin. This study show that Lactobacillus plantarum isolated from ugba has great potential for exploitation in food safety and preservation as a result of its bacteriocin content. Thus this locally fermented food plays a dual role of protection from pathogenic agents as well as serving as a functional food. It is therefore recommended that everybody should begin to consume ugba on a regular basis.*

Keywords— Ugba, Lactic acid bacteria, Bacteriocin.

I. INTRODUCTION

Ugba also called 'Ukpaka' in Eastern part of Nigeria is an indigenous fermented food, rich in protein and produced by a solid state fermentation of seeds of African oil bean tree (*Pentaclethra macrophylla*), a perennial legume tree. It is a popular food delicacy in Nigeria especially among the Ibo ethnic group where it serves as snack or as a food condiment. It is an important food item for various traditional celebrations where it is mixed with slices of boiled stock fish, garnished with vegetables and consumed. The unfermented seeds are bitter to taste as a result of anti-nutrition substances inherent in them. The natural fermentation of the seeds still done at the domestic level renders the product nutritious, palatable and non-toxic.

Diverse groups of microorganisms including *Bacillus, Micrococcus, Leuconostoc, Staphylococcus, Enterobacteriaceae and lactic acid bacteria* (LAB) have been reported to play active roles in the process of ugba fermentation (Enujiugha 2009). They produce antimicrobial substances such as organic acids, diacetyl, hydrogen peroxide and bacteriocins (Ogunbanwo *et al*. 2003) which are alleged to be connected with the preservation of many fermented food condiments in Nigeria. The preservation and safety are currently the two major challenges of the food industry as huge economic losses are sustained yearly due to food

spoilage while many consumers have been reported to develop adverse sensitivity reactions to chemical based preservatives. The Lactic acid bacteria (LAB) which are used generally as starter cultures for food fermentation are considered as having the potential to bridge this gap (Budde *et al*. 2003; Delves-Broughton 2005). They produce antimicrobial compounds that demonstrate antagonism toward the closely related species. The LAB in 'ugba' improve fermentation and also enhance the product storage, quality and safety by restricting the spoilage microflora from spoiling the food condiment and at the same time eliminating the pathogens such as *L.monocytogenes, S. aureus and E.coli* (Alor and Okonkwo 2021).

Lactic acid bacteria (LAB) are heterogenous group of bacteria which play a significant role in a variety of fermentation processes. They ferment food carbohydrates and produce lactic acid as the main product of fermentation. In addition, their degradation of proteins and lipids and production of various alcohols, aldehydes, acids, esters and sulphur compounds contribute to the specific flavour development in different fermented food products.(Sindhu and Khetarpaul 2001)

While many scientific reports are available on 'ugba', such studies have thus far addressed mainly the microorganisms associated with the fermentation processes of the seed as well as systems for optimization of the fermentation conditions (Odibo *et al*. 2008). The effect of processing techniques on the nutritive quality of ugba has also been reported by Okonkwo and Alor 2021 while the proximate composition and properties of the seeds have also received modest scientific attention (Odibo *et al* 2008). There is presently, paucity of scientific information on the ecological contribution of the LAB and bacteriocins for the safety and bio preservation of the food condiments.

Bacteriocins are extracellularly produced primary compounds of bacterial ribosomal synthesis which have a somewhat narrow range of bactericidal activity (Caplice and Fitzgerald 1999). They are active against other bacteria despite varying greatly in the chemical nature and mode of action. Bacteriocins have significant advantage over the established antibiotics in being easily degraded by the digestive enzymes without the risk of interference with the normal tract ecology (Caplice and Fitzgerald 1999). Bacteriocin producing LAB have been generally recognized as safe (GRAS) status and have been shown to fortify the barrier function of the gut microflora as well as promote the non-specific improvement of the immune system of man and animals (Tome *et al*. 2008).

The problem of chemotherapy and its associated side effects together with multiple drug resistance by infectious agents has led to the search for local foods that can serve as functional foods with dual role of protection from pathogenic agents as well as high nutrient based diet. Hence ugba (*P macrophylla*) which is generally consumed particularly in southeast Nigeria is investigated for its role in the isolation of lactic acid bacteria for bacteriocin production.

II. MATERIALS AND METHODS

2.1 Sample Collection:

Five samples each of traditionally fermented ‘ugba’ were randomly purchased from different sellers at different locations in Anambra state. This include ‘Awka’, ‘Ontisha’. ‘Nnewi,’ representing the three senatorial zones, in Anambra state. All the samples were transported to Microbiology laboratory of Nnamdi Azikiwe University Awka and stored at 3±1°C for a maximum of 24 hours prior to analysis.

2.2 Isolation of Lactic Acid Producing Bacteria (LAB):

Ten gram of each sample was homogenized separately in a porcelain mortar and then transferred into 250 ml flask containing 90 ml of buffered peptone water as diluents. The samples were mixed thoroughly by agitation for 2 min before serial 10-fold dilution was made in 0.1 % peptone water. For the isolation of the LAB, 0.1 ml supernatant of appropriate dilution was inoculated into de Mann, Rogosa and Sharpe (MRS) agar plates containing 0.5% nystatin using pour plate method. The plates were incubated anaerobically at 30 °C for 48 hours and the isolates purified by successive subculture. (Enujiugha *et al.*, 2002).

2.3 Characterization and Identification of Lactic Acid Bacteria:

The morphological, biochemical and molecular characteristics of the isolates were determined using standard microbiological methods.

The antibiogram profile of the isolates was determined using the method of Vlková *et al*. (2006).

The molecular identification of the best LAB isolate with the best antimicrobial activity was done by 16s rDNA sequencing method. (Murray 2010).

2.3.1 Bacteriocin Production by the Lab Isolates:

Bacteriocin production was performed by growing the isolates in MRS broth at 30ºC for 48 hours. This was done according to the method of Joshi *et al.,* (2006). After incubation, the broth was centrifuged at 5000xg for 10mins to obtain a cell free supernatant. The cell-free supernatant was saturated with 60% ammonium sulphate. After stirring on a magnetic stirrer, it was kept undisturbed in the refrigerator at the temperature of 25^0c. Precipitates formed were collected by centrifugation at 10,000 x g for 10mins and redissolved in 20mmol sodium phosphate buffer with pH 6.0.

2.3.2 Sensitivity of the Bacteriocin to Various Treatments:

The CFS (cell free supernatant) was divided into 2.0 ml portions before subjecting them to treatments in organic solvents (chloroform and ethanol), proteolytic action (pepsin and trypsin) and heat in order to evaluate the effect of the different factors on the activity and stability of bacteriocin. The solvents and enzymes were filtered, sterilized through Millex GV 0.22 µm filters before the addition of CFS. The controls consist of distilled water and untreated CFS in 0.1 M phosphate buffer. The samples and controls were incubated at 30 °C for 2 hours before the remaining bacteriocin activity were determined by the agar-well diffusion (AWD) assay against the indicator strains. (Azizpour *et al.,* 2009).

2.3.3 Detection of Antimicrobial Activity of The Bacteriocin:

A modification of the agar-well diffusion (AWD) method was employed in this assay (Azizpour *et al*, 2009). A loopful of the indicator strains *E. coli, S. aureus* and *C. albicans* were inoculated separately into 5ml normal saline for 5h. Then sterilized nutrient agar was each seeded with 1.0 ml each of the indicator strains at a concentration of 10^8 CFU/ml gently mixed and then dispensed into sterile Petri-dishes. The plates were left to solidify under a laminar airflow. Thereafter, wells (8 mm diameter) were made in each of the plates using a sterile cork borer. A 0.1 ml of the CFS was introduced into the different wells and left for 1 h in a refrigerator to allow for diffusion of the metabolite before incubating at 30 °C. The plates were examined for the development of translucent halos in the bacterial lawn surrounding the wells after 24 h incubation. the haloes (zones of clearing) produced by the different strains were compared. The percentage bacteriocin activity were calculated from the diameter of zones of clearing measured after each treatment relative to the halos produced from the positive control (untreated CFS) against each target organism. (Azizpour *et al.,* 2009)**.**

2.4 Characterization of Bacteriocin:

2.4.1 Effect of Temperature on the antimicrobial activity of the Bacteriocin:

Test tubes containing 5 ml of the partially purified bacteriocin was overlaid with paraffin oil to prevent evaporation and then heated at 40, 60, 80, 100°C for 30 min, and at 121°C for 15 min. The heat-treated bacteriocin and the control were incubated at 37ºC for 2 h. The antimicrobial activity was determined by agar-well diffusion (AWD) assay against the indicator strains (Okpala *et al.,* 2003).

2.4.2 Effect of pH on the antimicrobial activity of the Bacteriocin:

A 5ml aliquot of the partially purified bacteriocin was transferred into test tubes and the pH values of the contents were adjusted to 2–9 individually, using 1 M NaOH and 1 M HCl. After allowing the samples to stand at room temperature (30ºC) for 2h, the antimicrobial activity of the bacteriocin and the control were determined by agar-well diffusion assay (Joshi *et al*., 2006).

2.4.3 Effect of proteolytic enzyme on the antimicrobial activity of the Bacteriocin:

Test tubes containing 5ml aliquot of the partially purified bacteriocin was treated with pepsin and trypsin (1 mg/ml) each. The test tubes with and without the enzyme (controls) were incubated for 2 h at 30 °C and heated for 3 min at 100°C to denature the enzyme. Both the controls and the bacteriocin were assayed for antimicrobial activity by agar well diffusion method (Joshi *et al*., 2006).

III. RESULTS

3.1 Isolation and Characterization of LAB from Ugba:

A total of 25 organisms were isolated and screened. Six LAB isolates were obtained based on their catalase spot test reaction and colony morphologies on De- Mann Rogosa and Sharpe (MRS) agar. Table 1 shows the lactic acid bacteria isolated from each homogenized ugba. The biochemical identification and characterization of the isolates using conventional methods are shown in Table 2. The results obtained shows that all isolates were gram positive, non-motile, catalase negative and have varying carbohydrate (sugar) fermentation profile which presumptively made them Lactic acid bacteria.

TABLE 1
NUMBER OF ORGANISMS ISOLATED FROM UGBA MADE FROM DIFFERENT LOCATIONS IN ANAMBRA STATE

Ugba purchase	Number of organism isolated	Number of lactic acid isolated	Isolate code
From Awka	7	2	A1, A2
From Nnewi	8	2	N1,N2
From Onitsha	10	2	O1,O2

TABLE 2
BIOCHEMICAL IDENTIFICATION AND CHARACTERIZATION OF THE LAB ISOLATES

Isolate code	Cultural Characteristics	Cell Morphology	Gram Reaction	Catalase Reaction	Motility	Gas from glucose	Glucose	Lactose	Sucrose	Fructose	Galactose	
A1	Punctiform, glistening, smooth whitish colony	coccobacilli	+	-	-	-	+	+	+	+	+	*Lactobacillus* sp
A2	circular raised cream white colony	cocci in tetrads	+	-	-	-	+	+	+	+	-	*Pediococcus* sp
N1	Circular, flat translucent whitish colony	cocci in pairs and short chains	+	-	-	-	+	+	-	-	+	*Leuconostoc* sp
N2	Circular, flat off white colony	cocci in chains	+	-	-	+	+	+	-	+	+	*Lactococcus* sp
O1	Punctiform, smooth raised cream colony	long rods in chains	+	-	-	-	+	+	+	+	-	*Lactobacillus* sp
O2	Circular, flat off white colony	cocci in tetrads	+	-	-	-	+	+	+	+	+	*Pediococcus* sp

3.2 Antibiogram of the LAB Isolates:

Some of the LAB isolates were sensitive to all the antibiotics except A1, O1, N1 which showed varying degrees of susceptibility to antibiotics such as Erythromycin, Streptomycin, Ciprofloxacin and Zinnacef as shown in plate 1.

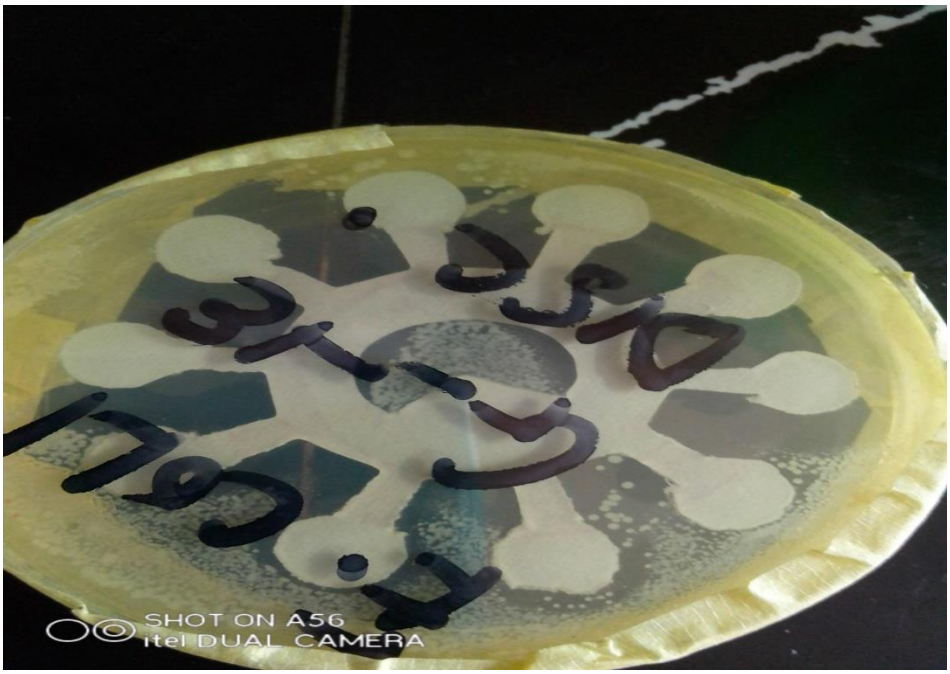

PLATE 1: Antibiogram of LAB isolate with designated code A1

Key: S = Streptomycin, SXT= Septrin, E = Erythromycin, PEF = Pefloxacin CN = Gentamycin APX =Amplicox, Z = Zinnacef, AM= Amoxicillin, R = Rocephin, CIP = Ciprofloxacin.

3.2.1 Antimicrobial Activities of the Cell Free Supernatant from the LAB Isolates against test organisms (*Escherichia coli, Staphylococcus aureus and Candida albicians)*.

The cell free supernatants of the LAB isolates examined for antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus and Candida albicians yielded* zones of inhibition ranging from 13-20mm, 12- 18 mm and 10-19mm respectively. The inhibition zones showed that all of the isolates have antimicrobial effect on the indicator microorganisms with isolate A1 having the highest zone of inhibition (20 mm for *Escherichia coli*,18 mm for *Staphylococcus sp* and 19mm for *Candida albicians)* as seen in Table 3.

TABLE 3

ANTIMICROBIAL ACTIVITY OF THE CELL FREE SUPERNATANT FROM LAB ISOLATES AGAINST *ESCHERICHIA COLI*, *STAPHYLOCOCCUS AUERUS* AND *CANDIDA ALBICIANS*

Isolate code	Zone of inhibition (mm) (Mean ± S.E)		
	Escherichia coli	Staphylococcus auerus	Candida albicians
A1	20.00±0.05	18.00±0.00	19.17±0.00
A2	15.15±0.03	12.10±0.01	15.00±0.00
N1	15.25±0.05	15.10±0.02	15.17±0.00
N2	16.00±0.00	16.28±0.03	16.00±0.00
O1	18.16±0.03	16.00±0.00	16.20±0.00
O2	15.00±0.01	12.22±0.02	10.6±0.00

3.2.2 Antimicrobial Activities of the Bacteriocin from the LAB Isolates against test organism (*Escherichia coli, Staphylococcus aureus and Candida albicians*)

The result obtained from the antimicrobial activities against *Escherichia coli*, *Staphylococcus aureus* and *Cadida albicians* as seen in table 4, showed that the isolate with designated code A1 had the highest zone of inhibition for *Escherichia coli* (18.36mm), *Staphylococcus auerus* (17.33mm) and *Candida albicians* (19mm) while the isolate with the code N1 and N2 had no inhibitory effect on the test organisms. Isolate O2 had no effect on *Escherichia coli* but had inhibition zones of 13.13mm 14.0mm on *Staphylococcus aureus* and *Candida albician* respectively. Also isolate O1 had zone of inhibition of 16.33mm for *Escherichia coli* and 11.00mm for *Candida albician* but no activity was detected for *Staphylococcus aureus.*

TABLE 4

ANTIMICROBIAL ACTIVITY OF THE BACTERIOCIN FROM THE LAB ISOLATES AGAINST *ESCHERICHIA COLI, STPHYLOCOCCUS AUERUS* AND *CANDIDA ALBICIANS*

Isolate code	Zone of inhibition (mm) (Mean ± S.E)		
	Escherichia coli	Staphylococcus auerus	Candida albicians
A1	18.36±0.03	17.33±0.01	19.00±0.00
A2	13.65±0.05	12.00±0.00	15.00±0.00
N1	NI	NI	NI
N2	NI	NI	NI
O1	16.33±0.03	NI	11.003±0.00
O2	NI	13.13±0.02	14.00±0.02

Key

NI= no zone of inhibition

The LAB isolate code A1 with the Best Antimicrobial Activity was identified as *Lactobacillus plantarum*

3.3 Characterization of the Bacteriocin of *Lactobacillus plantarum* (A1)

The sensitivity of the bacteriocin produces by *Lactobacillus plantarum* (A1) to various treatments were shown in table 6-8. The antimicrobial activity of the heat treated bacteriocin was found to be slightly stable at 40 and 60°C with a continuous decrease at 80 and 100°C for 30 mins. However, after incubation for 15 min at 121°C, there was complete loss of activity. The effect of pH on partially purified bacteriocin showed maximum activity against *Escherichia coli, Staphylococcus auerus and Candida albicians*. at pH 5.0 (18 mm ,17mm and 15mm respectively) and a continuous decrease as the pH increased from 6 to 9. The antimicrobial activity of bacteriocin was lost after treatment with proteolytic enzyme: trypsin and pepsin as seen in table 5.

TABLE 5

EFFECTS OF PH ON BACTERIOCIN FROM *LACTOBACILLUS PLANTARUM* (A1) AGAINST *ESCHERICHIA COLI, STAPHYLOCOCCUS AUERUS* AND *CANDIDA ALBICIANS*

pH	Inhibition zone diameter (mm)		
	Escherichia coli	Staphylococcus auerus	Candida albicians
2	7	5	6
3	11	10	9
4	15	14	13
5	18	17	15
6	10	9	8
7	8	7	6
8	5	4	5
9	3	NI	2
Control (without pH treatment)	18	17	18

Key

NI=No inhibition zone

TABLE 6

EFFECTS OF PROTEOLYTIC ENZYMES ON BACTERIOCIN FROM *LACTOBACILLUS PLANTARUM* (A1) AGAINST *ESCHERICHIA COLI*, *STAPHYLOCOCCUS AUERUS* AND *CANDIDA ALBICIANS*

Proteolytic enzyme	Inhibition zone diameter (mm)		
	Escherichia coli	Staphylococcus auerus	Candida albicians
Trypsin	NI	NI	NI
Pepsin	NI	NI	NI
Control (without Proteolytic enzyme)	18	17	16

Key:
NI=No inhibition zone

IV. DISCUSSION

Out of 15 isolates obtained from ugba, 6 Lactic acid bacteria were found and characterized (Tables 1 and 2). The presence of LAB in these samples is due to the lactic acid fermentation which is a principal production step. It also confirms the reports of Njoku and Okemmadu (2008) on the active roles lactic acid bacteria play in the spontaneous fermentation of ugba.

The susceptibility of the isolates tested against 10 different antibiotics in this research showed that six isolates were susceptible to all of the antibiotics used while the remaining four isolates (O1, A1 and N1) had varying degrees of susceptibility to antibiotics such as Erythromycin, Septrin, Ciprofloxacin, Zinnacef and Rocephine as shown in plates 1. LAB isolates with designated code O1, A1, N1 were resistant to Amoxicillin, Gentamycin, Streptomycin, Pefloxacin and Amplicox; and susceptible to Erythromycin and Ciprofloxacin. O1 was resistant to Septrin and susceptible to Rocephine and Zinnacef while A1 and N1 were resistant to Rocephine and susceptible to Septrin. These results are in agreement with the research conducted by Pundir *et al.* (2013) where all LAB isolates were sensitive to Erythromycin. *Lactobacillus plantarum* (A1) which gave the best antimicrobial and bacteriocin like activity was resistance to Amoxicillin, Gentamycin, Streptomycin, Pefloxacin, Amplicox, Zinnacef and Rocephin and was susceptible to Erythromycin, Septrin and Ciprofloxacin. This agrees with the reports of Zhou *et al.* (2005) who stated that Lactobacilli are usually sensitive to inhibitors of protein synthesis and more resistant to aminoglycosides. Resistance to common antibiotics by lactic acid bacteria could be intrinsic (naturally owned) or acquired (Mathur and Singh, 2005). Intrinsic resistance of lactic acid bacteria to many antibiotics is an advantage for isolates with bacteriocin genic/probiotic potential because it could be helpful for sustainable utilization of the strains in human intestinal microflora during antibiotic therapy (Ketema *et al*., 2010).

The cell free supernatants of the LAB isolates examined for antimicrobial activity against pathogenic bacteria *Escherichia coli*, *Staphylococcus auerus* and *Candida albicians* yielded zones of inhibition ranging from 13-20mm for *Escherichia coli*, 12- 18 mm for *Staphylococcus auerus* and for *Candida albicans* as seen in Table 3. The diameter of inhibition zones showed that all of the isolates have antibacterial effect on the indicator microorganisms. The highest diameter (20 mm for *Escherichia coli*, 18 mm for *Staphylococcus auerus and Candida albicians*.) was recorded for *Lactobacillus plantarum* (A1) which was the same with the report of Kaushik *et al.* (2009) where *Lactobacillus plantarum* had 20 mm zone of inhibition for *Escherichia coli*. However, this result was not in agreement with the reports of Mobolaji and Wuraola (2011), Adejumo *et al.* (2014) who reported no antagonistic effect on *Escherichia coli* and *Staphylococcus auerus by Lactobacillus. plantarum* while Oluwajoba *et al.* (2014) reported 15, 21 and 22 mm zone of inhibition for *Escherichia coli Staphylococcus auerus* and *Candida albicians respectively*. The antimicrobial activity exhibited by these LAB isolates may be due to decrease in pH, depletion of nutrients and production of antimicrobial compounds including bacteriocins and various organic acids such as lactic acid, acetic acid (Adejumo, 2014)

Bacteriocin from gram positive organisms such as lactic acid bacteria have attracted much attention and have been the subject of intensive investigation due to their ability to act as bio-preservative agents. The data obtained for *Lactobacillus plantarum* (A1) which showed the highest zone of inhibition against the target microorganisms in this study, is in agreement with that of Okpara *et al.* (2014) and Sankar *et al.* (2012) where the bacteriocin produced by *Lactobacillus. plantarum* was active against *Escherichia coli* giving an inhibition of 18mm. However this result did not agree with the reports by Falegan *et al.* (2014), Okoro *et al.* (2011), Ogunbawo *et al.* (2003), having zones of inhibition 7, 28, 8, 12 mm for *Escherichia coli* and 7mm for *Staphylococcus* auerus. The variations in the inhibition zones by the bacteriocin against *Escherichia coli* may be as a result of range of inhibition, assay method, concentration and purity of the inhibitor, the sensitivity of the indicator species, the density of the cell suspension used and the type of buffer or broth used (Sourau and Arijit, 2010). Also the variations in the inhibition zones by bacteriocins produced by

Lactobacillus. plantarum could also be as a result of the genetic diversity of *Lactobacillus plantarum* observed by Oguntoyinbo *et al.,* (2015).

The stability pattern of the bacteriocin produced by *Lactobacillus plantarum* was found to agree with the works of Joshi *et al.* (2006), Sankar *et al.* (2012) and Okpara *et al.* (2014) where there was a partial loss in activity as temperature was increased to 100ºC for up to 15mins and a complete loss of activity at 121°C. The thermo stability of bacteriocins is of great importance in its application in food system, especially when they are to be used together with pasteurization in a multiple hurdle approach to food preservation. The bacteriocin showed maximum activity against *Escherichia coli* at pH 5.0 (18 mm) after which the activity gradually decreased. This finding indicated that increased pH affected the stability of the bacteriocin. The antimicrobial activity of bacteriocin was lost after treatment with proteolytic enzyme: trypsin and pepsin. These results confirmed the proteinaceous nature of the bacteriocin. Studies done by Joshi *et al.* (2006), Elhag *et al.* (2015) revealed that bacteriocins of different LAB species lost their proteinaceous nature when treated with proteolyic enzymes.

The food borne pathogens isolated are the common pathogens that have been reported in works on microbiological analyses of Ugba by Ogbonna *et al.* (2011) and Gyar *et al.* (2014). These organisms of public health concern might have been introduced as a result of processing and packaging procedures such as poor handling and sanitation conditions. This could be due to the antimicrobial metabolites produced by *Lactobacillus plantarum*. These include many organic acids such as lactic, acetic and propionic acids produced as end products which provide an unfavourable acidic environment for the growth of many pathogenic and spoilage microorganisms. Acids are generally thought to exert their antimicrobial effect by interfering with the maintenance of cell membrane potential, inhibiting active transport, reducing intracellular pH and inhibiting a variety of metabolic functions (Ross *et al.,* 2002). Another possible explanation is the production of bacteriocins.

V. CONCLUSION

This study shows that *Lactobacillus plantarum* isolated from ugba has great potential for exploitation in food safety and preservation as a result of its bacteriocin content. Thus this locally fermented food plays a dual role of protection from pathogenic agents as well as serving as a functional food. It is therefore recommended that everybody should begin to consume ugba on a regular basis.

REFERENCES

[1] Adejumo, T.O. (2014). Antimicrobial activity of lactic acid bacteria isolated from fermented milk products. *African Journal of Food Science,* **8(**10): 490-496.

[2] Azizpour. K., (2009) Biochemical Characterization of Lactic Acid Bacteria Isolated from Rainbow Trout (*Oncorhynchus mykiss*) of West Azarbaijn, Iran. *Research. Journal of Biological. Science.* 4 -324-326. 8.

[3] Budde B B, Hornbaek T, Jacobsen T, Barkholt V, Koch A G.; (2003) *Leuconostoc carnosum* 4010 has the potential for use as aprotective culture for vaccum - packed meats: culture isolation, bacteriocin identification and mean application experiments. *International Journal of Food Microbiology*.83: 171-184.

[4] Caplice, E. and Fitzgerald, G.E. (1999). Food fermentation: role of microorganism in food production and preservation. *International Journal of Food Microbiology*, **50**: 131- 149.

[5] Elhag, N.B., Babiker, E.B. and Mahdi, A.A. (2015). Isolation and characterization of bacteriocin- producing lactic acid bacteria against indicator organisms. *Journal of Agri-Food andApplied Sciences,* **3**(1); 6-13.

[6] Enujiugha V N. (2009) Major fermentative organisms in some Nigerian soup condiments. *Pakistan Journal of Nutrition.* 8: 279-283.

[7] Enujiugha, V., C. Amadi and T. Sanni, (2002). Amylase in raw and fermented African oilbean seed (Pentaclethra macrophylla Benth). *Journal of European Food Research*. *Technology*, 214: 497-500.

[8] Falegan, C.R., Akoja, S.O., Ogunjobi, L.P. and Obaro, C.E. (2014). Assessment of the antimicrobial activity of cell free crude bacteriocin produced by *Lactobacillus* species isolated from ogi (fermented maize) on *Escherichia coli* a food borne pathogen *US Open GeneralMicrobiology Journal*,**1**(2): 1-8.

[9] Joshi, V. K., Sharma S. and Rana N. S. (2006). Production, Purification, Stability and Efficacy of Bacteriocin from Isolates of Natural Lactic Acid Fermentation of Vegetables. *Food Technology and Biotechnology***, 44**(3): 435-439.

[10] Kaushik, J.K., Kumar, A., Duary, R.K., Mohanty, A.K., Grover, S., *et al.,* (2009). Functional and Probiotic Attributes of an Indigenous Isolate of *Lactobacillus plantarum*. PLoS ONE 4(12): e8099. doi:10.1371/journal.pone.0008099.

[11] Ketema, B., Tetemke, M., Mogessie, A. (2010). Antibiotic susceptibility patterns of LAB isolated from *Wakalim*, a traditional Ethiopian fermented beef sausage. *Journal of Food Safety***30**: 213-223.

[12] Mathur, S. and Singh, R. (2005): Antibiotic resistance in food lactic acid bacteria - a review. *International Journal of Food Microbiology*,**105:** 281-295.

[13] Mobolaji, O.A. and Wuraola, F.O. (2011). Assessment of the antimicrobial activity of lactic acid bacteria isolated from two fermented ugba *Malaysian Journal of Microbiology*, **7**(3): 124-128.

[14] Murray (2010). The human microbiome jumpstart on DNA

[15] Njoku, H.O. and C.P. Okemadu, (2003). Biochemical changes during the natural fermentation of the African oil bean (Pentaclethra macrophylla) for the production of Ugba. *Journal of Science Food and Agriculture*, 49: 457-465.

[16] Odibo F.J.C, Ezeaku E.O, Ogbo F.C. (2008) Biochemical changes during the fermentation of Prosopis africana seeds for 'ogiri-okpei' production. *Journal of industrial Microbiological Biotechnology*.; 35: 947-952.

[17] Ogunbanwo, S.T., Sanni A.I. and Onilude A.A. (2003). Characterization of bacteriocin produced by *Lactobacillus plantarum* F1 and *Lactobacillus brevis* OG1. *African Journal of Biotechnology*, **2(**8): 219-227.

[18] Okpara, A.N., Okolo, B.N. and Ugwuanyi, J.O. (2014). Antimicrobial activities of lactic acid bacteria isolated from akamu and kunu-zaki (cereal based non alcoholic beverages) in Nigeria. *African Journal of Biotechnology*, **13** (29): 2977-2984.

[19] Pundir, R.K., kashyap, S., R., N. and Kaur, A. (2013). Probiotic Potential of Lactic acid Bacteria Isolated from Food Samples – an in Vitro study. *Journal of Applied Pharmaceutical Science*, **3** (03): 085- 093.

[20] Ross, R.P., Morgans, S. and Hill, C. (2002). Preservation and fermentation: Past present and future. *International Journal of Food Microbiology*, **79**: (1-2): 3-16.

[21] Sankar, N.R., Priyanka V.D., Reddy, R.S, Rajanikanth, P., Kumar, V.K. and Indira, M. (2012). Purification and characterization of bacteriocin produced by *Lactobacillus plantarum* isolated from cow milk. *International Journal of Microbiological Research*,**3** (2): 133-137.

[22] Sindhu, S.C. and Khetarpaul, N. (2001). Probiotic fermentation of indigenous food mixture: effect on antinutrients and digestibility of starch and Protein. *Journal of Food Composition and Analysis*, **14**: 601-609.

[23] Sourau, B., and Arijit, D. (2010). Study of physical and cultural Parameters on the bacteriocins produced by lactic acid bacteria isolated from traditional Indian fermented foods. *American Journal of Food Technology*, **5**(2): 111-120.

[24] Tome E, Pereira VL, Lopes CI, Gibbs PA, Teixera C. (2008); 'In vitro' tests of suitability of bacteriocin - producing lactic acid bacteria, as potential biopreservation cultures in vacuum - packaged cold - smoked Salmon. *Food Control*. 19: 535-543.

[25] Vlková, E., Rada, V., Popelářová, P., Trojanová, I. and Killer, J. (2006). Antimicrobial susceptibility of bifidobacteria isolated from gastrointestinal tract of calves. *LivestockScience* **105:** 253-259**.**

[26] Zhou, J. S., Pillidge, C.J., Gopal, P. K. and Gill, H.S. (2005). Antibiotic susceptibility profiles of new probiotic *Lactobacillus* and *Bifidobacterium* strains. *International Journal of Food Microbiology* **98**: 211-217.