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PRODUCTION AND CHARACTERIZATION OF BACTERIOCIN FROM LACTIC ACID BACTERIA ISOLATED FROM FERMENTED FOOD PRODUCTS

[1]Abiom, LA., [1]Ire, F.S*. and [1]Ahaotu, I.
[1]Department of Microbiology, Faculty of Science, University of Port Harcourt, Port Harcourt, Choba, Nigeria.
*Corresponding author: francis.ire@uniport.edu.ng; Orcid ID: 0000-0001-5599-1292

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ABSTRACT

Bacteriocin is a natal antimicrobial which can be exploited to evade the inevitability of adding chemical preservatives to food products. This study is aimed at the production and characterization of bacteriocin from Lactic acid bacteria (LAB) isolated from fermented food products. Sixteen (16) samples of various fermented food products (abacha, fufu, ogi and yoghurt) were screened for the presence of bacteriocin producing LAB using agar well diffusion method. The strain designated C_6 was selected for further studies due to its broad inhibitory effect on the growth of all tested organisms (Enterococcus faecalis ATCC 33090, Pseudomonas aeruginosa MN 515053, Listeria innocua ATCC 2127, Staphylococcus aureus, Escherichia coli, Candida spp and Aspergillus terrus MN907795). Molecular characterization spotted C_6 isolated from yoghurt as Lactobacillus fermentum (accession no. MN907811). Purification of the antimicrobial substance was achieved using High performance liquid chromatography (HPLC) with nisin as Standard which revealed it to be bacteriocin a proteinaceous antimicrobial that is stable to heat and organic solvents. Effect of substrate level on the growth of Lactobacillus fermentum and optimization of physicochemical parameters on bacteriocin production revealed optimum condition of 1.0 % glucose, 0.5 % sodium nitrate, pH of 6.0, incubation temperature of 35°C for 72h. This makes Lactobacillus fermentum and its bacteriocin a good antagonistic agent for exploitation in pharmaceutical and food processing industries as well as in the field of biotechnology.

Keywords: Fermented foods, Lactic acid bacteria, Bacteriocin, *Lactobacillus fermentum*.

INTRODUCTION

Lactic acid bacteria (LAB) represent a group of heterotrophic bacteria that produce lactic acid from the fermentation of carbohydrates as offshoot of their inaptitude in other biosynthetic processes (Jay, 2000). LAB are repeatedly isolated from soils, water, plants, silages, waste products, sour dough, fermented vegetables and also from animals and human intestinal, genital and respiratory tracts (Tserovska *et al*., 2002). LAB represents the highest percentage of bacteria producing probiotic properties (Rine *et al.*, 2019). Their use in the manufacturing of fermented milk products (yogurt, sour milk, cheese and butter) confer them more shelf-stability with characteristic aromas and flavors, although spoilage can ensue if their proliferation is unchecked. A preponderance of bacteriocins from gram-positive bacteria

comes from LAB (Ennahar *et al.,* 2000). They have extensively been sourced as replacement for chemical preservation in food (Gao *et al.,* 2010). Bacteriocins are bacteriostatic or bactericidal proteins which antagonize other species with the same nutritive requirements (Bella, *et al.,* 2002; Cotter *et al.,* 2013). LAB has drawn momentous interest because of its potential as both natural food additive and as therapeutic antibiotics (Van Heel *et al.,* 2011). As an antimicrobial peptides bacteriocins from LAB exhibit broad spectrum effect extending to fungi (Vanne *et al.;* 2000). Bacteriocins from LAB are very resistant when exposed to high thermal stress, they remain potent even at different pH spectrum, and they have a fast-acting mechanism that opens tiny spaces in the target bacterial membrane, even at very low concentrations, they are easily broken down by enzymes that lyse protein (Cleveland *et al.,* 2001). All these characteristics combine to make LAB effective in food preservation. LAB is biotechnologically at present seen as a biopreservative, with additional benefit of enhancing the organoleptic, nutritional, and shelf-life of foods (Lennox and Efiuvwevwere, 2013). Antibiotic resistance by bacteria has become widespread with continual use of conventional antibiotics (Yoneyema and Katsumeta, 2006). Development of novel groups of antimicrobials is progressively becoming more important in medical field (Fishe et al., 2005). Using bacterial peptides in foods can cut any possible issue a rising from drug resistance. Bacteriocins of LAB origin has potential for such application; this has drawn attention from researchers globally.

The number of pathogens believed to be transmitted by water and food keeps on growing increasing as are water and food-related illnesses (Choffnes et al., 2012). Regulation of these food-borne pathogens is a real challenge for food safety due to increase in resistance to drugs. Acute foodborne illnesses and intoxications are more of a problem for the government and food manufacturing industry today than they were decades ago hence the need to embark on this research.

Application of biotechnology in food processing can help increase food safety by substituting chemical preservatives with biopreservatives proven to be non-toxic. To this end the production of bacteriocin- a natural preservative from a non-pathogenic LAB isolated from local (indigenously) fermented food could prove beneficial to food manufacturing industry as a different approach to mitigate microbial spoilage and ensure food safety while improving microbial quality of food. Therefore, this present investigation was aimed at the production and characterization of bacteriocin from Lactic acid bacteria isolated from locally fermented food products (*abacha, fufu, ogi* and yoghurt).

MATERIALS AND METHODS

Sample collection

Sixteen (16) food samples, four samples each from abacha, fufu, ogi and yoghurt were bought from various locations in Port Harcourt, aseptically sealed in food-grade sampling bags and transported carefully in containers with ice packs to maintain acceptable condition for transportation of samples to laboratory for further analysis.

Isolation of bacteriocin producing organisms

The methods of Anupama (2020) were adopted with slight adaptation. Fifty grams (50g) of each solid food sample was weighed out and placed in blender beforehand sterilized with 70% alcohol and doused with de-ionized water, where they were then pulverized. Twenty-five grams (25g) of the blend was aseptically poured into a 225ml of sterile peptone in a 500ml sterile flask for enrichment of organism, after which the homogenates were left to stand for half an hour and robustly shaken for approximately 2-3 minutes, from which dilutions in ten-folds up to 10-5 was prepared. For the liquid samples, 1ml of the

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various samples was transferred into 9mls of sterile distilled water from which a ten-fold serial dilution was made. Spread plate was used for solid samples while pour plate was used for the liquid sample inoculation of the samples on de Mann-Rogosa Sharpe (MRS) agar, using dilution factor of 10-3, all in triplicate and incubated anaerobically (without oxygen) using candle jar. Growing discrete colonies observed in the petri dish were transferred by means of a sterile grease free inoculating loop to plates having freshly prepared basal agar (nutrient agar) and MRS agar and incubated at 370C for 24-48h, respectively. Upon successful incubation, the colonial cultural characteristics of the distinct isolates were documented. The isolates were stocked on nutrient agar slant for further analysis. Selected isolates were examined microscopically for their morphological, physiological, biochemical features. The cultural features were contrasted with features documented in Bergey's Manual of Determinative Bacteriology Cheesbrough, (2000).

Screening for bacteriocin producers

The method described in Egbe and Lennox (2018) was adopted in the screening of bacteriocin producing organisms: Seventy five morphologically distinct colonies were screened by the crowded plate technique against the test organisms as described by Willey et al., (2008). Each isolate was first diluted in sterile water after which 0.1ml was spread on nutrient agar plates in triplicates and each lactic acid bacterial isolate was inoculated on the plates by agar disk diffusion method. The plates were then incubated at 28^{o}C for 24 to 48 h. The plates were observed at different intervals for colonies with clear zones around them. The lactic acid bacterial isolate that show zones of inhibition were regarded as bacteriocin producing isolates. The positive isolates were further screened for those with antagonistic effect against target organisms using agar-well diffusion assay

Agar well diffusion method proposed by Mounyr and Saad (2016) was followed for susceptibility test. About 3-5 colonies of test organism were selected using a sterile inoculating needle and suspended in saline, after which the inoculum grew producing a turbidity equivalent to 0.5 McFarland standards which corresponds to approximately 1.5 x 10^{8}CFU/ml. The mixture well vortexed to ensure it was well-mixed and allowed to stand. A fresh, sterile cotton-tipped was inserted into the suspension, the superfluous liquid from the swab removed by pressing it against the tube wall. Subsequently, the swab was inoculated unto a plate containing freshly prepared Muller Hinton Agar (MHA) starting at the tip and covering the full plate by spreading backward and forward from edge to edge, ensuring the plates were rotated for approximately 60°. Four wells were prepared in the agar using sterile grease free cork borer (6mm). The wells were filled with 80µl of the broth culture of test isolates and all plates were later incubated using incubator set at 37oC and allowed to stand for 24h. The plates were monitored for clear areas around the wells. Diameters of this areas referred to as inhibition zones were measured. All antimicrobial tests were done in triplicates. Lactic acid bacteria that tested positive for production of inhibitory substance (bacteriocin) were grown in MRS broth (pH 6.5) infused with 10 % inoculum, held at optimized culture condition for 48h, after which it was centrifuged at11180g for 15min, before adding 1 mol/L NaOH to bring the pH to 6.0 (Ogunbanwo et al., 2003).

Optimization of production parameters

Optimization of production parameter was achieved by varying the pH, temperature and incubation time of production medium (one factor at a time) then recording the effect on bacteriocin production by LAB, at varying pH values from 5.5 to 7.5 and temperatures ranging from 25 to 45oC and incubation times ranging from 12 to 72 h, and the growth was calculated at 600nm and cell-free culture medium was used to quantitate protein concentration (Ramachandran et al., 2012).

Purification and antimicrobial activity assay of bacteriocin

Procedures and steps for crude extract of bacteriocin were followed sequential beginning from centrifuging primed culture (48 h old) using centrifuge set at 20,000 rpm, (≤4 ºC) for 30 min. Supernatant attuned to pH of 7.1 was filter sterilized using Whiteman membrane nylon filter (0.2µm) before the addition of 5mg/ml catalase (C-100 bovine liver) to cancel out peroxides and lactic acids effect (Kacem et al. 2005). This was used as bacteriocin crude extract (BCE) for bacteriocin antimicrobial assays (Messaoudi et al., 2012). High performance liquid chromatography of the bacteriocin-like product (BLP) was eluted with mobile phase consisting of 1% trifluoroacetic acid in a mixture of water (eluent A) and acetonitrile (eluent B), a gradient was followed, elution program was as follow: an initial from 0 - 60% of solvent B (0.1% acetic acid in acetonitrile) from 0 to 20 minutes, immediately followed by gradient from 60 to 100% of solvent B from 20 to 25 minutes, and finally using 100% of solvent B for a duration of 5 minutes. The flow rate was maintained at 1ml per minute, the absorbance was monitored at 215nm using UV detector and column temperature was kept at 35ºC. Data analysis was performed with agilent open lab software; the model of the HPLC is agilent 1100 coupled with UV and FLD detectors. HPLC chromatograms of the bacteriocin-like product were examined and each peak identified was named by direct comparison of their chromatographic retention times, retention indices, and mass spectra with that of the standards which was Nisin (Sigma, USA). The fractions were obtained and analyzed for antimicrobial activity against the test organisms. the partially-purified bacteriocin (100 µL) was incorporated into 10 mm wells on nutrient agar plates seeded beforehand with 100 µL (2×108 CFU/mL) suspension of each indicator strain and the plates were afterward incubated for 48 h.

Nisin standardization protocol

Following the methods of Papagianni et al., (2006), Nisin Standard were prepared by introducing 0.1g of nisin to solution containing the following; 10 ml 0.02 N HCl and 0.75% NaCl. Steam sterilized the solution for 15minutes time interval, at a temperature value; 121ºC and pressure of 15psi.This solution is then termed standard nisin solution (105 UI/ml).

Effect of physio-chemical parameters and substrate concentration on bacteriocins production

The effects of physicochemical parameters and substrate concentration on bacteriocin production was achieved evaluating its activity at varying pH values from 5.5 to 7.5, temperatures ranging from 25 to 45ºC, incubation times ranging from 12 to 72 h, carbon source (starch, sucrose, glucose, maltose), and nitrogen source (sodium nitrate, ammonium sulphate, beef extract, ammonium nitrite) in MRS broth, and the growth was measured at 600nm and cell free culture medium (crude bacteriocin) was used to quantitate protein concentration (Ramachandran, et al., 2012).

Characterization of bacteriocin

The crude bacteriocin of the isolates with best inhibition zone was characterized in line with stability to temperature and pH. Temperature effect was verified at 20 to 45ºC, pH between 5.0 to 8.0 (adding sterile 1mol/L NaOH or 1mol/HCL), and the enzymes α-amylase, catalase, proteinase K, chymotrypsin, trypsin and pepsin were used to treat the crude bacteriocin. Residual bacteriocin activity was noted against selected pathogenic bacteria at each of the treatments by using agar-well diffusion assay (Ogunbanwo et al., 2003) with minor adaptation.

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Characterization and identification of isolate

Morphological Identification

Selected isolates were examined microscopically for their morphological, physiological, biochemical features. The cultural features were contrasted with features documented in Bergey's Manual of Determinative Bacteriology Cheesbrough, (2000).

Molecular identification of isolates

Bacteriocin positive isolates were identified using a modified procedure described by Queipo-Ortuno et al., (2008) through the sequencing of the 16S rRNA section of the rRNA genes of the isolates. DNA extraction of the bacterial isolate was done by spinning overnight broth culture of bacterial isolates at 1400 rpm for 3 min, re-suspended the cells in 500ul of normal saline and heated at 95^0C for 20 min before suspension using ice and spinning again for 3 min at 14000rpm, afterward, the supernatant bearing the DNA was conveyed to a 1.5mlmicrocentrifuge tube and stored at -20^{o}C for other downstream reactions. The 16srRNA section of the rRNA genes of the isolates has been amplified using the 27F: 5'-AGAGTTTGATCMTGGC TCAG-3' and 1492R: 5'-CGGTTACCT TGTTACGACTT-3' primers on an ABI 9700 Applied Biosystems thermal cycler at a finishing concentration of 50 microliters for 35 cycles. The PCR mixt included: X2 Dream Taq Master Mix supplied by Inqaba, South Africa, (MgCl, Taq polymerase and DNTPs), 0.4 M primers and the DNA extracted as a Template. The PCR conditions were: initial denaturation, 95^{o}C for 5 min; denaturation, 95^{o}C for 30 seconds; annealing, 52^{o}C for 30 seconds; extension, 72^{o}C for 30 seconds for 35 cycles and final extension, 72^{o}C for 5 minutes (Queipo-Ortuno et al., 2008). Genomic DNA extracted was quantified using Nano Drop 1000 spectrophotometer (Inqaba Biotec South Africa). The product of the PCR amplification was resolved with 1% agarose gel at 120V for 15 minutes and viewed on a UV transilluminator. Sequencing was performed with Big Dye Terminator package on the 3510 ABI sequencer from Inqaba Biotechnological, South Africa. Evolutionary history was deduced using the Neighbor-Joining method in MEGA 6.0 and the distances were computed using the Jukes-cantor Method.

RESULTS AND DISCUSSION

Lactic acid Bacteria can antagonize and out-compete other bacterial within the same niche through their metabolites predominantly bacteriocin, with longer shelf life of products as upshot (Deegan et al., 2006). Table 1 shows high counts (9.8 x 105 CFU/ml) of LAB were detected in food samples analyzed this result is in agreement with observations of Diop et al., (2007) who isolated 220 strains of LAB from conventional fermented foods. A plausible reason could be that natural fermentation was ongoing within the selected food which is caused by LAB inherent in the food. Fluctuated numbers of LAB in the sampled food types might have been influenced by methods of handling and storing of this product. The isolation of LAB species from fermented foods in this work is in agreement with works of Adetunji and Adegoke (2007) who isolated *Lactobacillus plantarum*, *Lactococcus lactis*, *Streptococcus lactis*, *Lactobacillus brevis* and *Lactobacillus fermentum* from soft cheese. The abundance of bacteriocin producing strains in food samples as documented is consistent with the findings of Egbe and Lennox (2018) that isolated a total of 26 bacteriocin positive strains from some fermented food and D'Aimmo et al. (2007) who reported the presence bacteriocin positive *Lactobacillus* spp., from dairy products. Similarly, Sharpe (2009) detected bacteriocin-positive strains in fresh-cut vegetable products, whereas Salasiah et al. (2001) isolated bacteriocin-positive LAB from traditionally fermented foods. It can be adduced that selected food samples are excellent sources of potentially useful bacteria with bacteriocin producing capability.

Table 1: Distribution of bacteria in samples

S/N	Sample code	Mean count of culturable bacteria (cfu/ml)	Log cfu/ml
1.	AB	8.6×10^5	5.93
2.	FF	9.8×10^5	5.99
3.	OG	1.12×10^6	6.05
4.	YO	1.46×10^6	6.16

Key: AB-Abacha, FU-Fufu, OG-Ogi, YO-Yoghurt

Tables 3 shows inhibitory activity of cell free supernatant of the four isolates with best zone of inhibition (C_6, C_7, C_{13} and C_{15}) after eliminating organic acid and H_2O_2 effect by acid neutralization and treatment with catalase. For cell free of organic acid and H_2O_2, isolate C_{13} showed maximal activity against *Staphylococcus aureus* (33.7 mm) while isolate C_6 showed the least activity against *Candida* spp (11mm). Adebayo and Aderiye, (2010) similarly reported catalase-treated, neutralized, CFS filtrate of Lactic acid bacteria did not show any significant effect on the inhibitory activity on the test isolates, a different report is seen in the work of Yang *et al.* (2012); upon neutralization and elimination of H_2O_2 effect, isolated LAB species lost their inhibitory effect.

Using 16S rRNA gene technique, isolate C_6fromyoghurt registered as Lab 2 in Molecular Laboratory was identified as *Lactobacillus fermentum*. The obtained 16S rRNA sequence from the isolates produced was submitted in NCBI database, it matched with already existing sequences showing 100% similarity with available nucleotide sequence of *Lactobacillus fermentum* with accession number MK893985. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of 16S rRNA of the isolates LAB 2 within the *Lactobacillus* and revealed a closely relatedness to *Lactobacillus fermentum* than other *Lactobacillus* sp (Fig. 1). The 16S rRNA sequence of Lab 2 was registered in GenBank under the accession number MN907811. Plate 1 depicts the agarose gel electrophoresis of 16S rRNA gene of the bacterial isolates, Lab 2 and Lab 3 lane represent the 16S rRNA gene bands (1500bp), and lane L represents the 100bp molecular ladder. Plate 2 and 3 showed the Zone of clearance of the bacteriocin against *Listeria innocua* ATCC 2127 and *Enterococcus faecalis* ATCC 33090, respectively.

Table 3: Inhibitory activity against test isolates after eliminating effect of organic acid and hydrogen peroxide (H_2O_2) using agar well diffusion assay

	Zone of clearance (millimeter, mm)						
Isolate Code	*Listeria innocua* ATCC 2127	*Enterococcus faecalis* ATCC33090	*Pseudomonas aeruginosa MN515053*	*Staphylococcus aureus*	*Escherichia coli*	*Candida* spp	*Aspergillus* terrus MN907795
C_6	13.8	11.2	12.0	21.0	16.2	11.0	11.2
C_7	7.4	-	-	12.3	15.0	-	-
C_{13}	-	8.3	19.8	33.7	-	-	-
C_{15}	7.0	-	10.0	28.8	12.5	-	-

Key: -: No activity

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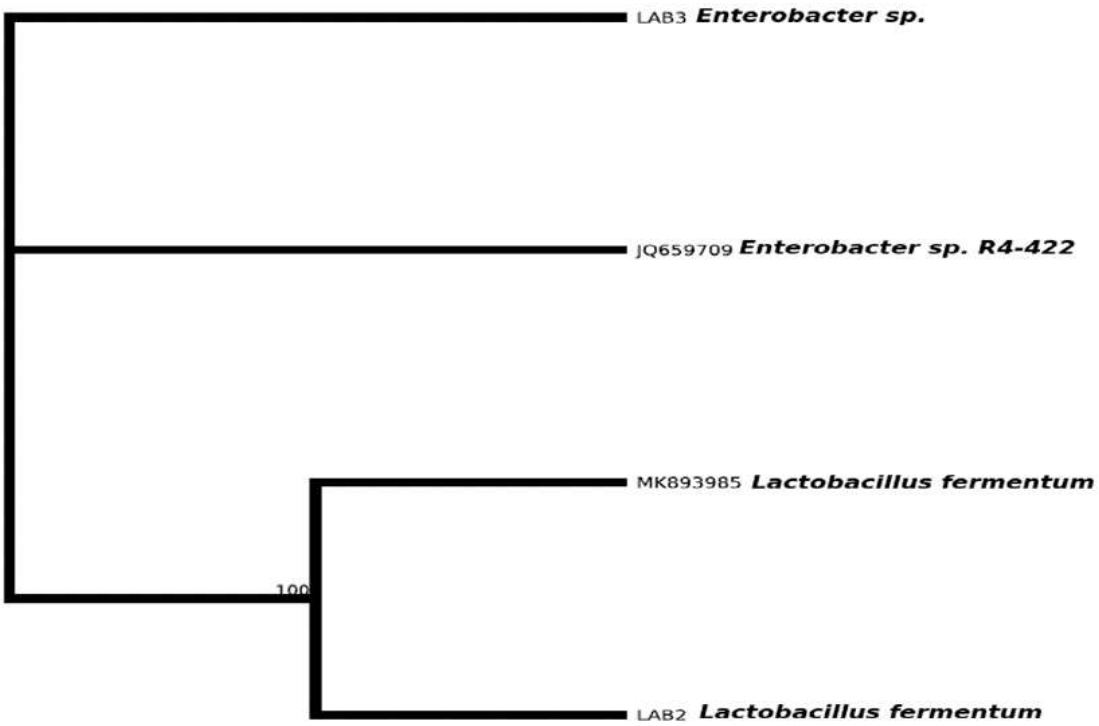

Fig. 1: Phylogenetic tree of evolutionary distance between the bacterial isolates

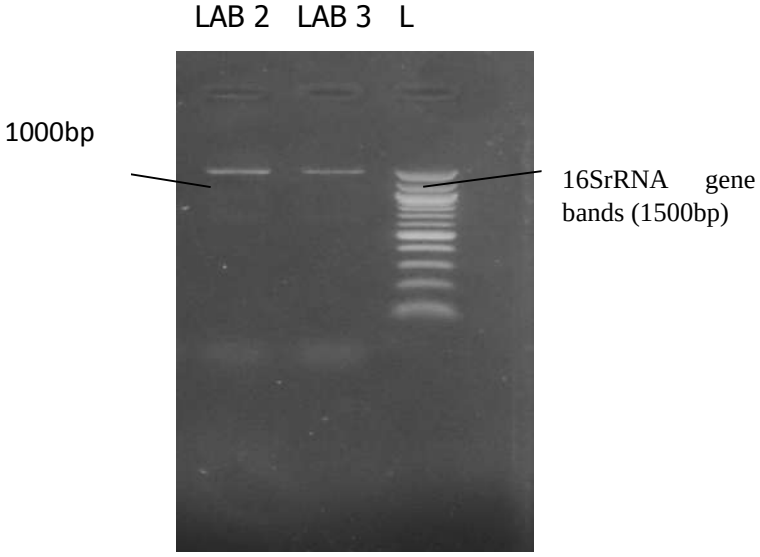

Plate 1: Agarose gel electrophoresis of the 16SrRNA gene of selected bacterial isolates.

Bacteriocins being protein in nature can be affected when exposed to proteolytic enzymes causing them to denature (Wataru *et al.,* 2018). This study showed that α-chymotrypsin, protease and trypsin had positive effect on bacteriocin of *L. fermentum* (Table 4) affirming that the antimicrobial compounds, produced by the bacterium is protein in nature which is consider a main characteristic of any bacteriocin while catalase and α-amylase showed no effect indicating that the inhibition was not from carbohydrate moieties or hydrogen peroxide. Todorov and Dick (2006) also reported that α-amylase and catalase treatment of inhibitory compounds did not alter the activity of bacteriocin produced in their study. Hartnett *et al.,* (2002) reported similar result where inhibition was not as a result of carbohydrate moieties or hydrogen peroxide.

The results obtained in this study also revealed that optimal parameter for growth of bacteriocin producing *Lactobacillus fermentum* occurs at glucose 1.0% as source of carbon, 0.5% sodium nitrate as source of nitrogen, pH 6, temperature of 35ºC and at 72h of incubation (Figs.2-6), this observation is in agreement to the report of (Sharma *et al*., 2011; Amortagui *et al*., 2014) who recorded optimum production of bacteriocin from *Bacillus subtilis*R75 at 72h of incubation. Similarly, Dicks and Todorov (2007) reported 12800 AU/mL of bacteriocin at 30°C. Many studies have documented maximum bacteriocin production between pH 4-5 (Abo-Amer, 2007; Anamalai *et al.*, 2009).

Antimicrobial activity of *L. fermentum* in this study was totally lost upon treatment with α-chymotrypsin, protease and trypsin, an indication that the inhibitory compounds formed by *L. fermentum* are proteinaceous, a unique feature of bacteriocin. Khalil *et al.*, (2009) reported that bacteriocin activity of *Bacillus megaterium*19 strain was impeded by pepsin and trypsin treatment.

Table 4: Action of proteolytic enzymes on activity of bacteriocin from *Lactobacillus fermentum*

S/N	Enzymes	Bacteriocin Activity
1	α-Amylase	+
2	Catalase	+
3	Proteinase K	-
4	α-Chymotripsin	-
5	Trypsin	-
6	Pepsin	-

Key
+: Activity, -: No activity

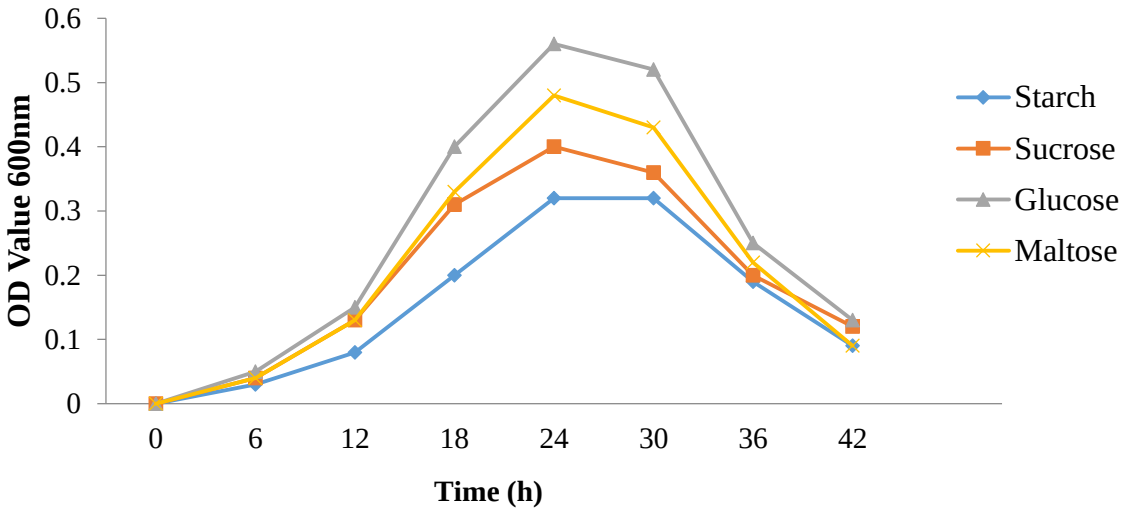

Figure 2: Effect of Carbon source on growth of *Lactobacillus fermentum*

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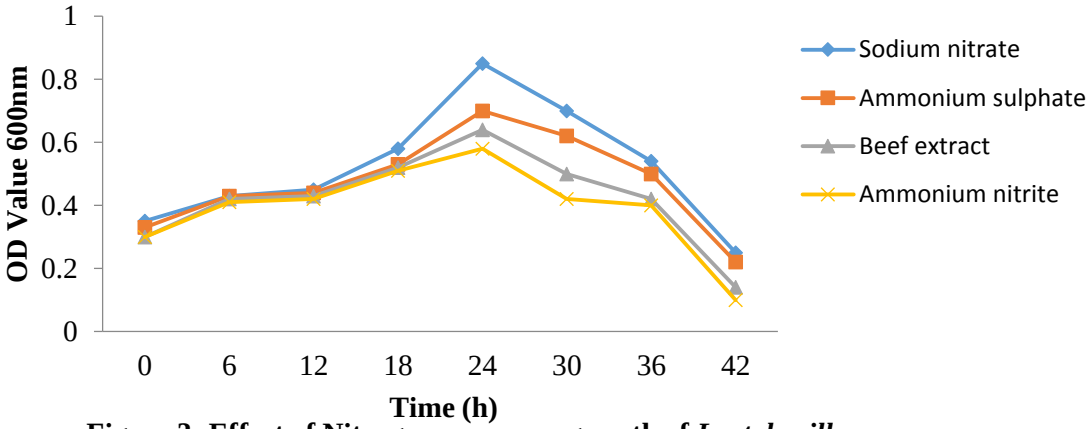

Figure 3: Effect of Nitrogen source on growth of *Lactobacillus fermentum*

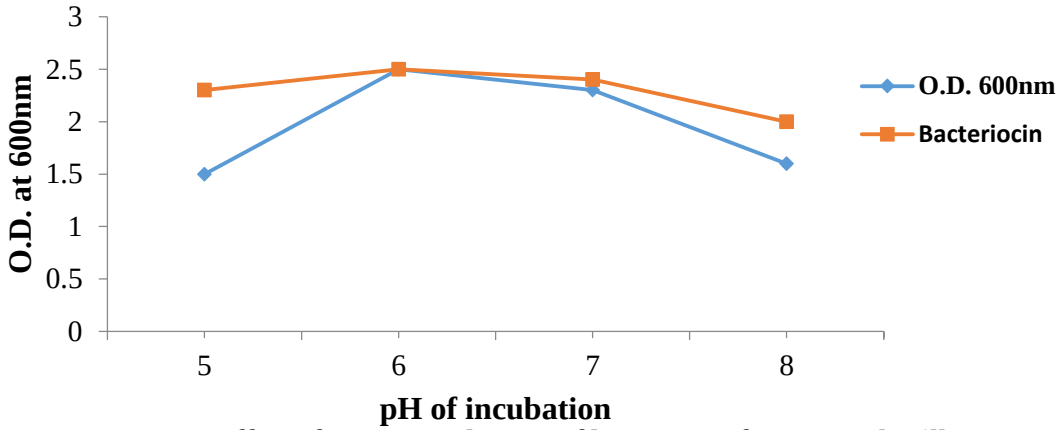

Figure 4: Effect of pH on production of bacteriocin from *Lactobacillus fermentum*

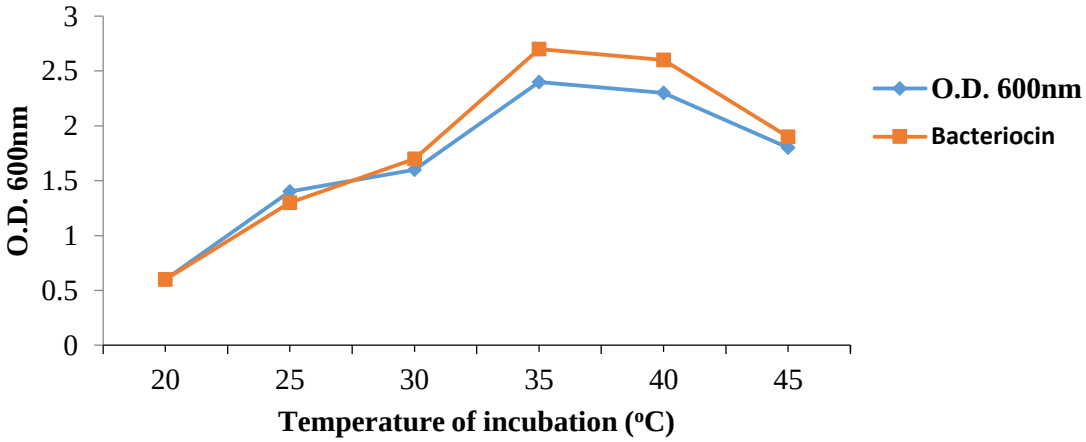

Figure 5: Effect of Temperature on production of bacteriocin from *Lactobacillus fermentum*

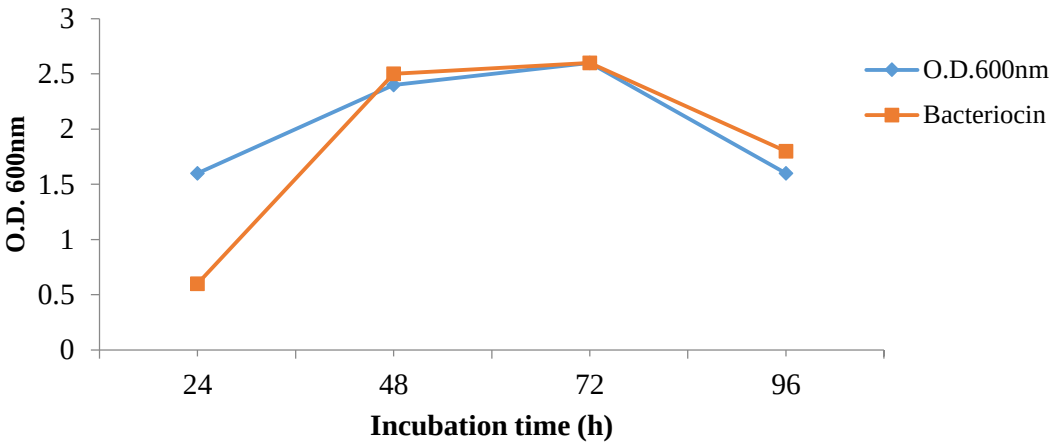

Figure 6: Effect of incubation time on production of bacteriocin from *Lactobacillus fermentum*

Culture supernatant of bacteriocin producing strain of *L. fermentum* was analysed by High Performance Liquid Chromatography profile, various peaks were derived; some were peaks of impurities derived from the medium of growth (MRS) of the bacterium. Active fraction from *Lactobacillus fermentum* examined revealed a unique peak with antimicrobial activity detected at 2.46 min elution period (Fig 7) which confirms the antagonistic substance to be bacteriocin by direct comparison of their chromatographic elution period with those of standards which was Nisin (Sigma, USA). This result is in agreement with the work of Balogu *et al.*, (2017) who carried out a study on the bacteriocin found in Fresh Cow Milk and Meat Samples obtained from Lapai Market in Niger State using Nisin as a standard. This result support the observations of Pravin and Prakash (2018) bacteriocin sample from *Lactobacillus zymae* WHL-7 isolated from whey showed active fraction from HPLC analysis with antimicrobial activity detected at 6.36 min elution period.

Table 4-5 shows the temperature and pH stability studies; partially purified bacteriocin remained stable at a wide temperature range (20-100^{o}C) and pH range (4-7). Similar results were reported in cases of bacteriocins produced by *L. plantarum* LE7 and LE5 (Amortegui *et al.*, 2014; Shama *et al.*, 2011). Partially purified bacteriocin in this study showed activity after treatment at 80^{0}C, indicating its suitability for use in food processing requiring high temperatures (20-100^{o}C).

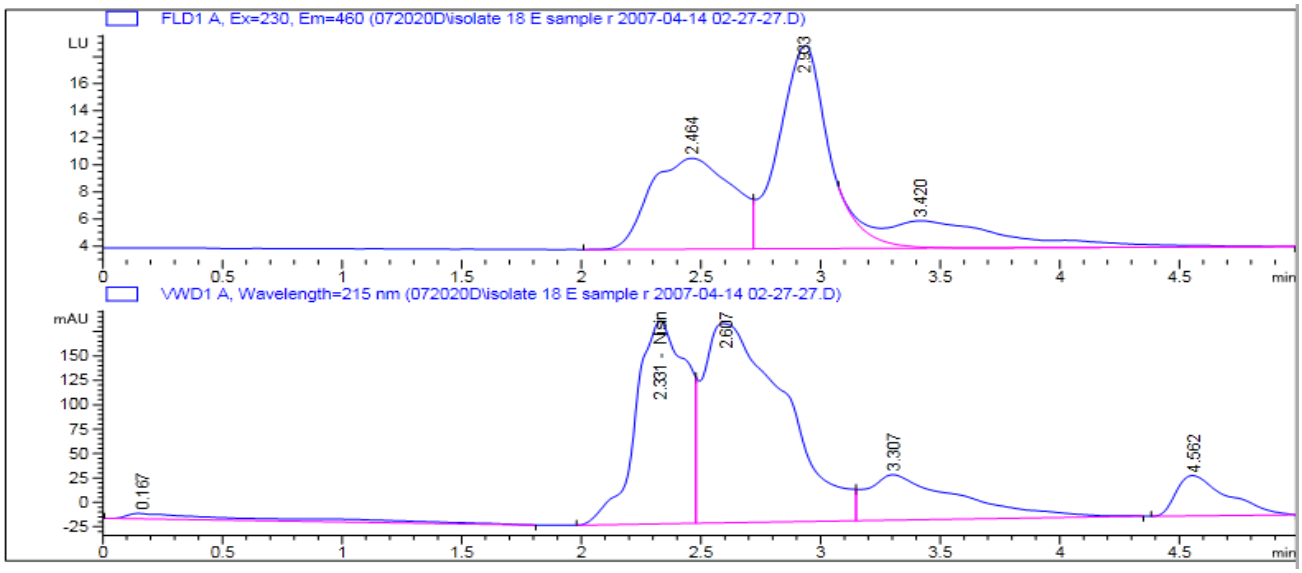

Figure 7: High Performance Liquid Chromatography (HPLC) profile of active faction isolated from *Lactobacillus fermentum* with antimicrobial activity detected during 2.46minute elution period

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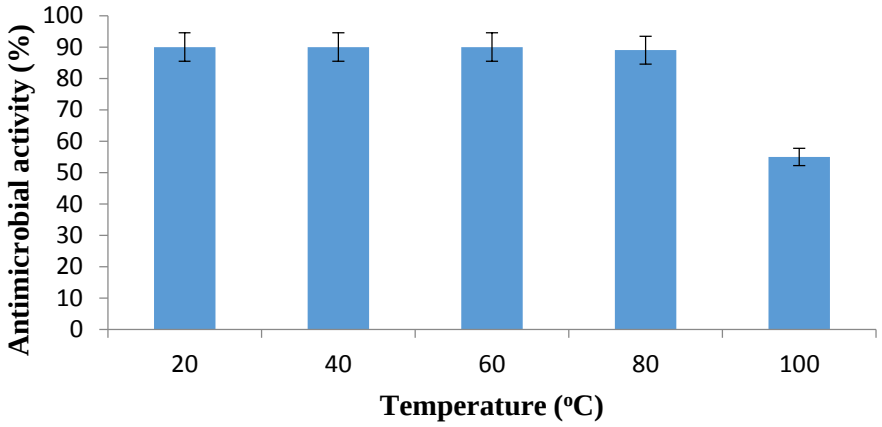

Figure 8: Effect of Temperature on the activity of partially purified bacteriocin from *Lactobacillus fermentum*

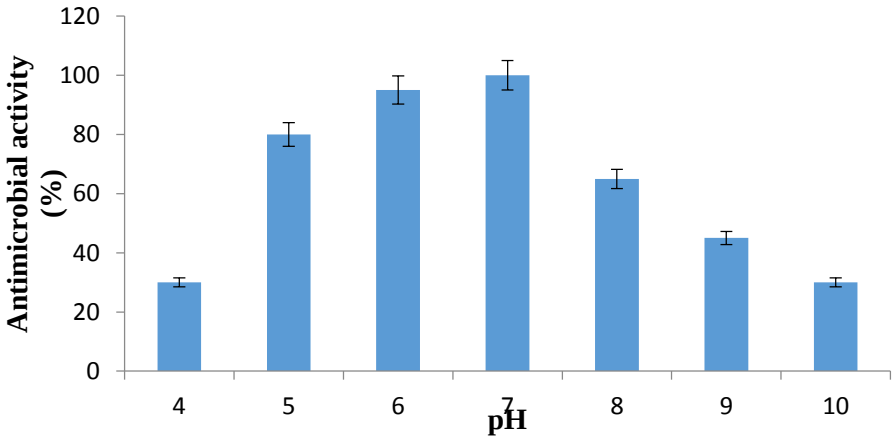

Figure 9: Effect of pH on the activity of partially purified bacteriocin from *Lactobacillus fermentum*

Plate 2: Zone of clearance of bacteriocin against *Listeria innocua* ATCC 2127

Plate 3: Zone of clearance of bacteriocin against *Enterococcus faecalis* ATCC 33090

CONCLUSION

This research revealed that LAB carefully isolated from locally fermented foods (abacha, fufu, ogi and yoghurt) can synthesize inhibitive bioactive proteins effective against some Gram-negative bacteria and Gram-positive bacteria, though the later were more susceptible to the same bacteriocin compared

to former. This research broadens our understanding of widespread distribution of beneficial bacteria in foods. The extent of this distribution allows for researchers to isolate bacteriocin positive bacteria readily from available market sources. This information may assist future work on isolating natural sources of antimicrobials that may be used against spoilage organism that may also include foodborne pathogens. It was observed from this study that culture supernatant from *lactobacillus fermentum* did not lost its antimicrobial activity after neutralization of the effect of organic acid, elimination of hydrogen peroxide and treatment with α-amylase, but its antimicrobial activity was lost when treated with α-chymotrypsin, protease and trypsin indicating that inhibitory compound produced by *Lactobacillus fermentum* is proteinaceous in nature which is consider the main characteristic of bacteriocin, hence it can be summarized that inhibitory compound produced from *Lactobacillus fermentum* is bacteriocin. The bacteriocin producing strains meets several criteria needed for it to be used as an effective bio-preservative, which includes pH tolerance and stability to temperature. The isolated Lactobacilli exhibited excellent probiotic characteristics which in use can control and enhance intestinal microbiota and thus can contribute in health benefits to consumers. This makes the bacteriocins producing LAB a good antagonistic agent for exploitation in the food processing industries, biotechnology and in pharmaceutical industries.

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