



Antimicrobial Properties of Purified Bacteriocins Produced from *Lactobacillus casei* and *Lactobacillus fermentum* against Selected Pathogenic Microorganisms

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Authors' contributions

Authors ORA and AKA designed and supervised the study. Author FAA performed the statistical analysis, wrote the protocol, and also wrote the first draft of the manuscript. All authors managed the analyses of the study. Author FAA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: (1) To isolate bacteriocin samples produced from *Lactobacillus* using natural fermented foods which include: palm-wine, milk, locust beans, fufu (white solid food made from cassava), ogi (known as pap) and dairy fermented product (Yogurt); (2) extraction and purification of these bacteriocin samples using centrifugation and ammonium sulphate respectively and removal of impurities using dialysis. (3) to confirm the production of bacteriocin by performing antimicrobial assay against some selected pathogenic microorganisms. (4) to examine the effects of pH, heat and storage stability as well as biopreservative efficiency of the bacteriocin samples in pap, kunu (made from millet) and fresh orange juice; (5) to investigate the effect of viable antibiotics on the growth of isolates.

Study Design: Data were analyzed using the statistical software package SPSS version 16.0 and standard errors of mean (SEM) for all the graphs plotted were represented with error bars while their characterization was designed using reciprocal of the highest dilution (2^n) that resulted in the inhibition of the indicator lawn. Thus, the arbitrary units (AU) of the bacteriocin activity per milliliter (AU/ml) were defined as $2^n \times 1000 / 10\mu\text{l}$.

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Place and Duration of Study: Microbiology Laboratory, Sacred heart Hospital, Abeokuta and Department of Microbiology, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria, between March 2010 and November 2012.

Methodology: Bacteriocins, otherwise known as the antimicrobial compounds were produced from the *Lactobacillus* strains and then isolated from Nigerian fermented foods which include: palm wine, milk, yoghurt, locust beans, ogi and fufu. These foods were isolated using de Mann Rogosa and Sharpe medium. The isolated microorganisms (*L. fermentum* and *L. casei*) were identified phenotypically after isolation. Bacteriocins were extracted and purified from the *Lactobacillus* strains by centrifugation, followed by ammonium sulphate precipitation and dialysis. The antimicrobial activities of the crude bacteriocins were tested against nine selected pathogenic clinical isolates collected from the University College Hospital, Ibadan. The tested isolates were *Shigella dysenteriae*, *Shigella flexneri*, Enteropathogenic *Escherichia coli* type 1, Enteropathogenic *Escherichia coli* type 2, Enterohaemorrhagic *E. coli* type 3, *Salmonella typhi*, *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Klebsiella pneumoniae*.

Results: The bacteriocins of *Lactobacillus fermentum* and *Lactobacillus casei* showed a broad range of activities and had higher significant effect ($P < 0.05$) on the selected pathogenic microorganisms. The effects of pH on the bacteriocins were active in range of 2 to 6. Bacteriocins produced by *Lactobacillus casei* were stable at 80°C for 15 minutes while bacteriocins produced by *Lactobacillus fermentum* were stable at 100°C for 15 minutes. It was observed that these bacteriocins can be stored between -20°C and 4°C and they had significant difference on the selected pathogenic microorganisms ($P < 0.05$). The preservative activities of the bacteriocins tested on different foods showed that the bacteriocin of *Lactobacillus fermentum* had maximum reduction on bacterial population. *Lactobacillus fermentum* and *Lactobacillus casei* isolates were resistant to erythromycin of 70% and 100% for cotrimoxazole, ciprofloxacin, augmentin and amoxicillin.

Conclusion: This study showed that bacteriocins from fermented foods could be used as an effective control for pathogenic microorganisms as they were able to exhibit antimicrobial activity against the test organisms when investigated for bacteriocin production and when characterized.

Keywords: *Lactobacillus fermentum*; *Lactobacillus casei*; bacteriocin; antimicrobial properties and pathogenic microorganisms.

1. INTRODUCTION

Lactobacilli are considered as beneficial bacteria because they have the ability to break down proteins, carbohydrates and fats in food and help in absorption of necessary elements and nutrients such as minerals, amino acids and vitamins required for the survival of humans and other animals [1]. *Lactobacilli* have an antagonistic effect on different microorganisms due in part to the production of bacteriocins [2].

Lactobacillus casei (*L. casei*) produces lactic acid which helps lower pH levels in the digestive system and impedes the growth of harmful bacteria. *L. casei* may be found in raw or fermented dairy products as well as fresh or fermented plant products [3]. These sources may include yogurt, cheese, and other types of food sources such as fermented green olives [4].

Lactobacillus fermentum otherwise known to be a hetero-fermentative lactic acid bacterium is frequently isolated from mucosal surfaces of healthy humans and fermented foods [5]. Several research studies have confirmed that some strains for *Lactobacillus fermentum* have natural resistances to certain antibiotics and chemotherapeutics [5]. Also, it has beneficial effects on the health of the gastrointestinal tract without any adverse action as it has been used as an alternative treatment to prevent or treat urogenital infection based on its probiotic properties and production of bacteriocins [6,7].

These microorganisms have been used in food and feed preservatives for centuries, since they can produce a variety of antimicrobial agents, including organic acids like lactic and acetic acid, ethanol, carbon dioxide, diacetyl, hydrogen peroxide and bacteriocins [8,9,10].

Bacteriocins differ from most therapeutic antibiotics because they possess proteinaceous agents that are rapidly digested by proteases in the human digestive tract. They are ribosomally synthesized peptides, and this fact creates the possibility of improving their characteristics to enhance their activities and spectra of action [11].

Both gram positive and gram negative bacteria produce bacteriocins, which constitute a heterologous subgroup of ribosomally synthesized antimicrobial peptides. In general, these substances are cationic peptides that produce hydrophobic and amphiphilic properties and the bacterial membrane is the target for their activity in most cases [12].

The industrial importance of the lactic acid bacteria (LAB) has been proven to be generally safe, due to their ubiquitous appearance in food and their contribution to the healthy micro flora of human mucosal surfaces [13,14].

With respect to medical applications, antimicrobials produced by probiotic LAB might play significant roles during *in vivo* interactions occurring in the human gastrointestinal tract, hence contributing to gut health [15].

For the past two decades, most reported research articles have focused on how to replace chemical antibiotics to natural peptides using LAB. However, very little research papers have discussed the purification and characterization of these bacteria. Thus, the aim of this study includes the following: (1) isolate bacteriocin producing LAB from local fermented foods which include: palm-wine, milk, locust beans, fufu (white solid food made from cassava), ogi (known as pap) and dairy fermented food (Yogurt), (2) extraction and purification of the bacteriocin using centrifugation and ammonium sulphate respectively and removal of impurities using dialysis, (3) to confirm the production of bacteriocin by performing antimicrobial assay against some selected pathogenic microorganisms. (4) to examine the effects of pH, heat and storage stability as well as biopreservative efficiency of the purified bacteriocin in pap, kunu (made from millet) and Orange juice; (5) to investigate the effect of viable antibiotics on the growth of isolates.

2. MATERIAL AND METHODS

2.1 Sample Collection

The samples investigated in this study were obtained from local fermented foods which include palm-wine, milk, locust beans, fufu, Ogi and dairy fermented food, Yogurt. These food samples were collected in sterile bottles from commercial producers in different locations of western region in Nigeria. All the microorganisms investigated in this study were

obtained from the University Teaching Hospital in the Department of Microbiology, Ibadan, Nigeria. The tested isolates include: *Shigella dysenteriae*, *Shigella flexneri*, Enteropathogenic *Escherichia coli* type 1, Enteropathogenic *Escherichia coli* type 2, Enterohaemorrhagic *E. coli* type 3, *Salmonella typhi*, *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. Biochemical tests were carried out on each of the pure isolates to confirm their identity before use.

2.2 Isolation and Identification of Bacteria

10g of each sample was added to 90ml of normal saline solution. Serial dilutions of homogenized palm wine, locust beans, "fufu", yoghurt, "ogi" and milk samples were used for the isolation. Aliquots of 10^{-4} and 10^{-5} (0.1ml) dilutions were aseptically dispensed on sterile plates and MRS (De Man Rogosa Sharpe) agar with adjusted pH 5.5 was poured onto it and allowed to set. The plates were incubated at 37°C for 48 hours under anaerobic conditions using anaeroGen, then placed and closed immediately in the anaerobic jar. Discrete colonies were streaked onto fresh agar to obtain pure cultures of each isolate. The pure colonies were characterized using colonial, morphological characteristics and biochemical tests which include gram reaction, catalase (as shown in Table 1). Non spore forming bacilli, non capsule, catalase negative and gram positive isolates were maintained on MRS agar slants and stored at 4°C for further tests. The identification of the cultures to species level was based on the phenotypic characteristics of the lactobacilli as described in Bergey's manual of determinative bacteriology. Phenotypic characterization was conducted twice for each strain [16].

2.3 Extraction of Crude Bacteriocin

To determine bacteriocin production, the *Lactobacillus fermentum* and *Lactobacillus casei* were grown in 500ml MRS broth at 37°C for 48 hours anaerobically in triplicates. The cultures were centrifuged at 4,400 rpm for 15 minutes at 4°C. To eliminate growth inhibition caused by organic acids, the resulting cell free supernatant fluids were adjusted to pH 7.0 with 1N NaOH. Inhibitory activity of hydrogen peroxide was eliminated by adding 5mg/ml catalase and sterilized by filtration through 0.22µm Millipore filter [17]. The crude bacteriocin were then assayed using disc diffusion method.

2.4 Preparation of Discs

Paper discs were prepared from Whatman No.1 filter paper. The discs were autoclaved for 15 minutes at 121°C and allowed to cool [18].

2.4.1 Impregnation of discs

Sterile discs were placed in Petri-dishes containing 0.02ml of crude bacteriocin. The discs were allowed to dry in a clean oven at 35°C for 2 hours and stored in sterile air tight containers [18].

2.5 Antimicrobial Assay

The antimicrobial activity of the crude bacteriocin was determined using the disc diffusion method [18]. The impregnated discs were placed on solidified Muller-Hinton agar seeded with 14 hours cultures of tested microorganisms. The plates were kept at 4°C for 3 hours to

permit diffusion on the assay material, and incubated at 37°C for 24h using discs containing MHA broth as control. The antimicrobial activity was done in triplicates. Zones of inhibition were then measured and the values were recorded.

2.6 Purification of Bacteriocin

The crude supernatant was further purified by treating with 80% Ammonium sulphate for 6 hours at 4°C with gentle stirring. Bacteriocin precipitate was extracted by centrifugation at 4,400rpm for 15 minutes [19], the surface pellets and bottom pellets were decanted and dialysis (purification) was followed in a tubular cellulose membrane: the extracted bacteriocin was poured in cellulose membrane tube against 2 liters distilled water for 24 hours so as to remove impurities that might be present in the extracted bacteriocin. Each bacteriocin was then tested for activity using Agar well diffusion method.

2.7 Bacteriocin Activity

Two fold serial dilutions of bacteriocin samples were made in saline solution. The bacteriocin activity was determined using Agar well diffusion with slight modification. 100µl culture of the tested organisms was added into 20ml Muller Hinton Agar (MHA) and swirled for even distribution. Wells (4mm diameter) were made within the inoculated agar. Thereafter, aliquots of 10µl of bacteriocin were poured in the wells. Then they were incubated and the inhibition zones were observed after 24hours. It should be mentioned that the bacteriocin activity was defined as the reciprocal of the highest dilution (2^n) that resulted in the inhibition of the indicator lawn. Thus, the arbitrary units (AU) of the bacteriocin activity per millilitre (AU/ml) were defined as $2^n \times 1000 / 10\mu\text{l}$ [20].

2.8 Characterization of Bacteriocin

The bacteriocin samples were characterized with respect to their effects on pH, heat stability, storage stability, and their biopreservative efficiency in local pap, kunu and fresh orange. Also, the effects of viable antibiotics on the growth of isolates were examined.

2.8.1 Effects of pH

To test the stability at different pH, aliquots of bacteriocin were adjusted to pH values ranging from 2, 4, 6, 8, 10 and 12 using 4M HCl and 4M NaOH, respectively and subsequently incubated for 1hr at 37°C. The residual activities were then assayed, using treated bacteriocin of *Lactobacillus fermentum* against *Staphylococcus aureus*, and *Lactobacillus casei* against *Klebsiella pneumoniae* while untreated bacteriocin samples were used as control [21].

2.8.2 Heat stability

The effects of temperature on the bacteriocin were tested by heating the bacteriocin from 40°C to 100°C with 20°C increment and autoclaving at 121°C, a control was maintained by incubating the bacteriocin sample at 37°C. Aliquots of each treatment were taken after 15, 30 and 45 minutes [21,22]. The residual activities were then assayed, using treated bacteriocin of *Lactobacillus fermentum* against *Staphylococcus aureus* and *Lactobacillus casei* against *Klebsiella pneumoniae* while untreated samples were used as control.

2.8.3 Storage stability

The storage stability of the bacteriocin was investigated by incubating the bacteriocin at 37°C, and refrigerated at 4°C and -20°C respectively. After 7, 15 and 30 days, the residual activities were then assayed, using treated bacteriocin of *Lactobacillus fermentum* against *Staphylococcus aureus* and *Lactobacillus casei* against *Klebsiella pneumoniae* while untreated samples were used as control [21].

2.8.4 Biopreservative efficiency of bacteriocin in pap, kunu and fresh orange juice

The biopreservative efficiency of the bacteriocins obtained from *Lactobacillus fermentum* and *Lactobacillus casei* were determined using Joshi *et al* protocol with slight modification. Each food product which include pap, kunu and fresh orange juice (5ml each) was added to one milliliter of each tested bacteriocin and refrigerated for 2, 4, 6, 8 and 10 days respectively. The residual activities were taken every 2 days interval and serially diluted at 10^{-6} were made and the plated on nutrient agar and was incubated at 37°C for 24 hours. The colony count was recorded and compared with the control (without bacteriocin) [23].

2.9 Effect of Viable Antibiotics on the Growth of Isolated *lactobacillus* Strains

An overnight culture of the isolated *Lactobacillus* strains was grown on MRS Agar plates. Antibiotics bio-discs viz: Gentamycin (1µg), Cotrimoxazole (1µg), Chloramphenicol (1µg), Augmentin (1µg), Amoxillin (1µg), Erythromycin (1µg), Tetracycline (1µg) and Ciprofloxacin (1µg) were placed on the agar surface and the plates were kept at 4°C for 1h for diffusion, and incubated at 37°C for 24 hours. Growth inhibition was recorded by measuring the diameter of the zones and compared with standard antibiotics sensitivity chart [24]. The results were interpreted as percentage using the CLSI guidelines [25]

2.9.1 Statistical analysis

To determine the effect of the bacteriocins of *Lactobacillus* strains against test microorganisms, the zone of inhibition data were analyzed using one-way analysis of variance (ANOVA). Following ANOVA, and where means were significant, means were separated (Post Hoc test) by using Tukey's test. The Statistical Package for the Social Sciences (IBM SPSS statistics 22) was used for both ANOVA and means separation. The extents of standard errors of mean (SEM) for all the graphs plotted were represented with error bars. The level of significant was set at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Isolation and Identification

Predominant colony types were selected and purified by continuous streaking on MRS agar. The morphology of these isolates was characterized by microscopy, macroscopic and gram staining. Seventeen strains of Lactic acid bacteria strains isolated from Nigerian fermented foods were identified phenotypically. Isolates that were gram positive and catalase negative, as well as those showing absence of spore formation and those exhibiting absence of motility and rods shape were further identified. The identification of *Lactobacillus* strains to species level was based on their morphology, physiology and cultural characteristic. *Lactobacillus fermentum* and *Lactobacillus casei* were referred as the genus *Lactobacillus* (Table 1). The isolated *Lactobacillus* strains identified were one *Lactobacillus fermentum* and one *Lactobacillus casei*.

Table 1. Biochemical, morphological and physiological characteristics of isolates

Isolate	Citrate	Catalase	Motility	Sucrose	Lactose	Glucose	Voges proskauer	Methyl red	Arginine hydrolysis	Capsule	Spore formation	Gram reaction	Opacity	Elevation	Edges	Consistency	Colour	Size (mm)	Shape
<i>Lactobacillus fermentum</i>	+	-	-	A	A	A	-	+	+	-	-	+	opaque	Raised	smooth	wet	Creamy white	1-2	Round
<i>Lactobacillus casei</i>	-	-	-	A	A	A	+	-	-	-	-	+	opaque	Raised	smooth	wet	Creamy white	2-3	Round

+ = positive, - = negative, A = acid production

3.2 Antimicrobial Properties of Crude Bacteriocins

Bacteriocins obtained from *Lactobacillus casei* and *Lactobacillus fermentum* were used for assay study. These bacteriocins presented broad spectrum of antimicrobial activities when used against some selected microorganisms. The bacteriocins of *Lactobacillus fermentum* and *Lactobacillus casei* had significant effect ($P < 0.05$) on the nine tested microorganisms, Bacteriocins of *L. fermentum* and *L. casei* had higher effect on the tested microorganism. The highest zone of inhibition was observed for crude bacteriocins of *Lactobacillus fermentum* (9mm) against *Staphylococcus aureus* while the least zone was observed for crude bacteriocins of *Lactobacillus casei* (4mm) against *Klebsiella pneumoniae* (Table 2). The inhibitory activities showed on these bacteriocins against selected microorganisms reveal the presence of bacteriocin in the *Lactobacillus* strains. Earlier reports [26-30] revealed the presence of the compound bacteriocins in the *Lactobacillus* strains and bacteriocins have inhibitory effect against several bacteria. Possession of bacteriocin in *Lactobacilli* strains indicates their probiotics potentials.

3.3 Effects of pH

The bacteriocins of *Lactobacillus* strains used in this study were active over a wide range of pH (2-6) and it reduces to alkaline pH 7 to 12., indicating strong probiotic potential, because most of the bacteriocins are resistant to acidic pH more than basic pH. The purified bacteriocin of *Lactobacillus fermentum* showed a maximum activity at an initial pH of 2 and 4 with activity unit of 1600AU/ml against *Staphylococcus aureus* and bacteriocin of *Lactobacillus casei* showed a maximum activity at an initial pH of 2, 4 and 6 with activity unit of 1600AU/ml against *Klebsiella pneumoniae* (Fig. 1). Similar results were reported by Ben-Yahia et al. [31]. They reported that the bacteriocin of *Lactobacillus* were active over a wide range of pH 4 to 9 and is the optimum pH concentration for good inhibitory activity of bacteriocin from *Lactobacillus* strains against a wide spectrum of various pathogenic organisms for example *Staphylococcus aureus* and *Bacillus cereus*.

Table 2. Antimicrobial activity (mm) of crude bacteriocin against test organisms

Organism	<i>L. fermentum</i>	<i>L. casei</i>
Control	-	-
<i>S. typhi</i>	6.00±1.00 ^a	7.00±0.00 ^{cd}
<i>Sh. dysenteriae</i>	8.00±0.00 ^c	6.00±1.00 ^{bc}
<i>Sh. Flexneri</i>	5.67±0.58 ^{ab}	8.00±1.00 ^d
<i>EPEC-2</i>	5.00±1.00 ^a	6.67±0.58 ^{cd}
<i>E. coli 0157</i>	5.67±0.58 ^{ab}	5.67±0.58 ^{bc}
<i>EPEC-4</i>	7.33±0.58 ^{bc}	7.67±0.58 ^d
<i>St. pneumoniae</i>	5.00±1.00 ^a	5.00±0.00 ^{ab}
<i>K. pneumoniae</i>	5.00±0.00 ^a	4.00±0.00 ^a
<i>S. aureus</i>	8.67±0.58 ^c	7.00±0.00 ^{cd}

Values are mean ± SD of three determinations.

Each superscript letters (^{a, b, c} and ^d) depicts the expression of significance of crude bacteriocin of *L. fermentum* and *L. casei* against the tested microorganisms ($P < 0.05$).

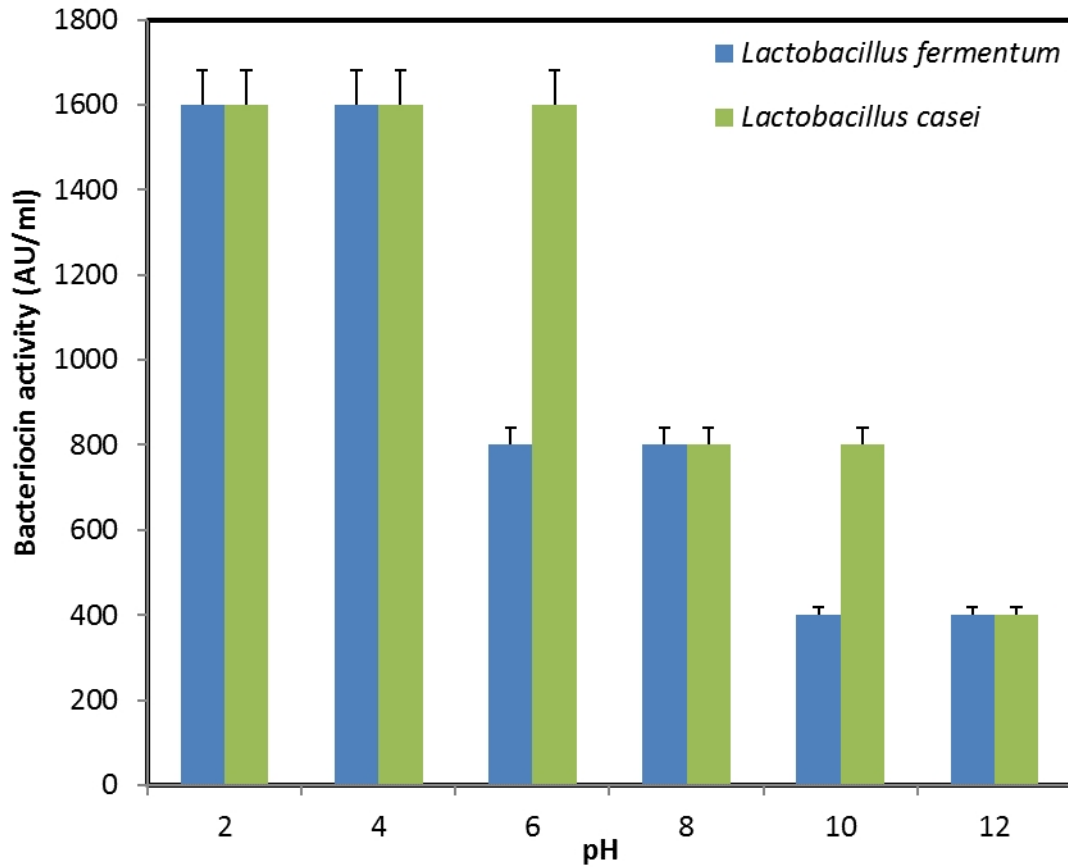


Fig. 1. Effect of pH on antimicrobial activity (AU/ml) of bacteriocin from *Lactobacillus spp* with error bars (n=5), estimated from statistical analysis

3.4 Heat Stability

Figures 2 and 3 show that bacteriocin of *Lactobacillus fermentum* had the maximum activity after heating at 100°C for 15 minutes (6400AU/ml) but it declined after 121°C for 45 minutes (400AU/ml) and bacteriocin of *Lactobacillus casei* had maximum activity after heating at 80°C for 15 minutes (3200AU/ml) but it declined after 100°C for 45 minutes.. Similar results were recorded for a number of bacteriocins produced by *Lactobacillus* strains which was resistance at 100°C for 15 minutes [23]. The phenomenon of heat stability of LAB bacteriocins have been reported earlier in literatures [21, 32, 33]. This present research is also in agreement with the above mentioned reports as it was observed that heat stability of the bacteriocin used in this study still retain its activity after heating at 121°C for 45 minutes, which means it could be placed within the heat stable low molecular weight group of bacteriocins. This quality of the bacteriocin makes it superior in processed foodstuffs where high heat is applied. Heating stability is a very useful characteristic in case of using bacteriocin as a food preservative, because many food processing procedures involve a heating step [34].

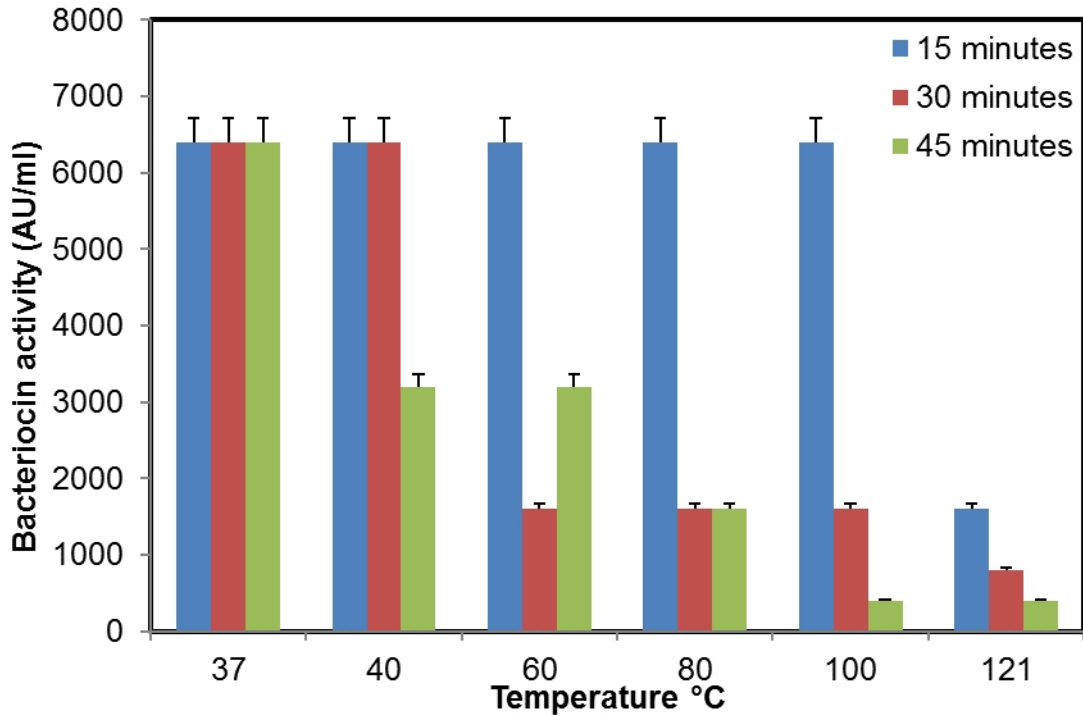


Fig. 2. Effect of temperature on antimicrobial activity (AU/ml) bacteriocin of *Lactobacillus fermentum* with error bars (n=6), estimated from statistical analysis.

3.5 Storage Stability

Figs. 4 and 5 show that the days and temperature at which the bacteriocins could be stored as the maximum zone of inhibition was observed at 4°C for 7 days (9mm) from bacteriocin of *Lactobacillus fermentum* while bacteriocin of *Lactobacillus casei* had inhibition zone of 7mm at 4°C, which means the bacteriocins can be stored at -20°C and 4°C indicating that cold temperature may be the most appropriate preservation technique for storing bacteriocins when compared with the control. The statistical analysis of the shows that the bacteriocin of *Lactobacillus fermentum* while bacteriocin of *Lactobacillus casei* had significant effect ($p < 0.05$) on both *Staphylococcus aureus* and *Klebsiella pneumoniae* respectively. Similar results was reported in literature [35] that the high stability of bacteriocin during prolong storage makes them superior and can have a positively impact on their use as biopreservative, with a view to improving the hygiene and safety of food products especially processed foods.

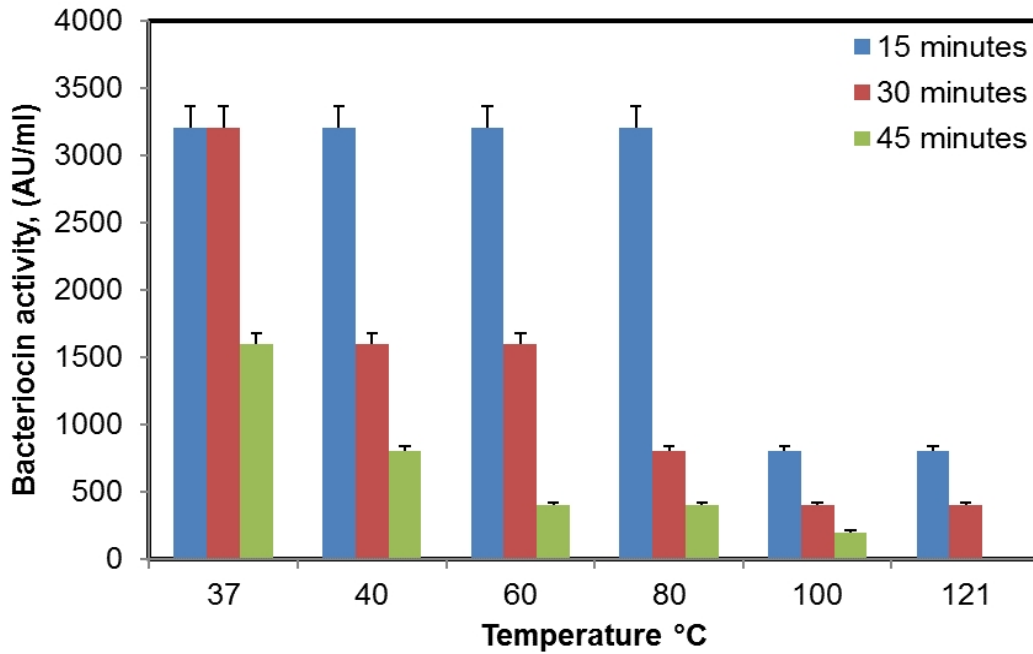


Fig. 3. Effect of temperature on antimicrobial activity (AU/ml) bacteriocin of *Lactobacillus casei* with error bars (n=6), estimated from statistical analysis.

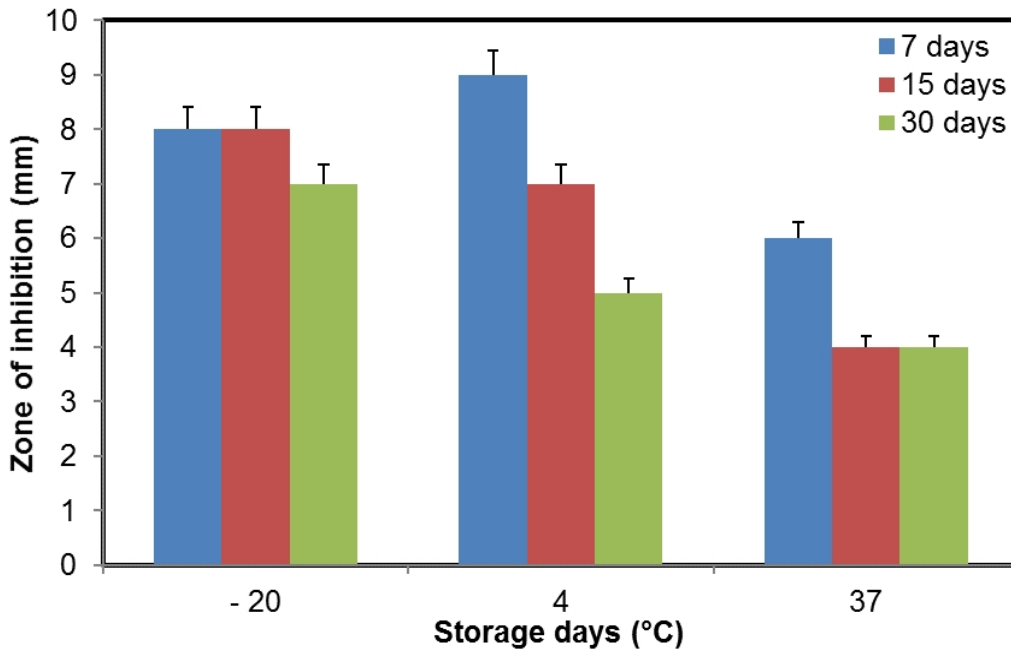


Fig. 4. Effect of time on bacteriocin activity (mm) of *Lactobacillus fermentum* during different storage condition with error bars (n=3), data are mean \pm standard deviation estimated from statistical analysis.

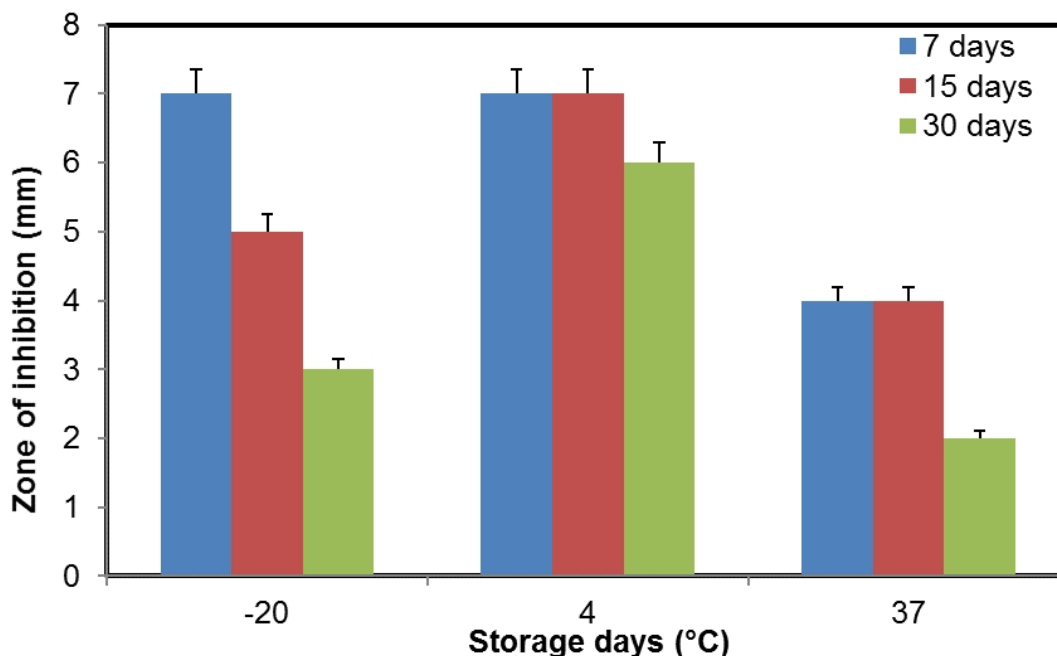


Fig. 5. Effect of time on bacteriocin activity (mm) of *Lactobacillus casei* during different storage condition with error bars (n=3), data are mean \pm standard deviation estimated from statistical analysis.

3.6 Biopreservative Efficiency of Bacteriocin in pap, Kunu and Fresh Orange Juice

Bacteriocin produced by the *Lactobacilli* used in this study when applied to pap, kunu and fresh orange juice inhibited the multiplication of aerobic bacteria when compared to the untreated control, the bacteriocin of *Lactobacillus fermentum* had maximum reduction on bacterial population (Figs. 6-8). These results further revealed that microbial count drastically decreased in both the treated and untreated sample. Similar results were reported in published articles [23,36]. Their findings show that Lactic acid bacteria have been demonstrated to cause a significant reduction in the bacterial population in meat.

3.7 Effect of Viable Antibiotics on the Growth of Isolated *Lactobacillus* Strains

Most of the *Lactobacillus* isolates were resistant to erythromycin of 70% and 100% for cotrimoxazole, ciprofloxacin, augmentin and amoxicillin which is in accordance with the findings of Voravuthikunchai et al. [37]. The outcome of their investigations revealed that *L. plantarum* (B14) and *L. rhamnosus* (B13, C5, G4, and G10) were sensitive to tetracycline but resistance to the remaining 7 antibiotics in the octa disc [38, 39] also reported that the resistance of Ciprofloxacin was a variable 17 to 95.3% from isolates of *Lactobacilli*. Figure 9 indicates the growth of isolate of *Lactobacillus fermentum* was not inhibited by Augmentin, Cotrimoxazole, Amoxicillin, Erythromycin, and Ciprofloxacin. Zone of inhibition was sensitive to Gentamycin, Chloramphenicol, and Tetracycline while growth of isolate of *Lactobacillus*

casei was not inhibited by Augmentin, Cotrimoxazole, Amoxicillin, and Ciprofloxacin. Zone of inhibition was sensitive to Gentamycin, Chloramphenicol, Tetracycline and Erythromycin. Resistance of the Lactobacilli strains to some antibiotics could be used for both preventive and therapeutic purposes in controlling intestinal infections.

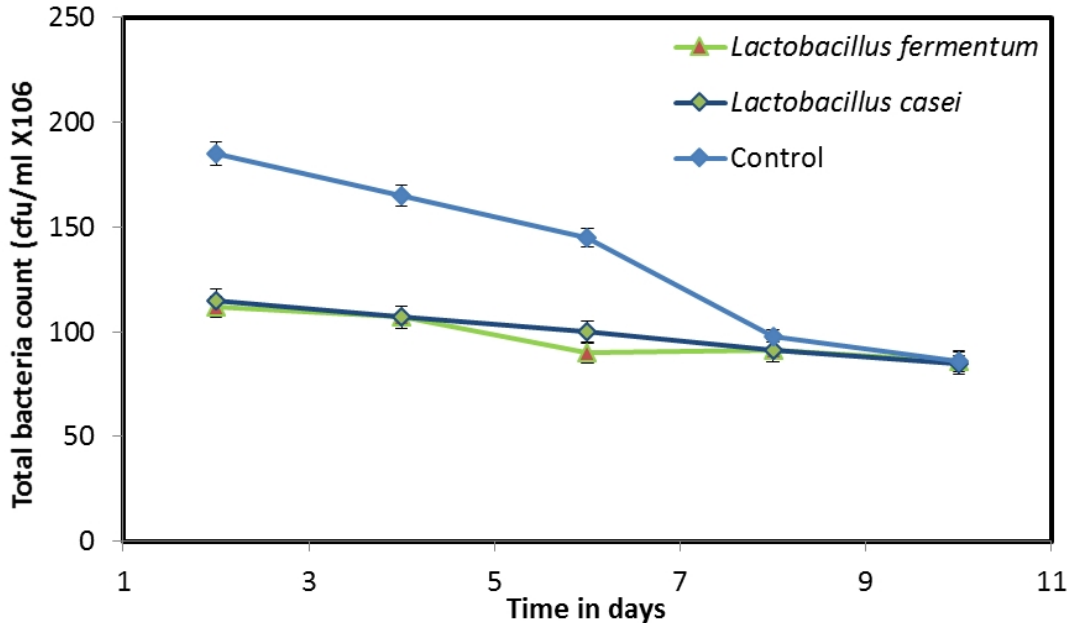


Fig. 6. Biopreservative efficiency of purified bacteriocin in pap with error bars (n=5), estimated from statistical analysis.

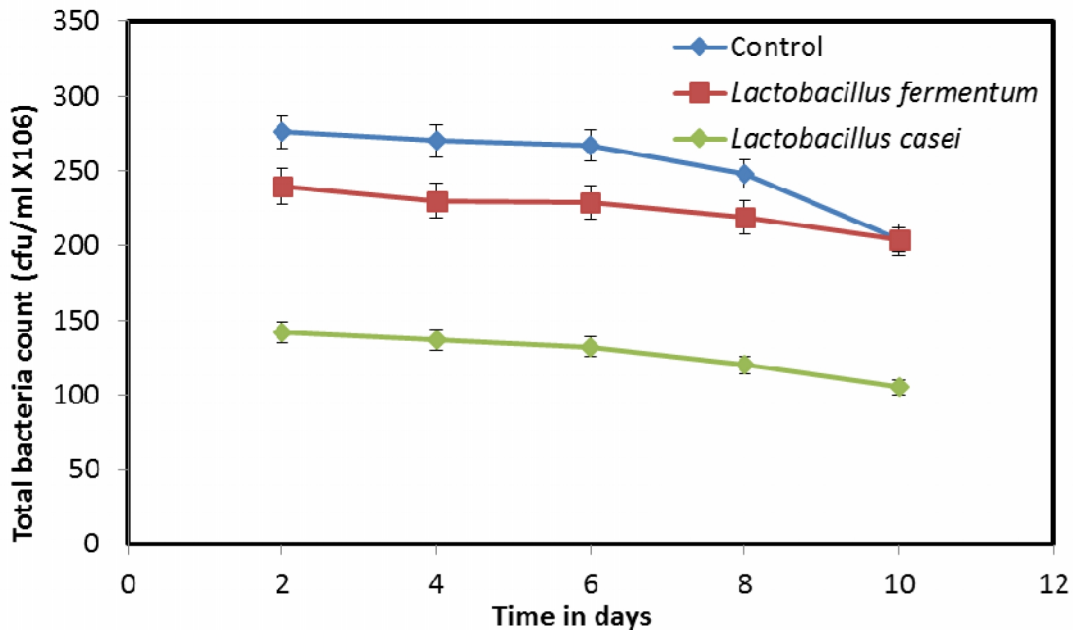


Fig. 7. Biopreservative efficiency of purified bacteriocin in fresh orange juice with error bars (n=5), estimated from statistical analysis.

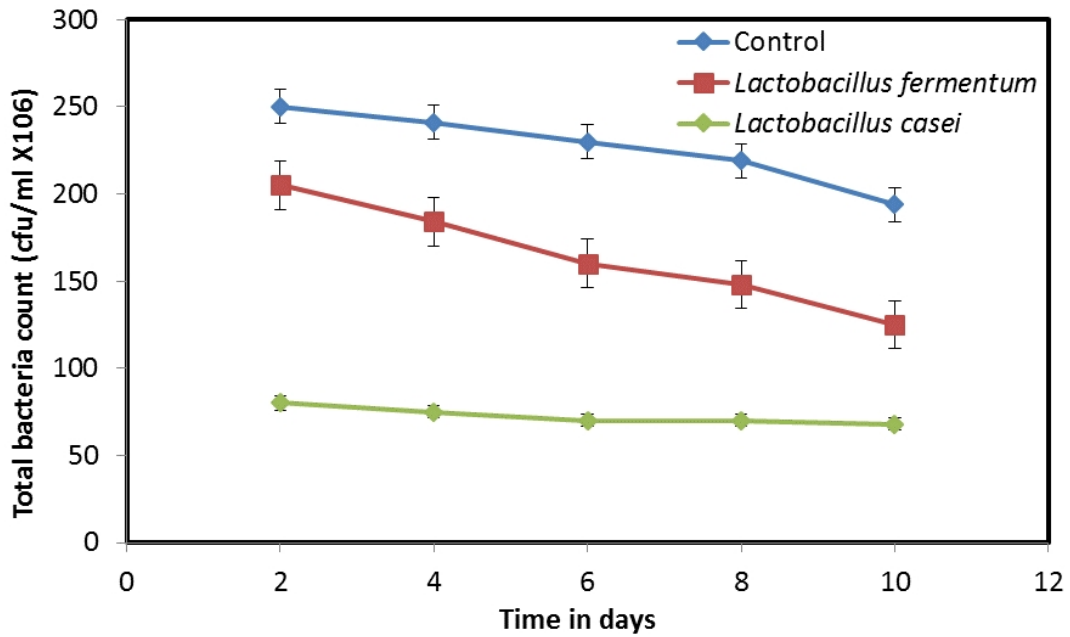


Fig. 8. Biopreservative efficiency of purified bacteriocin in Kunu with error bars (n=5), estimated from statistical analysis.

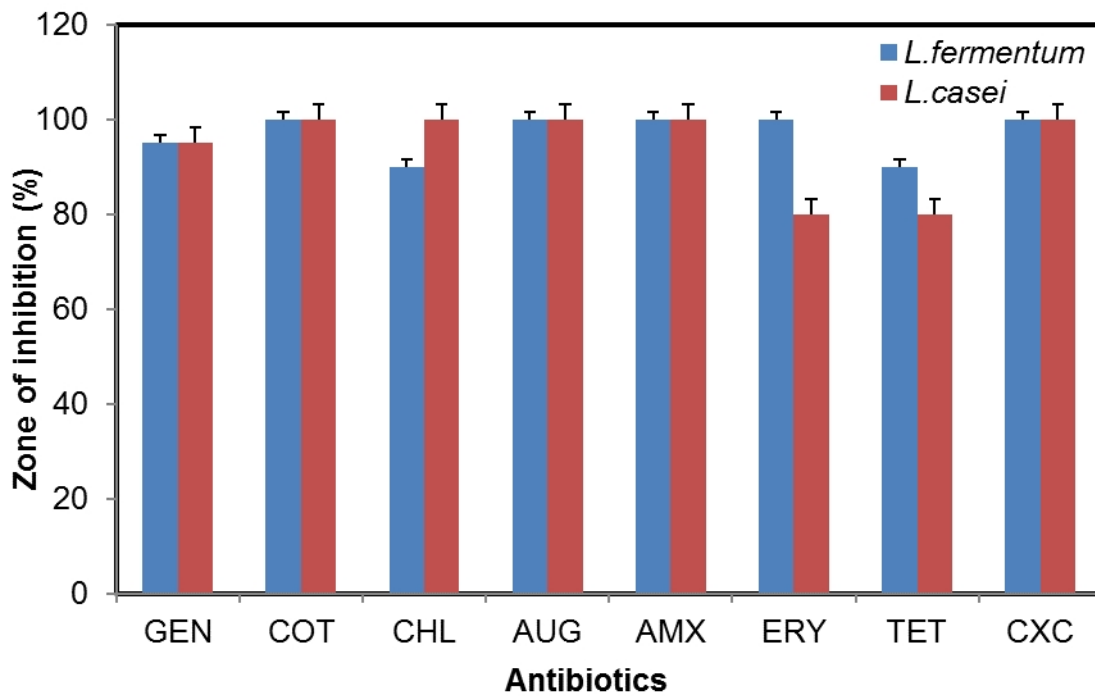


Fig. 9. Sensitivity of isolated strains of *Lactobacilli* against antibiotics discs (%) with error bars estimated from statistical analysis.

4. CONCLUSION

The *Lactobacilli* strains used in this study exhibited antimicrobial activity against the tested organisms. The bacteriocins were stable over a wide range of pH and heat. The bacteriocins could be freeze and refrigerated for storage purpose. The maximum reduction of bacterial population was observed when added to natural food products. The resistance of the probiotic strains to antibiotics could be used for both preventive and therapeutic purpose in controlling intestinal infection, which indicated that the isolated *Lactobacillus* strains meet several criteria that can make them useful for applications in probiotic.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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