EFFECT OF BACTERIOCIN FROM SELECTED LACTIC ACID BACTERIA ON SOME TEST ORGANISMS

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ABSTRACT

Lactic acid bacteria (LAB) are a group of Gram positive, fastidious bacteria having some members capable of producing bacteriocin-a ribosomally synthesized antimicrobial peptide. The study is aimed at determining the effect of bacteriocin from selected LAB isolates on some test organisms. Samples (cow and goat milk, kunu, nunu, cheese and fermented locust beans) were obtained from Malete, Kwara State. LABs were isolated under aseptic condition using spread plate method. After biochemical characterization, the isolates obtained were screened for their ability to produce bacteriocin using standard procedures. The isolates that produced bacteriocin with the widest zone of inhibition were then identified using 16SrRNA gene sequencing analysis. The antibacterial activity of the bacteriocin was tested against *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus cereus*. A total of thirty-six isolates were obtained out of which nine produced bacteriocin, however, two of these isolates produced bacteriocin with the widest zone of inhibition against the test organism and these were identified as *Lactobacillus plantarum* SJC103 and *L. apis* HBAm1. Using bacteriocin from *L. apis* the highest mean zone of inhibition of 15.00 ± 1.00 mm was obtained against *S. aureus* and the least of 10.00 ± 0.00 mm against *P. aeruginosa* and *Salmonella typhi* while for bacteriocin from *L. plantarum* the highest mean zone of inhibition of 17.00 ± 0.00 mm was obtained against *S. aureus* and the least of 7.50 ± 0.50 mm against *Salmonella typhi*. It was concluded that LAB bacteriocin produced inhibitory effect on the test organisms.

Keywords: Antibacterial activity, bacterioicin, characterization, isolation, lactic acid bacteria
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INTRODUCTION

All organisms, both eukaryotic and prokaryote are able to produce ribosomal antimicrobial peptides [1]. In bacteria, such compounds are referred to as bacteriocins. The focus of bacteriocins in bacteria has mostly been on lactic acid bacteria (LAB) as they are considered as GRAS (Generally Regarded as Safe) organisms by The Food and Drug Agency [1]. Bacteriocins exhibit antibacterial activity towards closely related strains [2] however, there has been an increasing number of reports on bacteriocins with broad range of antimicrobial effect [3]. In the past decade, interest in bacteriocin research is growing rapidly, especially those from lactic acid bacteria due to its potential as both a natural food preservative and as therapeutic antibiotics [4].

LAB bacteriocins are inherently tolerant to high temperature and are known for their activity over a broad pH range. They are also colourless, odourless, and tasteless, which further enhance their potential usefulness. There have been no reports on the development of resistant bacteria despite the long history of bacteriocin use; a reason for this could be attributed to its fast-acting mechanism, which forms pores in the target membrane of bacteria, even at extremely low concentrations. They are easily degraded by proteolytic enzymes due to their proteinaceous nature. Thus, bacteriocin fragments do not live long in the human body or in the environment, this minimizes the opportunity of target strains to interact with the degraded antibiotic fragments: which is the common starting point in the development of antibiotic resistance [5]. This research is aimed at studying the effect of LAB bacteriocin on selected pathogenic bacteria with the objectives of isolating bacteriocinogenic LAB from food samples and determining the antibacterial activity of the bacteriocin.

MATERIALS AND METHODS

Sample collection

Fresh samples of raw cow and goat milk, nunu, kunu, cheese and fermented locust beans were obtained from Malete, Kwara State and placed in clean ice packs and was immediately transported to the laboratory. Typed culture of *Staphylococcus aureus* ATCC 25923 was obtained from Federal Institute of Industrial Research, Oshodi; *Pseudomonas aeruginosa*, *Salmonella typhi*, *Bacillus cereus* and *Escherichia coli* were obtained from University of Ilorin, Microbiology Department, Ilorin, Kwara State. The isolates were confirmed by sub-culturing on selective media and through biochemical tests.

Preparation of McFarland Standard

McFarland 0.05% standard was prepared by introducing 0.05 ml of 1% BaCl in 9.95 ml of 1% H_2SO_4, the solution was shaken well.

Standardization of inocula

The inocula were standardized by introducing 10 ml of peptone water into sterile test tubes which were then inoculated with the test organisms. The turbidity of the media containing the isolates was adjusted to 0.05% McFarland standard.

Isolation of lactic acid bacteria (LAB)

One gram/ml of each food sample was aseptically introduced into 9 ml of 0.1 % peptone water in sterile test tubes. Serial dilution was performed and 0.1 ml of appropriate dilutions were plated on sterile DeMann Rogosa Sharpe (MRS) Agar plates and the plates were incubated at 37^0C for 48-72 hours under aerobic condition while anaerobic incubation was performed using an anaerobic jar.

Morphological screening

Pure culture of each isolate was obtained by repeated subculturing. Each pure isolate was morphologically screened through physical observation of the size, shape, colour, consistency and edge of the colonies.

Staining and biochemical screening

Biochemical analysis such as oxidase, catalase test, sugar fermentation and citrate utilization test, haemolytic activity and staining techniques-Gram staining, spore staining were conducted following standards methods of Olutiola *et al*. [6].

Screening of lactic acid bacteria isolates for bacteriocin production

Screening of isolates was initially conducted using the agar spot method [7]. Ten (10) µL of overnight broth cultures of each isolate was spotted onto the surface of MRS agar plates and incubated for 24 hours at 37^0C to allow colonies to develop. Hundred (100) µL of 18 hours culture of *Stapylococcus aureus* ATCC 25923 was mixed with 3.5 mL of soft (0.75%) MRS agar and poured over the plates. After incubation at 35 ^{0}C for 18 hours the plates were then checked for the formation of inhibition zones around the bacteriocin producer colonies, experiments were conducted in duplicates. Isolates that showed inhibitory activity using the initial procedure described above were further analysed to know whether the inhibitory effect was due to bacteriocin production using the agar diffusion bioassay as described by Herreros *et al*. [8] and Yang *et al*. [9] to screen for bacteriocin producing LAB among the isolates. Staphylococcus aureus ATCC 25923 was used as indicator organism. One ml of the indicator organism (as adjusted to 0.05% McFarland and equivalent to 1 x 10^8 cfu/mL) was used to inoculate 15 ml of molten MRS agar and poured into a Petri dish. After solidification, three wells (6 mm diameter) was cut and 100 µL of cell-free supernatant (CFS) from each LAB isolate and appropriately adjusted CFS; using 0.1M NaOH to pH 6 in order to rule out organic acid was added to the first well while CFS without NaOH treatment was added to the second, sterile MRS broth was introduced into the third well to serve as control, the plates were incubated for 24 hours at 35^0C, experiments were conducted in duplicate and the zone of inhibition observed was recorded.

Extraction of crude bacteriocin

The isolates were cultured in MRS broth and incubated at 37^0C for 48 hrs. After the incubation period, the broth culture of the isolates was centrifuged at 4000 rpm for 1 hour. The cell free supernatant was collected by decantation and neutralized to pH 6 using 1M NaOH. This was taken as crude bacteriocin [10].

Antibacterial activity of bacteriocin from selected LAB isolates

Under aerobic condition, the antibacterial activity of the crude bacteriocin from the two isolates that produced the highest zone of inhibition after screening was tested against some pathogenic and spoilage organisms by using the agar well diffusion method. In performing growth inhibition by agar well diffusion method, MRS agar plates were seeded with 0.1mL of 1 x 10^8 cfu/mL of the test organisms. The restrictive activity of the crude bacteriocin against selected pathogenic microorganisms (*Salmonella typhi*, *Escheriachia coli*, *Pseudomonas aeroginosa and Bacillus cereus*) was performed on MRS agar plate, wells were made by using a sterile cork borer (6 mm in diameter) under sterile condition and 100 µL of the crude bacteriocin was added to each of the wells. The plates were incubated at 37^0C for 24 hours for further examination. The antibacterial activity of the bacteriocin was determined by measuring diameter of the zone of inhibition around the wells [9]. The tests were conducted in duplicate.

Identification of Selected Bacteriocin Producing LAB using 16S rRNA Gene Sequencing

Identification of bacteriocin producing LAB using 16SrRNA gene sequencing was done following standard methods [9]. A homology search of the sequences was then conducted using the BLAST program at the NCBI database.

Characterization of Bacteriocin

Effect of Heat

Five ml of the crude bacteriocin was taken into different test tubes and heated at 60, 90 and 100^0C for 1 hour. The treated bacteriocin was examined for antibacterial activity using *S. aureus* ATCC 25923 as indicator organism via agar well diffusion technique [9], crude bacteriocin at a temperature of 30^0C without heat treatment served as control.

Effect of pH

The crude bacteriocin (5 mL) was adjusted to pH 3, 5, 7, 9 and 11 with hydrochloric acid (1M HCl) and sodium hydroxide (1M NaOH), incubated for 1 hour at 30^0C and similarly assayed for antibacterial activity using *S. aureus* ATCC 25923 as indicator organism [10] crude bacteriocin (pH 5.9) without pH treatment served as control.

RESULTS

Isolation and preliminary identification of lactic acid bacteria

A total of 36 isolates were obtained in all as shown in Table 1. After preliminary identification, 11 isolates were Gram positive and catalase positive while 25 were Gram positive and catalase negative.

Cellular, biochemical and sugar fermentation analysis

The 25 isolates that were Gram positive and catalase negative were subjected to further analysis, result presented in Table 2.0 and 2.1. The isolates were all non-sporing, non-motile and oxidase negative. However, their sugar fermentation ability varies.

Screening for bacteriocinogenic lactic acid bacteria

Antibacterial activity of LAB using agar spot technique

None of the isolates from goat and cow milk produced an observable zone of inhibition against the indicator organism. However, 1 isolate from Kunu, 3 isolates from Nunu, 4 isolates from fermented Locust beans and 4 isolates from Cheese produced a measurable zone of inhibition as presented in Table 3.

Antibacterial activity of LAB using agar well diffusion

Lactobacillus apis produced a zone of inhibition of 15.00 ± 1.00 mm against the indicator organism when CFS (cell free supernatant) alone was used and 14.00 ± 0.00 mm when the CFS was neutralized with NaOH.

Effect of enzyme

The crude bacteriocin (2 mL) were treated with protease (a proteolytic enzyme), at a final concentration of 2 mg/mL in potassium phosphate buffer (pH 7.0). The mixtures were then incubated at 37^0C for 2 hours [11] and similarly assayed for antibacterial activity using *S. aureus* ATCC 25923 as indicator organism [9], crude bacteriocin without enzyme treatment served as control. Also, *L. plantarum* produced a zone of inhibition of 17.50 ± 0.50 when CFS alone was used and 16.00 ± 0.00 mm when the CFS was neutralized with NaOH as shown in Table 4.

Antibacterial activity of bacteriocin

The mean zone of inhibition (mm) obtained from the antibacterial activity of bacteriocin from *L. apis* and *L. plantarum* is presented in Figure 2. Using bacteriocin from *L. apis*, the highest mean zone of inhibition of 15.00 ± 1.00 mm was obtained against *Staphylococcus aureus* and the least of 10.00 ± 0.00 mm against *Pseudomonas aeruginosa* and *Salmonella typhi* while for bacteriocin from *L. plantarum* the highest mean zone of inhibition of 17.00 ± 0.00 mm was obtained against *S. aureus* and the least of 7.50 ± 0.50 mm against *Salmonella typhi* .

Characterization of bacteriocin

The zone of inhibition recorded during assay for thermal stability of bacteriocin from *L. apis* was highest (14.50 ± 0.50 mm) at the control temperature (30^0C) and least (5.50 ± 0.50 mm) at 100^0C while for bacteriocin from *L. plantarum* was highest (16.50 ± 0.50) at 30^0C and least (11.00 ± 0.00 mm) at 90^0C and 100^0C and as presented in Figure 3. The zone of inhibition recorded for the pH adjustment on bacteriocin activity from *L. apis* and *L. plantarum* at the lowest pH used (3) were 4.50 ± 0.50 mm and 8.00 ± 0.00 mm respectively and at the highest pH used (11) 5.50 ± 0.50 mm and 7.00 ± 0.50 mm respectively as shown in Table 5. No inhibition zone was observed when bacteriocin from *L. apis* and *L. plantarum* were treated with protease as presented in Table 6.

Table 1: Number of lactic acid bacteria isolates obtained from the samples

Samples	Cow milk	Goat milk	Kunu	Nunu	Locust beans	Cheese	Total
Number of Isolates	2	10	2	7	9	6	36

Table 2: Cellular, biochemical and sugar fermentation characteristics of lactic acid bacteria isolates

	Cell morphology			**Biochemical features**						**Sugar fermentation**					
Test/ Isolate	**GS**	**SP**	**MT**	**OX**	**Ca**	**HP**	**CU**	**GF**	**LF**	**GaF**	**SF**	**FF**	**MaF**	**MlF**	**Probable Organisms**
G1	+ve, Rod	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	*Lactobacillus* spp
G2	+ve, Rod	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	*Lactobacillus* spp
C1	+ve, Rod	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve Gas	+ve	-ve	-ve	-ve	+ve	*Lactobacillus* spp
C2	+ve, Rod	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	*Lactobacillus* spp
C3	+ve, Rod	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve Gas	+ve	-ve	+ve	-ve	+ve	*Lactobacillus* spp
C4	+ve, Rod	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve	*Lactobacillus* spp
C10	+ve, Rod	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	*Lactobacillus* spp
K1	+ve, Rod	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve	*Lactobacillus* spp
K2	+ve, Rod	-ve	-ve	-ve	-ve	-ve	+ve	+ve Gas	+ve	-ve	-ve	+ve	+ve Gas	+ve	*Lactobacillus* spp
N1	+ve, Cocci	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve Gas	+ve	-ve	+ve	-ve	*Lactococcus/Streptococcus* sp
N2	+ve Rod	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve	*Lactobacillus* spp
N3	+ve Rod	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	*Lactobacillus* spp
N4	+ve Cocci	-ve	-ve	-ve	-ve	-ve	+ve	+ve Gas	+ve Gas	+ve	-ve	+ve	-ve	+ve	*Lactococcus/Streptococcus* spp
N5	+ve Rod	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve	*Lactobacillus* spp
N6	+ve Rod	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	+ve	Lactobacillus spp
N7	+ve Cocci	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve	Lactococcus/Streptococcus sp
L3	+ve, Rod	-ve	-ve	-ve	-ve	-ve	+ve	+ve Gas	+ve	-ve	-ve	-ve	-ve	-ve	Lactobacillus spp
L4	+ve Cocci	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve	Lactococcus/Streptococcus sp
L5	+ve Cocci	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	Lactococcus/Streptococcus sp
L6	+ve Rod	-ve	-ve	-ve	-ve	-ve	+ve	+ve Gas	+ve	-ve	-ve	+ve	+ve Gas	+ve	Lactobacillus spp
L7	+ve Rod	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	*Lactobacillus* spp
L8	+ve Rod	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	*Lactobacillus* spp
CH1	+ve Cocci	-ve	-ve	-ve	-ve	-ve	+ve	+ve Gas	+ve Gas	-ve	-ve	-ve	+ve Gas	+ve	*Lactococcus/Streptococcus* sp
CH2	+ve Rod	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	*Lactobacillus* spp
CH3	+ve Cocci	-ve	-ve	-ve	-ve	-ve	+ve	+ve Gas	+ve	-ve	+ve	+ve	+ve	+ve	*Lactococcus/Streptococcus* sp
CH4	+ve Cocci	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	*Lactococcus/Streptococcus* sp

Keys: -ve= negative, +ve = positive, GS= Gram staining, SP = Spore staining, OX = oxidase, Ca= Catalase Test, MT= Motility Test, CU = Citrate utilization, GF= Glucose fermentation, LF = Lactose fermentation, GaF= Galactose Fermentation, SF = Sucrose fermentation, FF= Fructose fermentation, MF = Mannitol fermentation, MlF= Maltose fermentation, HP= Haemolytic pattern; Isolates- G1, G2 (from Goat milk), C1,C2,C4,C10 (from Cow milk), K1, K2 (from Kunu), N1-N7 (from Nunu), L3-L8 (from Locust beans), CH1-CH4 (from Cheese)

Table 3: Antibacterial activity of LAB isolates against *S. aureus* ATCC 25923 using agar spot technique

Isolate	Zone of inhibition (mm)
G1	0.00 ± 0.00
G2	0.00 ± 0.00
C1	0.00 ± 0.00
C2	0.00 ± 0.00
C4	0.00 ± 0.00
C10	0.00 ± 0.00
K1	0.00 ± 0.00
K2	14.00 ± 1.00^{af}
N1	1.50 ± 0.50^{b}
N2	0.00 ± 0.00
N3	0.00 ± 0.00
N4	0.00 ± 0.00
N5	4.00 ± 0.00^{bd}
N6	0.00 ± 0.00
N7	2.50 ± 0.50^{bd}
L3	15.5 ± 0.50^{ac}
L4	4.00 ± 0.00^{bd}
L5	1.00 ± 1.00^{b}
L6	3.50 ± 0.00^{bd}
L7	0.00 ± 0.00
L8	0.00 ± 0.00
CH1	6.00 ± 0.00^{de}
CH2	3.00 ± 1.00^{be}
CH3	11.00 ± 1.00^{f}
CH4	4.00 ± 0.00^{b}

Values are expressed as mean ± SEM (standard error of the mean) of the zone of inhibition produced by each isolate against the indicator organism, where n=2. Values with different superscript in a column are statistically significant at $p < 0.05$

Keys: Isolates- G1, G2 (from Goat milk), C1, C2, C4, C10 (from Cow milk), K1, K2 (from Kunu), N1-N7 (from Nunu), L3-L8 (from Locust beans), CH1-CH4 (from Cheese).

Table 4: Antibacterial activity of LAB isolates against *s. aureus* ATCC 25923 using agar well diffusion

	Bacteriocin samples/Zone of Inhibition (mm)		
Isolate	**CFS**	**CFS + NaOH**	**Control**
K2	15.00 ± 1.00[a]	14.00 ± 0.00 [a]	0.00 ± 0.00
N1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
N5	6.00 ± 0.00[b]	3.50 ± 0.50 [b]	0.00 ± 0.00
N7	4.00 ± 1.00 [c]	2.00 ± 0.00 [c]	0.00 ± 0.00
L3	17.50 ± 0.50[d]	16.00 ± 0.00 [d]	0.00 ± 0.00
L4	3.00 ± 1.00[e]	2.00 ±0.00[e]	0.00 ± 000
L5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
L6	1.00 ± 1.00[f]	0.00 ± 0.00[g]	0.00 ± 0.00
CH1	5.50 ± 0.00[h]	4.00 ± 0.00[h]	0.00 ± 0.00
CH2	4.00 ± 0.00[i]	4.00 ± 0.00 [i]	0.00 ± 0.00
CH3	9.00 ± 1.00 [j]	7.50 ± 0.00 [j]	0.00 ± 0.00
CH4	2.00 ± 0.00[k]	2.00 ± 0.00 [k]	0.00 ± 0.00

Values are expressed as mean ± SEM (standard error of the mean) of the zone of inhibition obtained from bacteriocin produced by each isolate against the indicator organism, where n=2. Values with the same superscript in a row are not statistically significant at $p < 0.05$

Keys: CFS= culture free supernatant, CFS + NaOH= culture free supernatant neutralized with sodium hydroxide, control= sterile MRS broth; Isolates: K2 (from Kunu), N1, N5, N7 (from Nunu), L3, L4, L5, L6 (from Locust beans), CH1-CH4 (from Cheese).

16sRNA sequencing analysis of bacteriocinogenic LAB

The LAB isolates that produced the highest zone of inhibition were subjected to gene sequencing analysis. After blasting the isolates were confirmed as *Lactobacillus apis* HBAm1 and *Lactobacillus plantarum* SJC103 for K2 and L3 respectively as shown in Figure 1.

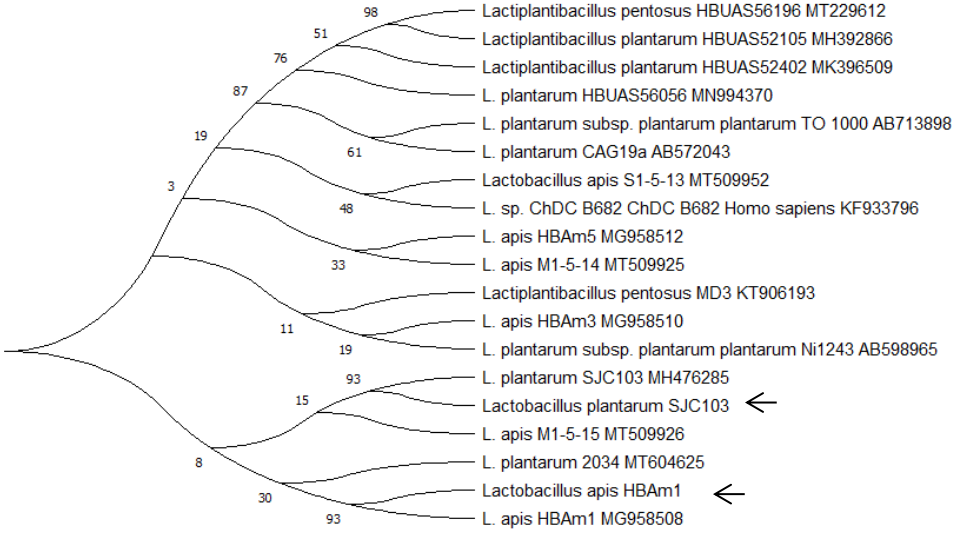


Figure 1: Phylogenetic Tree for *L. apis* HBAm1 and *L. plantarum* SJC103

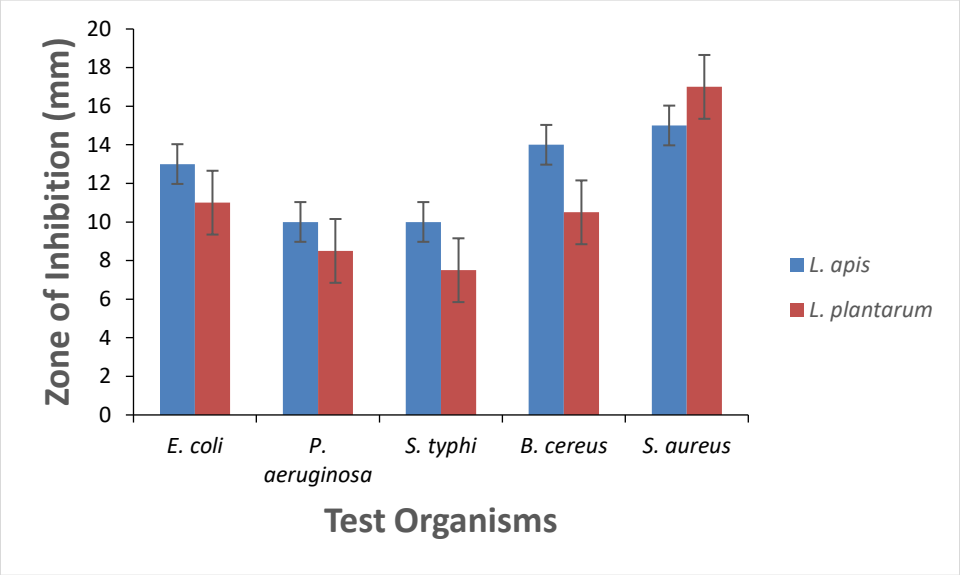


Figure 2: Antibacterial activity of bacteriocin from *Lactobacillus plantarum* SJC103 and *Lactobacillus apis* HBAm1

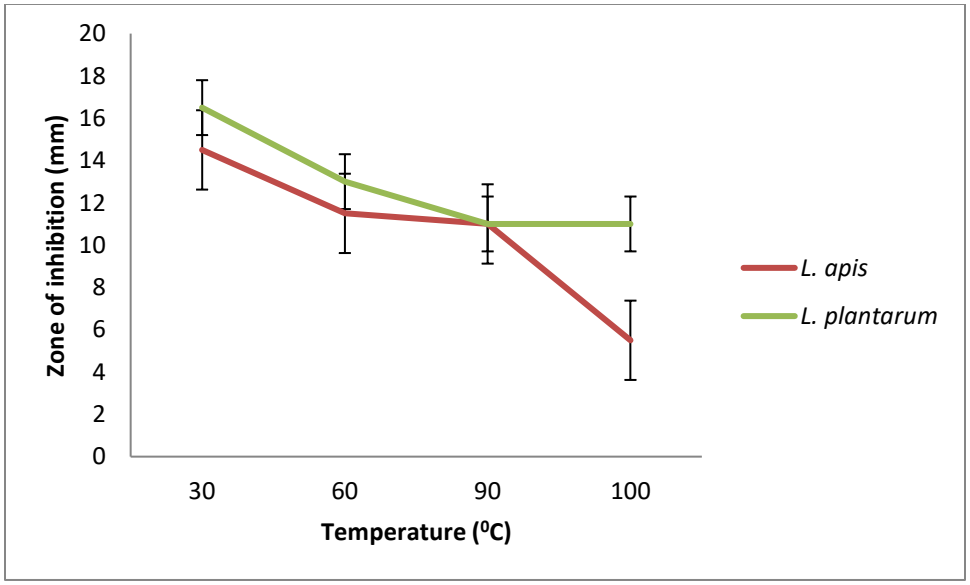


Figure 3: Thermal stability of bacteriocin produced by *Lactobacillus apis* HBAm1 and *Lactobacillus plantarum* SJC103

Table 5: Effect of pH adjustment on the activity of bacteriocin from *Lactobacillus apis* HBAm1 and *Lactobacillus plantarum* SJC103

	Isolates/Zones of Inhibition (mm)	
pH	***Lactobacillus apis* HBAm1**	***Lactobacillus plantarum* SJC103**
3	4.50 ± 0.50^{a}	8.00 ± 0.00^{b}
5	6.00 ± 0.00^{c}	8.00 ± 0.00^{c}
7	11.50 ± 0.50^{d}	13.50 ± 0.50^{d}
9	8.50 ± 0.50^{e}	9.50 ± 0.50^{e}
11	5.50 ± 0.50^{f}	7.00 ± 0.50^{f}
Control (5.9)	14.00 ± 0.00^{g}	15.00 ± 0.50^{g}

Values are expressed as mean ± SEM (standard error of the mean) of the zone of inhibition of the pH adjusted bacteriocin from each isolate, where n=2. Values with different superscript in a row are statistically significant at $p < 0.05$.

Table 6: Effect of Enzyme on the Activity of Bacteriocin from *Lactobacillus apis* HBAm1 and *Lactobacillus plantarum* SJC103

	Isolates/Zone of Inhibition (mm)	
Enzyme Treatment	***Lactobacillus apis* HBAm1**	***Lactobacillus plantarum* SJC103**
Protease (2mg/ml)	0.00 ± 0.00	0.00 ± 0.00
Control	12.50 ± 0.50	14.50 ± 0.50

Values are expressed as mean ± SEM (standard error of the mean) of the zone of inhibition of the enzyme treated bacteriocin from each isolate.

DISCUSSION

Lactic acid bacteria (LAB) are recognized for their fermentative ability and nutritional benefits, as they are able to exert strong antimicrobial activities against many pathogenic microorganisms. Moreover, LABs have been observed to compete for nutrients or space with spoilage microorganisms through production of diacetyl and bacteriocins [12]. Isolation of microorganisms from natural sources has always been one of the best ways to get organisms of interest in research studies. Lactic acid bacteria (LAB) used for this research were isolated from food samples (Table 1) and described in Table 2.

The bacterioicinogenic and antibacterial activity of the LABs were explored (Table 4 and 5); 36% of the isolates produced bacteriocin effective against *S. aureus* ATCC 25923 used as the indicator organism, contrary to the work of Goyal *et al*. [13], who reported that only 13 of the 145 potential LAB isolated from Indian diary product produced bacteriocin. Yang *et al*. [9] reported that only 20% of all the isolates (160) obtained from cheeses and yoghurts showed inhibitory activity against at least one indicator organism and Ayeni *et al.* [14] reported that 24 of the total isolates (96) obtained from dairy foods and cow's intestine produced the highest inhibition zones against indicator organism. Therefore, the choice of food samples is important for the successful isolation of bacteriocinogenic lactic acid bacteria.

The bacteriocin produced by two of the isolates produced the widest zone of inhibition against the indicator strain; using 16SrRNA sequencing these isolates were confirmed as *Lactobacillus apis* HBAm1 and *Lactobacillus plantarum* SJC103. The antibacterial activity of bacteriocin from these LAB isolates against *S. aureus*, *B. cereus*, *E. coli*, *S. typhi* and *P. aeruginosa* produced inhibitory effects. Both isolates produced inhibitory activity on all the test organisms used with no significant difference in the mean zone of inhibition produced by *Lactobacillus plantarum* SJC103 and *Lactobacillus apis* HBAm1 on the test organisms. This is similar to the work of Ali *et al.* [12] who reported that the LAB isolated had inhibitory effect on all the test organisms and the findings of Bartdoj *et al*. [15] who reported that the LAB isolated produced inhibitory effect not only against (*Lactobacillus bulgaricus* 340 i.e. a closely related species) but also against food-borne pathogens such as *E. coli* HB 101, *Candida pseudotropicalis*, *S. aureus* ATCC 25923 and *L. innocua* F.

The thermal stability of bacteriocin from *L. apis* was significantly reduced when heated at 100^{0}C for 1 hour as against the bacteriocin from *L. plantarum* which still retained its antibacterial activity after the same heat treatment; this may constitute an advantage for potential use as biopreservative where heat treatment up to 100^{0}C for 1 hour is required in order to preserve the food products. This is contrary to the work of Simova *et al*. [11] who reported that the thermal stability of bacteriocins from all the strains used were not reduced even at 100^{0}C for 1 hour as well as the findings of Yang *et al*. [9] who reported that the BLS (bacteriocin like substance) produced by the eight selected LAB isolates were thermally stable at 80^{0}C for 60 minutes and

100^0C for 90 mins as their inhibitory effects against *Listeria innocua* were retained.

Analysis of the effect of pH adjustment on bacteriocin activity revealed that bacteriocin from both organisms produced a better inhibitory effect at neutral pH (7) than at acidic and basic pH, this implies that the bacteriocin are most effective at neutral pH. This is contrary to the findings of Bonade *et al*. [16] who reported that the inhibitory activity of helveticin 51 (a bacteriocin) produced by *Lactobacillus helveticus* G51 was stable over the pH range from 3 to 7, showing a remarkable reduction above pH 7. However, the bacteriocin from *Lactobacillus plantarum* at pH 3 produced a higher zone of inhibition which was significantly higher than that produced by *Lactobacillus apis* at the same pH. This is contrary to the work of Batdorj *et al*. [15], who reported that the bacteriocin activity of *Enterococus durans* A511 was effective at a broad pH, ranging from 2 to 10 and the findings of Chahad *et al*. [12] who reported that the strains tested exhibited a broader pH range of activity, as the bacteriocins produced were effective against *Listeria monocytogenes* in the pH range of 2-8, with lowered activity at pH 6.

When the bacteriocin from *L. plantarum* SJC103 and *L. apis* HBAm1 were treated with protease, there was no inhibitory activity observed meaning that the compound (bacteriocin) is proteineous in nature. This tallies with the work of Ayeni *et al*. [13], Grosu-Tudor *et al*. [17] and Zhang *et al*. [18].

CONCLUSION

It was deduced that fermented products were the best source of lactic acid bacteria with bacteriocin potential and the LAB bacteriocin produced more inhibitory effect on the Gram-positive bacteria than the Gram-negative bacteria tested.

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