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Studies on Bacteriocin Production and Activities of *Lactobacillus Tucceti* CECT 5920 and *Lactobacillus Mindensis* TMW Isolated from Nigerian Traditional Fermented Foods

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ABSTRACT

Bacteriocin production and activities potentials of lactic acid bacteria (LAB) isolated from traditional fermented foods ("Ugba", pap, fermented cassava and 'Kunu zaki') were studied. Serial dilution of each of the samples was performed and 0.1ml of appropriate dilution was streaked on De Man Rogosa Sharpe (MRS) agar containing 50mg of nystatin for the isolation of LAB. Forty five LAB isolates were recovered from samples and were screened for bacteriocin production by the Agar Well Diffusion assay and two best bacteriocin producers characterized by molecular method as *Lactobacillus tucceti* CECT 5920 and *Lactobacillus mindensis* TMW were tested for their biotechnological potentials. *Staphylococcus aureus* NCTC 8325 and *Escherichia coli* 0157:H7 cultures were used as test pathogens. *L. tucceti* CECT 5920 and *L. mindensis* had the same level of bacteriocin production and antimicrobial activity ($P<0.05$). Temperature had more effect on bacteriocin activity on *L. tucceti* CECT 5920 against *S. aureus* NCTC 8325 and *E. coli* 0157:H7 while pH had same effects on both LAB isolates and pathogens. Both LAB isolates had highest effect from NaCl against *E. coli* 0157:H7 at 0.2% concentration. Crude bacteriocin samples treated with pepsin had no sensitivity against the test pathogens. Storage had decreasing effect on bacteriocin activity from both LAB isolates. *L. tucceti* CECT 5920 was sensitive to Cotrimoxazole while *L mindensis* TMW was resistant to all the antibiotics tested. *L. tucceti* CECT 5920 gave better results in all the biotechnological potentials tested, thus, the two LAB isolates may perform well in industrial processes.

Introduction

Traditional food fermentation is a food preservation method intended to extend shelf-life, improve palatability, digestibility and the nutritive value of food by the activities of naturally occurring microorganisms especially Lactic acid bacteria (LAB). Fermentation of various foods by lactic acid bacteria (LAB) is one of the oldest forms of bio-preservation practiced by mankind. Bacterial antagonism has been recognized for over a century but in recent years this phenomenon has received more scientific attention, particularly in the use of various strains of lactic acid bacteria. One important attribute of LAB is their ability to produce anti-microbicrobial compounds called bacteriocin (Lindgren and Dobrogosz, 1990).

Lactic acid bacteria produce bacteriocins which comprise a huge family of ribosomally synthesized peptides that have antibacterial activity towards closely related strains (Aderiye and Laleye, 2003), although there are an increasing number of bacteriocins reported to have broad range antimicrobial activity. In the past decade, interest in bacteriocin research, especially from lactic acid bacteria (LAB), has gained great momentum due to its potential as both a natural food preservative and as therapeutic antibiotics. Bacteriocins have a number of positive attributes that have made them especially attractive for various applications. LAB bacteriocins are inherently tolerant to high thermal stress and are known for their activity over a wide pH range. These antimicrobial peptides are also colourless, odourless, and tasteless, which further enhance their potential usefulness. Despite the long history of bacteriocin use, there have been no reports on the development of resistant bacteria. One possible reason is that bacteriocins have a fast acting mechanism by forming pores in the target membrane of bacteria, even at extremely low concentrations. They are also easily degraded by proteolytic enzymes due to their proteinaceous nature (Mokoena *et al.*, 2005). Therefore, bacteriocin fragments do not live long in the human body or in the environment, which minimizes the opportunity of target strains to interact with the degraded antibiotic fragments (a common starting point in the development of antibiotic resistance).

Perhaps, the most significant advantage of bacteriocins over conventional antibiotics is their primary metabolite nature since they have relatively simple biosynthetic mechanisms compared with conventional antibiotics, which are secondary metabolites. This fact makes them easily amenable through bioengineering to increase either their activity or specificity towards target microorganisms. Bacteriocins have been presented as a viable alternative to

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antibiotics due to the high specificity of some bacteriocins against clinical pathogens, including multi-antibiotic resistant (MDR) strains (Mokoena *et al.,* 2005).

Rationale

The remedy to the problem presented by chemically based food additives is the use of antimicrobial metabolites of fermentative microorganisms. Many antimicrobial substances have been in use for some time now without any known adverse effect. Many of these organic compounds which have stirred interest are bacterial metabolites used to produce or associated with fermented foods. Hence, it is strongly believed that microbial metabolites will become the next generation of food additives and the current interest in LAB is a step in the right direction.

Objectives

This research work was aimed at isolating bacteriocin producing lactic acid bacteria from some Nigerian fermented foods and determining some factors affecting the bacteriocin activity in foods.

Materials and Methods

Sample Collection and Analyses

Ten (10) samples of each traditional fermented food (ugba, pap, cassava and "kunu-zaki") were purchased from the retailers at Umuahia main market. The samples were packaged inside a cooler containing ice cubes and quickly transported to the laboratory for analyses. One gram each of the food samples was homogenized in 0.1% peptone water and serially diluted and 0.1ml aliquots of appropriate dilutions were inoculated onto De Man Rogosa and Sharpe (MRS, Oxoid, England) agar medium fortified with 50mg of nystatin (Roissart and Luguet, 1994) for the isolation of lactic acid bacteria (LAB). The plates prepared in triplicates were incubated at 35°C for 48 hrs anaerobically (using anaerobic gas packs) for isolation of mesophilic LAB. The mixed isolates were sub-cultured on MRS agar plates and the pure cultures were stored on MRS agar slants at 4°C. All the isolates recovered were maintained by by-weekly sub-culturing on MRS agar for 48hrs (Cheesbrough, 2004)

Culture Identification

The cultures were identified by observing the colonial morphologies, microscopy, biochemical, sugar fermentation tests (Cheesbrough, 2004) and by (GTG)5-PCR and 16S rDNA molecular characterization (data not shown).

Identification of Test Bacterial Pathogens

Cultures of *Staphylococcus aureus* NCTC 8325 and *Escherichia coli* 0157:H7 were used as test pathogens in this work. They were sub-cultured onto Mannitol Salt agar and McConkey agar respectively, gram stained and subjected to the necessary biochemical and sugar fermentation test to confirm their identity.

Preparation of Mcfarland Standard for Inoculum Preparation

To standardize the inoculum density for a susceptibility test, a $BaSO_4$ turbidity standard, equivalent to a 0.5 McFarland standard was used. It was prepared as follows:

1. A 0.5-ml aliquot of 0.048 mol/L $BaCl_2$ (1.175% w/v $BaCl_2.2H_2O$) was added to 99.5 ml of 0.18 mol/L H_2SO_4 (1% v/v) with constant stirring to maintain a suspension.
2. The Barium Sulfate suspension was transferred in 4 to 6 ml aliquots into screwed-cap tubes of the same size as those used in growing or diluting the bacterial inoculum. These tubes were tightly sealed and stored in the dark at room temperature.
3. The correct density of the turbidity standard was verified using a spectrophotometer with a 1-cm light path and matched cuvette to determine the absorbance. The absorbance at 625 nm was 0.008 to 0.10 for the 0.5 McFarland Standard.

Selection of Bacteriocin Producing Lactic Acid Bacteria

Forty-five LAB isolates recovered from the fermented food samples collected were screened and narrowed down to 10 LAB after gram staining, biochemical and sugar fermentation tests. They were screened for bacteriocin production by the Agar Well Diffusion (AWD) assay (Lasta *et al.,* 2008) and two LAB isolates were picked as the best bacteriocin producers. These two isolates were identified by (GTG)5-PCR and 16S rDNA as *Lactobacillus tucceti* CECT 5920 and *Lactobacillus mindensis* TMW and were used for further studies in the work.

Production and Purification of Bacteriocin Sample

The LAB isolates were each propagated in 1000ml MRS broth (pH 7.0; Oxoid, England). A cell-free solution was obtained by centrifuging the culture (10,000 rpm for 20 min, at 4 ^oC) and was adjusted to pH 7.0 by means of 1M NaOH to exclude antimicrobial effect of organic acids. The cell-free solution obtained was precipitated with ammonium sulphate (40% saturation). The mixture was stirred for 2 hr at 4^oC and later centrifuged at 20,000 rpm for 1 hr at 4^oC. The precipitates were re-suspended in 25ml of 0.05M Potassium phosphate buffer (pH 7.0). The new precipitates were collected and used for further analyses **(**Jimenez-Diaz *et al.,* 1993)

Determination of Bacteriocin Antimicrobial Activity

The test pathogens (*S. aureus* NCTC 8325 and *E. coli* 0157:H7) were grown in 100ml of peptone water for 18 hr and the concentration was matched against 0.5 Mcfarland Standard to obtain a concentration of $1.0x10^6$ CFU/ml. 18hr old culture broths of LAB isolates grown in MRS broth were centrifuged at 5000 rpm for 15 min and the pH of the cell free supernatant was adjusted to pH 6.5-7.0 with 1N NaOH to neutralize the effects of the organic acid. The LAB isolate was seeded on the surface of Mueller-Hinton Agar (Oxoid, England) using sterile swab sticks. 3mm deep wells were made on the Mueller-Hinton agar using sterile cork borer and the diluted test bacteria broths were placed into each agar well using sterile pipette. The plates were kept at room temperature for 2 hr and then incubated at 37°C for 24hr. The antagonistic activity of bacteriocins was determined by measuring the diameter of the inhibition zone around the wells (Vinod *et al.,* 2006).

Effect of Temperature on Bacteriocin Activity

The pH-adjusted culture supernatants of the LAB isolates were heated for 10 mins at 30, 40, 60, 80, 100^oC and for 15 mins at 121^oC respectively and the bacteriocin activity was tested against the pathogens as described earlier (Vinod *et al.,* 2006).

Effect of pH on Bacteriocin Activity

Culture supernatants of LAB isolates were adjusted to pH range of 2-12 using diluted 1M HCL and 1M NaOH solutions and were allowed to stand at room temperature for 2hr. The residual bacteriocin activity was then determined against the indicator organisms as described earlier (Vinod *et al.,* 2006).

Effect of NaCl on Bacteriocin Activity

MRS broths with 0.1-1.0% NaCl and distilled water (control) were sterilized by autoclaving and were inoculated with 10% of the overnight bacteriocin producing culture and incubated at 37°C for24 hr (Todorov and Dicks, 2004). The crude bacteriocin activity was assayed by inoculating the culture supernatant against indicator organism (Vinod *et al.,* 2006).

Table 1. Morphological and Biochemical Identification of Test Pathogens

S/N	Morphology	Gram reaction	Catalase	Coagulase	Glucose	Lactose	M-R	V-P	Indole	Citrate	Isolate
1	Yellow, discrete colonies on Mannitol Salt Agar	Gram + cocci in clusters	+	+	+/-	+/-	-	+	-	-	*S. aureus*
2	Pinkish raised colonies in singles on MacConkey Agar.	Gram + short rods in singles	-	-	+/+	+/+	+	-	+	-	*E. coli*

+/+ : Acid and gas production.

Table 2. Antimicrobial Activity of Crude Bacteriocin (Mm)

***Lactobacillus tucceti* CECT 5920**		***Lactobacillus mindensis* TMW**	
S. aureus	*E. coli*	*S. aureus*	*E. coli*
19^{a}±0.08	18^{a}±0.08	18^{a}±0.08	17^{a}±0.07

[a]Data values with the same letters are not significantly different ($p<0.05$; n=3).

*Control: MRS broths without LAB isolates

Interpretative Refrence Range

Sensitive	Intermediate	Resistant
≥17	11 – 15	≤10

Effect of Protease on Bacteriocin Activity

A 5-ml aliquot of adjusted LAB culture supernatant preparation was transferred into test tubes and treated with protease (0.1-1.0mg/ml) at pH 7. The test tubes with and without the enzyme (control) were incubated for 2hr at 37°C. Both the control and the samples were assayed for antimicrobial activity by using well diffusion method (Vinod *et al.*, 2006). The result is reported as either sensitive or resistant.

Effect of Storage on Bacteriocin Activity

Purified bacteriocin samples (0.1%, w/v) dissolved in peptone water were tested every 24hr for 14 days to determine their antimicrobial activities against the test isolates as described earlier (Vinod *et al.*, 2006).

Determination of Antibiotic Susceptibility of Lab

The LAB isolates were inoculated into MRS broth individually and incubated for 24 hrs. 25 ml of Muller Hinton agar was seeded with the pH-adjusted culture broth of LAB isolates (10^6 CFU/ml), mixed well, poured into sterile Petri plates and allowed to solidify. OCTA-antibiotic discs (8 antibiotics in a single ring) were placed upside down on the agar, firmly pressed and kept again at 4^{o}C for 1hr. The plates were then incubated at 37^{o}C over night and the zones of inhibition measured with a 15cm transparent rule (Vijai *et al.*, 2004).

Statistical Analyses

Data collected were subjected to Analysis of Variance (ANOVA). Mean separation was done using Duncan Multiple range test using Statistical Package for Social Sciences (SPSS) version 20. Differences in statistical significance were considered at $P \leq 0.05$ and n=3.

Results and Discussion

The present investigation was aimed at determining the biotechnological potentials of lactic acid bacteria isolated from traditional fermented food samples. Results showed that 45 isolates were recovered from the 40 samples of traditional fermented food collected and these were narrowed down to 10 isolates after morphological, biochemical and sugar fermentation tests. They were then screened for bacteriocin production and only two isolates showed best bacteriocin production. These two isolates were then identified by (GTG)5-PCR and 16S rDNA sequencing (data not shown) as *Lactobacillus tucceti* CECT 5920 and *Lactobacillus mindensis* TMW and were used for further studies in the work.

The two test pathogens used in this work: *Staphylococcus aureus* NCTC 8325 and *Escherichia coli* 0157:H7 were identified in Table 1

Assessment of bacteriocin production by the two isolates showed that they have statistically similar results (0.06^{a}±0.02 and 0.08^{a}±0.02mg/ml; Fig.1).

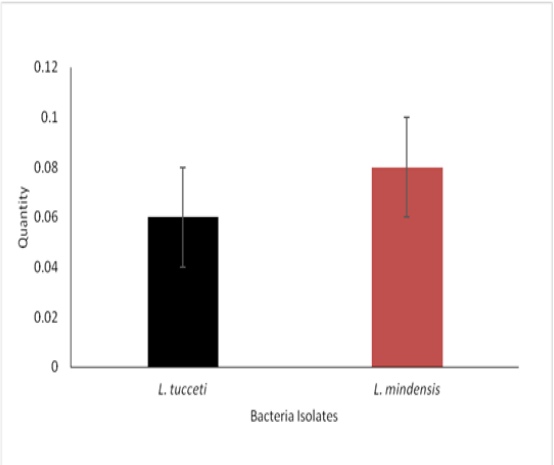

Fig 1. Bacteriocin production by LAB isolates (mg/ml)

Bacteriocin production is considered a very biotechnologically important attribute of lactic acid bacteria in the present search for an alternative to chemical food preservatives.

When the crude bacteriocin samples produced by the two isolates were tested for antimicrobial activities, *L. tucceti* CECT 5920 had statistically same inhibition on *S. aureus* and *E. coli* (19^a±0.08 and 18^a±0.08mm respectively, P<0.05) while *L. mindensis* TMW also had statistically same inhibitory effects on the two test pathogens (18^a±0.08 and 17^a±0.07mm, P<0.05) respectively (Table 2). This also revealed that the bacteriocins produced by these isolates have broad spectrum activity against gram positive and gram negative bacteria (*S. aureus* and *E. coli* respectively). Similar results were recorded by Adesokan, *et al.*, (2009) against *S. aureus, E. coli* and *Pseudomonas aeruginosa.* The result is a welcome development in modern food industry as the high level of susceptibility showed by the two test pathogens to the bacteriocins produced by the two LAB isolates is an advantage in food preservation and a possible good replacement for chemical preservatives. According to Ogunbanwo *et al.*, (2004), Lactic acid bacteria have potentials to inhibit the growth of pathogenic and spoilage bacteria and the possibilities exist for using them to improve the shelf life of different foods. Their antagonistic property is attributed to the low pH, the un-dissociated acid and production of other primary and secondary antimicrobial metabolites (Ten Brink *et al*., 1994).

Table 3. Effect of temperature on bacteriocin activity (mm)

Temp (for 10mins)	*L. tucceti* CECT 5920		*L. mindensis* TMW	
	S. aureus	***E. coli***	***S. aureus***	***E. coli***
30	18	17	17	17
40	-	-	-	-
80	-	-	-	-
100	-	-	-	-
121°C (for 15mins)	-	-	-	-
*Control	-	-	-	-

*Control: MRS broths without LAB isolates

Interpretative Refrence Range (mm)

Sensitive Intermediate Resistant

≥17 11 – 15 ≤10

The result of effect of temperature on bacteriocin activity showed bacteriocin activity up to the temperature of 30°C. *L. tucceti* CECT 5920 gave 18mm and 17mm zones of inhibition against *S. aureus* NCTC 8325 and *E. coli* 0157:H7 respectively while *L. mindensis* TMW had 17mm inhibition for the two test pathogens respectively. The LAB isolates exhibited mesophilic inhibitory activities (≤30°C, Table 3) and the mesophilic temperature of activity of the isolates showed that they could only be useful in food preservation in traditional food fermentation where the temperature is within that which the isolates can tolerate. However, this narrow range of temperature tolerance will be a disadvantage in modern food industries where higher temperatures are encountered. The increase in temperature beyond 30°C could have possibly destabilized the 3-Dimensional structure of the bacteriocins (which are proteins) resulting in their denaturation. Djadouni and Kihal (2013) reported two bacteriocins that were thermally stable over a wide temperature range up to 100°C for 15 mins. This could be due to the ecological conditions prevalent where the lactic acid bacteria were isolated. Significant reduction in the bacteriocin production as the temperature increased was reported by Meera and Devi (2012) and this is in agreement with the findings in this work. Growth temperature seems to play an important role in bacteriocin activity.

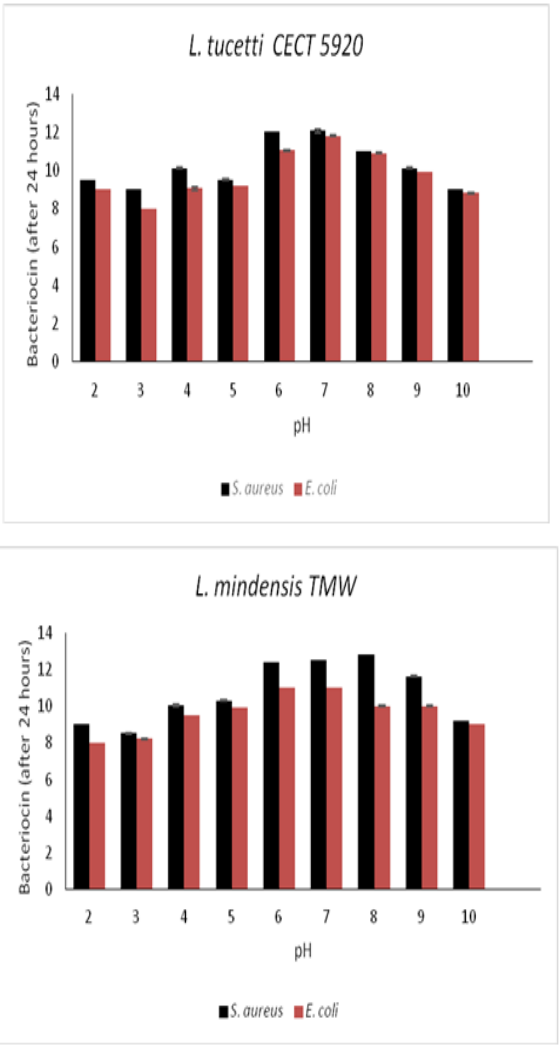

Fig 2. Effects of pH on bacteriocin activity (mm)

Result from Fig. 2 showed that when incubated at a range of pH, *L. tucceti* CECT 5920 had a maximum inhibitory activity of 12.06±0.08mm and 11.81±0.01mm against *S. aureus* NCTC 8325 and *E. coli* respectively at pH of 7, while *L. mindensis* TMW had maximum inhibitory activity (12.81±0.01mm) against *S. aureus* NCTC 8325 at pH of 8 and 11.02±0.01mm against *E. coli* 0157:H7 at pH of 7. However, both isolates showed wide range of pH tolerance and activity as they inhibited the test pathogens between the pH range of 2-10: an indication of acidophilic and slightly alkalinophilic activity. Both isolates showed optimum inhibitions at or near neutral pH. It has been reported that some bacteriocins retained their activity at pH 2.0 to 6.0 (Djadouni and Kihal, 2013) and this report is in agreement with the findings here. Djadouni and Kihal (2013) stated that microbial cells are significantly affected by the pH of their immediate environment because they apparently have no mechanism for adjusting their internal pH.

When the isolates were tested for bacteriocin activity in the presence of varying concentration of NaCl, *L. tucceti* CECT 5920 showed a higher inhibition (14mm) against *E. coli* 0157:H7 than *S. aureus* NCTC 8325 while *L. mindensis* TMW also showed higher activity (13mm, Table 4) against *E. coli* 0157:H7 than *S. aureus* NCTC 8325 both at 0.2% concentration. Both isolates showed bacteriocin activity between 0.1-0.3% of NaCl.

Table 4. Effect of Nacl on Bacteriocin Activity (mm)

Conc (%)	*L. tucceti* CECT 5920		*L. mindensis* TMW	
	S. aureus	*E. coli*	*S. aureus*	*E. coli*
0.1	6	7	6	7.5
0.2	10	14	11	13
0.3	9	12	9	12
0.4	8	10	7	8
0.5	R	R	R	R
0.6	R	R	R	R
0.7	R	R	R	R
0.8	R	R	R	R
0.9	R	R	R	R
1.0	R	R	R	R
*Control	N.I	N.I	N.I	N.I

[a,b,c,d,e]Data values with the same letters on the same columns are not significantly different ($p<0.05$), n=3)
*Control: MRS broth. N.I = NO INHIBITION

Beyond this concentration, bacteriocin activity was not recorded against any of the test pathogens. Mahrous *et al.*, (2013) reported that in MRS broth, 2% NaCl increased the activity of bacteriocins isolated from *Lactobacillus pentosus* CH2 against *E. coli* ATCC 25922 (14 mm zone of inhibition) while *L. fermentum* M1 had 15 mm zone of inhibition against *Bacillus subtilis* NCIB3610. The higher activity shown against *E. coli* 0157:H7 (a gram negative bacterium) by the two LAB isolates could be attributed to the nature of Gram negative cellwall which seems to be more susceptible to salt than gram positive cellwall possibly due to the thicker peptidoglycan nature of the latter. However, detrimental effects of high concentrations of NaCl, have been reported by Verluyten *et al.*, (2004). Some authors commented that growth of LAB in environments with high salt concentrations (NaCl concentrations higher than 3%), is inhibited, whilst lower amounts from 1% to 2% can exert a positive effect on the growth of the LAB isolates (Ganzle *et al.*, 1998; Korkeala *et al.*, 1992). It has also been reported that homo-fermentative microorganisms like *Lactobacillus delbrueckii* are in general more salt tolerant than hetero-fermentative LAB. While the majority of bacteriocins from LAB exhibit a bactericidal effect on target cells, bacteriocins that are bacteriostatic have also been reported (Hale and Hinsdill, 1973).

Table 5. Effect of Pepsin on Bacteriocin Activity (mm)

Pepsin (mg/ml)	*L. tucceti* CECT 5920		*L. mindensis* TMW	
	S. aureus	*E. coli*	*S. aureus*	*E. coli*
0.1	R	R	R	R
0.2	R	R	R	R
0.3	R	R	R	R
0.4	R	R	R	R
0.5	R	R	R	R
0.6	R	R	R	R
0.7	R	R	R	R
0.8	R	R	R	R
0.9	R	R	R	R
1.0	R	R	R	R
*Control	R	R	R	R

*Control: MRS broth without enzyme. R= Resistance and S= Sensitive

Interpretative Refrence Range

Sensitive Intermediate Resistant

≥17 11 – 15 ≤10

Result from Table 5 showed that there was no observed antimicrobial activity against the test pathogens when the LAB isolates were incubated with varying concentrations of pepsin enzyme. Purified pediocin SA-1 was reported resistant to treatment with trypsin, a-chymotryp-sin, pepsin and papain, but not to Proteinase K (Anastasiadou *et al.*, 2008). However, in agreement with the findings of this work is the result of Rajaram *et al.*, (2010) that Proteinase K and pepsin strongly inhibited bacteriocin production. Hence, the absence of bacteriocin activity recorded here could be due to the inhibition of the protease enzyme used. The bacteriocin pediocin ACH from *Pedicoccus acidilacti* was found sensitive to proteolytic enzymes and was completely inactivated by several proteolytic enzymes (Bhunia *et al.*, 1988; Bonade *et al.*, 2001). The stability of bacteriocin to different conditions reflects that such compounds can withstand the conditions normally encountered in food processing, so would remain effective during processing.

Evaluation of the effect of storage over a period of 14 days on bacteriocin's activity showed that there was a gradual decrease in the activity of the bacteriocin as storage progressed for both LAB isolates. However, there was bacteriocin activity recorded over the 14days of storage against the two test isolates. For *L. tucceti* CECT 5920, the highest bacteriocin activity ($19.01^{a}\pm0.01$) was recorded within the first 1-3days against *S. aureus* NCTC 8325 and 18.05^{a} ±0.07 against *E. coli* 0157:H7. The highest activity recorded from *L. mindensis* TMW against *S. aureus* NCTC 8325 was 18.02^{a} ±0.02 while it was $17.05^{a}\pm0.07$ against *E. coli* 0157:H7. There was about two-third loss in activity of bacteriocin of *L. tucceti* CECT 5920 on the 14^{th} day of storage compared with the first three days of storage. But for *L. mindensis* TMW, it was almost a four-fifth loss in activity (Table 6). Hamdi *et al.*, (2015) reported that extracted bacteriocin from *L. acidophilus* at 4^{o}C when fresh, after 15 days and 30 days of storage with the diameter of inhibition zone of 14, 12 and 10 against *Bacillus subtilis*, *S. aureus* and *E. coli*, respectively. However, the diameter of inhibition zone decreased to 11, 9.5 and 6.5 mm after 60 days of storage and reached to 7.5, 6 and 4mm of diameter after 90 days of storage for *Bacillus. subtilis*, *S. aureus* and *E. coli*., respectively. These results and the findings from this work are in accordance with those reported by Malini and Savitha (2012) as they found that the bacteriocin activity produced by *L. paracasei* subsp. *tolerans* isolated from locally available cheese was more stable at 4^{o}C for 30 days. By this result, it is found that bacteriocin will retain its antimicrobial activity when used as a bio-preservative in foods for up to 90 days in storage.

Table 6. Effect of Storage Time on Bacteriocin Activity (mm)

Storage time (days)	*L. tucceti* CECT 5920		*L. mindensis* TMW	
	S. aureus	***E. coli***	***S. aureus***	***E. coli***
1	19.01^{a}±0.01	18.05^{a}±0.07	18.02^{a}±0.02	17.05^{a}±0.07
3	19.01^{a}±0.01	18.02^{a}±0.02	18.05^{a}±0.07	17.01^{a}±0.01
7	10.05^{b}±0.07	11.01^{b}±0.01	10.01^{b}±0.01	10.05^{b}±0.07
10	7.01^{c}±0.01	8.05^{c}±0.07	4.05^{c}±0.07	5.01^{c}±0.01
12	6.05^{d}±0.07	7.06^{d}±0.08	4.06^{c}±0.08	5.06^{c}±0.08
14	6.02^{d}±0.02	6.01^{e}±0.01	3.01^{d}±0.01	3.07^{d}±0.09

^{a,b,c,d,e}Data values with the same letters on the same columns are not significantly different ($p<0.05$; n=3)

Interpretative Refrence Range

Sensitive Intermediate Resistant

≥17 11 – 15 ≤10

Table 7. Antibiotic susceptibility pattern of LAB isolates (mm).

	Cotrimoxazole (25µg)	**Cloxacilin (30µg)**	**Erythromycin (10µg)**	**Gentamycin (10µg)**	**Augmentin (30µg)**	**Streptomycin (10µg)**	**Tetracycline (30µg)**	**Chloramphenicol (30µg)**
L. tucceti CECT 5920	18.06^{a}±0.08	12.02^{b}±0.02	8.10^{d}±0.14	8.02^{d}±0.02	8.06^{d}±0.08	8.07^{d}±0.09	8.02^{d}±0.03	10.06^{c}±0.08
L. mindensis TMW	8.05^{b}±0.07	8.06^{b}±0.09	9.06^{a}±0.09	9.02^{a}±0.03	8.07^{b}±0.09	8.06^{b}±0.08	8.01^{b}±0.01	8.02^{b}±0.02

^{a,b,c,d}Data values with the same letters on the same columns are not significantly different ($p<0.05$; n=3)

Interpretative Reference Range

Sensitive Intermediate Resistant

≥15 13 – 14 ≤12

When the isolates were tested against their possible activity against selected eight antibiotics, result showed that *L. tucceti* CECT 5920 was sensitive to cotrimoxazole (18.06^{a} ±0.08mm) and resistant to other antibiotics namely (cloxacillin, erythromycin, gentamycin, augmentin, streptomycin, tetracycline and chloromphenicol). *L. mindensis* TMW was completely resistant to the eight antibiotics tested (Table 7). In their work, Lavanya *et al.*, (2011) reported that almost all the strains of lactic acid bacteria they tested were resistant to penicillin and 10% were susceptible to ampicillin, (β-lactam antibiotics). Zhou *et al*., (2000a) had earlier reported that *Lactobacillus* and *Bifidobacterium* strains were susceptible to β-lactam antibiotics (Penicillin, ampicillin) and this may be due to the difference in source of isolation. Among antibiotic resistances, vancomycin resistance is of major concern because vancomycin is one of the last antibiotics broadly efficacious against clinical infections caused by multidrug resistant pathogens. Some LAB however, including strains of *L. casei*, *L. plantarum* and *Leuconostoc* spp., *L. bulgaricus*, *L. fermentum* were found to be resistant to vancomycin. Such resistance is usually intrinsic, that is, chromosomally encoded and non-transmissible (Zhou *et al*., 2000b). One of the main criteria needed to be fulfilled by a probiotic organism is, it should be non-pathogenic (Dubois *et al*., 1956; Ljungh and Wadstrom, 2006). Result from this work showed that the LAB isolates will resist broad spectrum antibiotic therapy when they are used as probiotics in the intestine thus ensuring their survival and establishment as part of the microflora so as to elicit their antagonistic effects on the intestinal pathogens.

Conclusion and Recommendation

Much of the interest in the analysis of LAB produced bacteriocins and activity is driven by their potential applications. The properties of the bacteriocins studied, like the inhibition of pathogenic strains, their stability over a wide pH range, heat resistance and high salt tolerance makes them promising agents in food preservation.. In overall performance, *L. tucceti* CECT 5920 did better than *L. mindensis* TMW, however, both LAB isolates would make good research organisms of study in future works as regards the use of lactic acid bacterial metabolites as better substitutes in food preservation that the conventional chemical preservatives which have several health issues.

In a way of recommendation, the government and private bodies should invest more in researches involving the preservative and probiotic potentials of lactic acid bacteria that abound in our traditional fermented foods so as to encourage the application of the preservative and probiotic potentials of the organisms that emerged from this work in our local industries. Bacteriocins can be used to preserve our locally made fruit juices and vegetable pastes thereby prolonging the availability of such foods round the year and also improving our health status as the use of chemical preservatives which have been associated with several health challenges will be by-passed since bacteriocins are metabolically digested by the human body without any side effect.

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