academicJournals

Vol. 13(29), pp. 2977-2984, 16 July, 2014
DOI: 10.5897/AJB12.376
Article Number: 597FBCE46095
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Antimicrobial activities of lactic acid bacteria isolated from akamu and kunun-zaki (cereal based non-alcoholic beverages) in Nigeria

Okpara, A. N., Okolo, B. N. and Ugwuanyi, J. O.*

Department of Microbiology, University of Nigeria, Nsukka, Nigeria.

Received 23 January, 2012; Accepted 25 April, 2012

Three lactic acid bacteria (LAB) isolates designated AS_1, AS_2 and KN_4 isolated from kunun-zaki (a sorghum based non-alcoholic beverage widely consumed in Northern Nigeria) and identified as *Lactobacillus plantarum, Lactobacillus brevis* and *Lactobacillus delbruckii*, respectively, produced significant inhibitory compounds in broth. The partially purified inhibitory compounds were screened by agar spot assay method for antagonistic activity against target Gram positive and negative bacteria as well as yeasts associated with food spoilage. The partially purified compounds exhibited strong activity against *Staphylococcus aureus* ATCC 12600, *Bacillus cereus* and *Escherichia coli* ATCC 11775. The inhibitory compound produced by AS_1 and KN_4 inhibited *Bacillus subtilis.* Only the inhibitory compound produced by AS_1 affected *Candida albicans* and *Candida krusei.* Analysis of variance indicated that there was a significant difference ($P<0.05$) in the susceptibility of the different target organisms to partially purified inhibitory compounds. Gram positive bacteria were affected more than yeasts. Proteolytic enzymes, trypsin and pepsin, but not catalase and α-amylase, completely inactivated antagonistic activity of the compounds demonstrating their proteinacious nature. The inhibitory compounds were fairly heat stable and also stable over broad pH ranges. The use of these or related GRAS isolates in the production of this and related beverage may increase the safety, shelf life and marketing appeal of such beverages

Key words: Bacteriocins, lactic acid bacteria (LAB), target organisms, antimicrobial activity.

INTRODUCTION

Lactic acid bacteria (LAB) play essential roles in the fermentative production of many traditional foods. A wide variety of strains are routinely used as starter cultures in the manufacture of fermented dairy, meat, vegetable and bakery products (Lowe and Arendt, 2004). Many LAB strains are present as natural contaminants on a variety of foods such as cereals (De Martinis et al., 2001; kalolou et al., 2004), vegetables (Garcia-Graellis et al., 2000; Buddle et al., 2003; Ogunbanwo et al., 2004), milk (Kalchayanand et al., 1994; Gould, 1996) and meat (Moreno et al., 1999; Mataragas et al., 2003; Cadirci and Citak, 2005). In some parts of Africa, they play important parts in the production of alcoholic beverages and cereal based weaning formulae. Although, they may represent

*Corresponding author. E-mail: jerry.ugwuanyi@unn.edu.ng. Tel (+234) 0 803 306 6518.

undesirable contaminants in some food products, LAB, through their fermentative activities also exert a positive effect, imparting desirable flavours and inhibiting a variety of food spoilage and pathogenic organisms (Strom et al., 2002; Buddle et al., 2003).

The antimicrobial effect of LAB has been used by man through fermented foods for more than 10,000 years without any adverse effects (Shehane and Sizemore, 2002; Soomro et al., 2002) and this has enabled him to fortuitously improve the shelf life, safety and nutritional status of many foods. The preservative effect of LAB is partly due to production of a number of antimicrobial metabolites including organic acids, hydrogen peroxide and diacetyls (Adams and Moss, 1997; O'Keeffe and Hill, 1999; Vaughan et al., 2004). Among the various antimicrobial metabolites produced by LAB, bacteriocins are often the most potent inhibitors of bacteria (Klaenhammer, 1988; Deegan et al., 2006). Bacteriocins and bacteriocin-producing strains of LAB have been the focus of extensive research in recent years due to their food preserving potential (Toora, 1995; Savadogo et al., 2004). Although bacteriocins are produced by a broad spectrum of bacteria, those produced by LAB are of particular interest in the food industry because these bacteria have generally been recognized as safe (GRAS) (Nettles and Barefoot, 1993). Furthermore, as the majority of bacteriocin-producing LAB is natural food isolates, they are ideally suited to food applications (Deegan et al., 2006).

Although, various methods are employed for the preservation of foods, increasing consumer awareness of the uncertainties and potential health risks associated with the use of synthetic chemicals as preservatives in food has made it necessary to examine the possibility of using antimicrobial agents of biological origin as biopreservatives in food industries. Of particular relevance in this connection is the use of antimicrobial preservatives produced by the GRAS microorganisms employed in the production of the foods in question. This study is therefore, designed to screen Akamu and Kunun-zaki (cereal based non-alcoholic beverages) widely consumed in Nigeria for bacteriocin-producing LAB and to examine the partially purified bacteriocins for their capacity to inhibit the growth of selected spoilage and food-borne pathogens.

MATERIALS AND METHODS

Akamu was purchased from Nsukka market as produced and sold by local producers, while Kunun-zaki was purchased from hawkers in Enugu all in Enugu State of Nigeria. Both products were produced and marketed by peasant domestic producers. Five samples of each beverage were bought.

Target organisms

Spoilage and food-borne microorganisms (target organisms) used in this study were *Staphylococcus aureus*, ATCC 12600, *Escherichia coli*, ATCC 11775 obtained from Bioresources Development and Conservation Programme (BDCP) Centre, Nsukka. Untyped strains of *S. aureus* (4 strains), *Bacillus subtilis, Candida albicans,* and *Candida krusei* were obtained from the culture collection of the Department of Microbiology, University of Nigeria, Nsukka. Pathogenic organisms were grown aerobically in tryptic soy broth supplemented with 0.6% (w/v) yeast extract (TSB/YE) at 37°C. Food spoilage organisms were grown in nutrient broth under the same condition. The organisms were maintained by weekly subculturing on slants of appropriate media and stored at 4°C. Before each experiment, the microorganisms were activated by successive subculturing, and 18 to 24 h culture of each target organism was used for bioassay. Culture of target organism used in bioassay was standardized at approximately $5x10^7$cfu/ml determined by standard pour plate method on appropriate media.

Isolation of LAB from 'Akamu and kunun-zaki'

Sample homogenates of akamu and kunun-zaki were prepared and inoculated on de Mann, Rogosa and Sharp, (MRS) agar (Fluka) as described by Oxiod (1982). Inoculated plates were incubated at 37°C for a maximum of 72 h in a candle jar and the developed colonies counted. LAB colonies were purified by repeated subculturing on MRS agar. Pure cultures of LAB isolates were stored as frozen culture in MRS broth supplemented with 25% sterile glycerol, while working cultures were maintained on MRS agar slants at 4°C.

Screening and selection of LAB isolates with antagonistic activity

Pure cultures of LAB isolates were screened for antagonistic activity against target organisms by agar spot method. Overnight culture of each isolate of LAB was spotted onto MRS agar plate and incubated at 37°C for 24 h in a candle jar to allow colonies to develop. Seven milliliters of semi-solid TSB/YE (containing 0.7% agar) was incubated with approximately 5×10^7 cells/ml of target organism to be tested for sensitivity to LAB culture and overlaid on MRS agar plates on which LAB isolates were grown in triplicate. Another set of triplicate MRS agar plates containing LAB colonies were overlaid with 7 ml of sterile TSB/YE (0.7% agar) as control. Plates were incubated aerobically at 37°C for 24 h and examined for zones of inhibition. The colonies that showed inhibition zones were selected and characterized.

Characterization and identification of LAB Isolates

Pure cultures of selected LAB isolates were characterized as described by Batt (1999) and Teixeira (1999). The following standard microbiological tests were used for characterization of isolates, microscopic examination of cell morphology, physiological tests, biochemical tests, cultural growth conditions and carbohydrates (sugar) fermentation profile. Identification was based on comparison of observed characteristics of isolates with those of lactic bacteria as described in the Bergey's Manual of Determinative Bacteriology (Holt et al., 1994).

Production and assay of bacteriocin

For bacteriocin production, 1000 ml of MRS broth (without Tween 80) in 1.2L Erlenmeyer flasks were inoculated with 1% (v/v) of overnight culture of each LAB isolate and incubated without agitation at 37°C for 48 h in a candle jar. After incubation, cells were removed by centrifugation at 6000 *g* for 10 min at 4°C, followed

by filtration through 0.45 mm pore size cellulose acetate filter to obtain a cell-free supernatant (CFS). The crude bacteriocin present in the culture supernatant was precipitated by ammonium sulphate (40% saturation) at 4°C. The precipitate (crude bacteriocin) was partially purified by dialysis against 20 M sodium phosphate buffer (pH 7.0) at 4°C for 2 h using standard protein dialysis bags (BDH, Poole England). The partially purified bacteriocin was sterilized by passing through a 0.45 µm membrane filters and stored at 4°C for further use without any other treatment unless otherwise stated.

Determination of antimicrobial activity of bacteriocin

The inhibitory activities of bacteriocins against target microorganisms were determined by agar well diffusion method (Choi et al., 1999). Antimicrobial assay was performed under conditions that eliminated the inhibitory effects due to other compounds such as organic acids and hydrogen peroxide (also produced by LAB). Dialysis eliminated organic acids while the effects of hydrogen peroxide was excluded by addition of catalase (sigma LGI 026k 7049) in the concentration of 1mg per ml. To exclude the inhibition due to the presence of lytic bacteriophages, the reverse side technique was used according to Moreno et al. (1999). For bacteriocin assay, 7 ml of soft TSB/YE (0.7% agar) was inoculated with 100 µl of overnight culture containing 5 ×10 cells/ml of target organism and overlaid on TSB/YE (1.5% agar) plates in duplicate and allowed to gel.

Two wells of 5 mm diameter were cut in the agar plate using a sterile cork borer. Wells were filled with 100 µl of bacteriocin solution. Another set of duplicate TSB/YE agar plates inoculated in the same way except that their wells were filled with 20 M sterile sodium phosphate buffer (pH 7.0) in place of bacteriocin were prepared as control. After incubation, plates were examined for the presence of inhibition zones around the wells as an indicator of inhibitory activity. The diameters of the inhibition zones were measured in millimeters. Antagonistic activities were expressed and recorded as the mean of inhibition zone values.

Determination of total soluble protein in the bacteriocin solution

Total soluble protein in the bacteriocin solution was estimated by the method of Lowry (1951), using bovine serum albumin (BSA) as standard. A reagent blank containing 20 M sodium phosphate buffer in place of bacteriocin was also prepared. Colour development was measured at 595 nm in spectrophotometer (LKB Biochem Novaspec, Cambridge England), immediately after incubation.

Effects of hydrodytic enzymes on stability of bacteriocin

The nature of the inhibitory compound produced by LAB isolates was investigated by testing their sensitivity to some hydrolytic enzymes. The enzymes used were Trypsin from beef pancreas (EC No: 232-6508, Breckland Scientific, U.K), pepsin (EC: 232-629-3, Breckland Scientific, U.K), catalase from bovine liver (Sigma, I G, 026k 7049) and α-amylase (EC. No: 336-5656, Breckland Scientific, U.K).

Each inhibitory compound was treated with the various enzymes as follows: each enzyme was suspended in appropriate buffer solution at concentration of 5 mg/ml. Trypsin was suspended in 0.1 M tris hydrochloride buffer (pH 7.2), pepsin in 0.1 M citrate buffer (pH 6.2), catalase in 20 M sodium phosphate buffer (pH 7.0) and α-amylase in 0.1M citrate phosphate buffer (pH 6.2). Afterwards, each sample of bacteriocin was treated with the different enzymes solution to a final concentration of 1 mg/ml. Bacteriocin-enzyme mixture was incubated for 1 h at room temperature (≈28°C). Following enzyme treatment, the antagonistic activity of each inhibitory compound against a sensitive target organism, *S. aureus* ATCC 12600 was monitored by agar well diffusion assay as previously described. Untreated bacteriocin samples were used as control.

Effects of temperature on stability of bacteriocin

Each sample of bacteriocin was tested for stability at different temperatures. Aliquot of each sample freshly obtained from LAB isolates was heated at different temperatures ranging from 40 to 80°C for 30 min, with increment of 10°C and also at 100°C for 15 min. Following heat treatment, samples were cooled to room temperature. Afterwards, the residual activity of the heat treated samples against *S. aureus* ATCC 12600 was monitored by agar well diffusion assay as previously described and compared to the activity of the untreated samples used as control.

Effects of pH on stability of bacteriocin

Each partially purified bacteriocin sample was screened for stability at different pH values (2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10, 0). The initial pH of sample (pH 7.0) was adjusted to pH 2, 3, 4, 5 and 6 with 0.1 M hydrochloric acid (HCL) and pH 8, 9 and 10 with 1 M sodium hydroxide (NaOH) and subsequently incubated at room temperature (≈28°C) for 1 h. Thereafter, the residual activity of treated sample against *S. aureus* ATCC 12600 was monitored by agar well diffusion method as previously described. The activity of sample at initial pH (7.0) was used as a control.

Statistical analysis of data

The antagonistic activities of LAB isolates and partially purified bacteriocin from LAB isolates as well as the effects of various physicochemical parameters on the stability of bacteriocin were evaluated using the two-way analysis of variance (ANOVA) and student's t-test. Duncan's multiple range tests of variables was used for comparisons between antagonistic activity and susceptibility of a target organism. It was also used to identify means that differed significantly. The differences were considered significant at $p \leq 0.05$.

RESULTS

Prevalence of LAB in samples of akamu and kununzaki

Data obtained from this study indicate that LAB occurred in high numbers on cereals. The viable cell colony count on MRS agar ranged from 10^7 to 10^{10} colony forming unit per gram or millilitre (cfu/g or cfu/ml) of sample. A total of 13 presumed LAB isolates were obtained from the beverages sampled. Out of these, eight isolates were from akamu and five from kunun-zaki. Of the 13 isolates, three (designated AS_1, AS_2 and KN_4) were selected for further studies on the basis of appreciable antagonistic activity against target organisms on preliminary assays. Isolates AS_1 and AS_2 were obtained from Akamu while isolate KN_4 was from Kunun-zaki.

Table 1. Morphological, physiological and biochemical characteristics of isolates.

Test	AS_1	AS_2	KN_4
Gram reaction	+	+	+
Microscopic appearance	Bacilli	Short rods	Bacilli
Cellular arrangement	Chains	Pairs	Chains
Motility	-	-	-
Spore	-	-	-
Catalase	-	-	-
Hydrolysis of arginine	-	+	+
Growth in 18% NaCl	-	-	+
Growth in 6.5% NaCl	+	+	+
Growth at pH 9.6	-	-	-
Growth at pH 4.4	+	+	+
Growth at 10°C	-	-	-
Growth at 45°C	+	w	+
Fermentation of glucose with acid	+	+	+
Glucose with gas	-	+	-
Dulcitol	d	+	-
D-xylose	w	+	+
D-melobiose	-	+	-
Fructose	+	+	+
Galactose	+	+	D
L-Arabinose	-	+	-
L-sorbose	+	-	-
Lactose	-	+	-
Maltose	+	+	D
Mannitol	-	+	-
Raffinose	-	-	-
Rhmnose	-	+	-
Probable identity	*L. plantarum*	*L. brevis*	*L. delbrucki*

+, Positive reaction; -, negative reaction; w, weak reaction; D/d= variable.

Characterisation of isolates AS_1, AS_2 and KN_4

The morphological, physiological and biochemical characteristics as well as carbohydrate fermentation profile of the three selected LAB isolates (AS_1, AS_2 and KN_4) are shown in Table 1. Based on these biochemical data, isolates AS_1 was identified as *Lactobacillus plantarium,* AS_2 as *Lactobacillus brevis* and, KN_4 as *Lactobacillus delbruckii.*

Antagonistic activity of partially purified inhibitory compounds produced by isolates AS_1, AS_2 and KN_4

The antagonistic activities of the partially purified inhibitory compounds obtained from the isolates of LAB against the different target organisms based on agar well diffusion assay is shown in Table 2. The partially purified inhibitory compounds of isolates AS_1, AS_2 and KN_4 hadactivity against the different target organisms. The compounds inhibited the growth of the different organisms significantly ($P<0.05$). The three inhibitory compounds showed strong activity against *S. aureus*, *B. cereus* and *E. coli* ATCC 11775. The inhibitory compounds from isolates AS_1 and KN_4 were also active against *B. subtilis*. Only the inhibitory compound from isolate AS_1 affected *C. albicans* and *C. krusei*. The activities of all three inhibitory compounds were strongest against *S. aureus* ATCC 12600.

Total soluble protein in the partially purified inhibitory compounds

Partially purified inhibitory compounds produced by isolate AS_1 had 2.34 mg/ml protein while AS_2 and KN_4 had 1.6 and 2.13 mg/ml, respectively.

Effect of hydrolytic enzymes on stability of inhibitory compounds

The residual activity of the three inhibitory compounds

Table 2. Antagonistic activities (as zone of inhibition) of partially purified inhibitory compounds.

Target organism	Inhibition zone values (mm) mean ± S.D		
	AS_1	AS_2	KN_4
S. aureus ATCC 12600 (S1)	22.0±2.8	20.0±0.7	22.0±0.5
S. aureus (S2)	20.0±0.7	18.0±1.4	18.0±0.5
S. aureus (S3)	20.0±2.8	15.0±0.1	20.0±1.0
S. aureus (S4)	20.0±0.1	15.0±0.7	22.0±0.5
B. cereus (B1)	12.0±1.4	18.0±0.7	15.0±0.1
B. cereus (B2)	12.0±0.7	18.0±0.1	17.0±0.1
B. sublitis	15.0±1.4	0	16.0±0.7
E. coli ATCC 11775	18.0±0.7	15.0±1.4	18.0±1.0
Candida albicans (C1)	10.0±0.1	0	0
C. krusei	10.0±0.7	0	0

0 = No antagonistic activity detected.

Table 3. Residual activity of compounds after enzyme treatment.

Enzyme	Inhibition zone values (mm) mean ± S.D		
	AS_1	AS_2	KN_4
Trypsin	0	0	0
Pepsin	0	0	0
α-Amylase	22.0±2.9	20.0±0.9	22.0±0.6
Catalase	22.0±2.6	20.0±1.1	22.0±0.2
Control	22.0±2.8	20.0±0.7	22.0±0.5

0 = No activity detected.

was completely destroyed after treatment with proteolytic enzymes trypsin and pepsin. The activities were however, not affected by either α-amylase or catalase (Table 3).

Effect of heating on the activity inhibitory compounds

Results obtained after the partially purified inhibitory compounds heated at different temperatures indicate that these compounds were thermostable. There was no reduction in the residual activities of the various compounds, after heat treatment at 80°C for 30 min. However, thermostability of the compounds declined as the heating temperature was increased to 100°C for up to 15 min, and the variations in heat stability were significant ($P<0.05$). The activity of inhibitory compound produced by AS_2 was lost completely after heat treatment at 100°C for 15 min, while there was up to 60% reduction in the activity of the inhibitory compound from KN_4 after heating at 100°C for 15 min. The inhibitory compound obtained from AS_2 was the most stable to heat treatment, maintaining 100% activity after 15 min at 100°C.

Effect of pH on stability of inhibitory compounds

Each inhibitory compound was assayed for activity after 1 h incubation at different pH levels (pH 2.0-10.0). Data obtained indicates that pH affected the stability of the various inhibitory compounds differently (Table 4). The inhibitory compounds were shown to be stable between pH 5.0 and 8.0, (when activity at pH 7.0 was used as reference). However, there was approximately 33.4% decrease in the residual activity of the inhibitory compound produced by isolates KN_4 following treatment at pH 3.0, 4.0 and 9.0. Activity of this compound was completely destroyed following treatment at pH 2.0 and 10.0. Similarly, there was 40% reduction in the residual activity of inhibitory compound produced by isolates AS_2 following treatment at pH 4.0, while complete inactivation occurred at pH 2.0, 3.0, 9.0 and 10.0. The inhibitory compound produced by isolate AS_1, was stable between pH 3.0 to 8.0. Slight decrease in activity was detected at pH 2.0, 9.0 and 10.0. However, this decrease was not significant ($P>0.05$). Analysis of variance data confirm that stability of the inhibitory compounds produced by isolates AS_2 and KN_4 depended significantly on hydrogen ion concentration ($P<0.05$) as shown in Table 4.

DISCUSSION

This study was intended to isolate bacteriocin producing LAB from fermented traditional cereal based non-alcoholic beverages, and to test the capacity of partially

Table 4. The effect of pH on the stability of inhibitory compounds.

pH	Inhibition zone value (mm) mean ±S.D		
	AS_1	AS_2	KN_4
2.0	19.0±0.7	0	0
3.0	20.0±0.4	0	19.0±1.4
4.0	22.0±0.7	17.0±0.7	20.0±0.9
5.0	22.0±1.4	20.0±0.7	22.0±0.7
6.0	22.0±2.8	20.0±1.4	22.0 ±0.7
7.0 (control)	22.0±2.8	20.0±0.7	22.0±0.7
8.0	22.0±1.4	20.0±0.7	22.0±1.4
9.0	19.0±0.7	0	18.0±1.4
10.0	18.0±0.7	0	0

purified bacteriocins to antagonize food spoilage and pathogenic micro-organisms. This should serve as guide to the potential applicability of LAB and their elaborated inhibitory compounds in commercial biopreservation of foods, particularly those traditionally produced, improved or preserved using lactic fermentations. This should help base the application of these organisms in food presservation on empirical knowledge. The population of LAB on the tested foods was high. Kunun-zaki and akamu had populations of the order of 10^9 and 10^{10} cfu/g/ml, respectively. This is understandable considering that these beverages had undergone lactic fermentation as principal production step. Some strains produced antimicrobial principles that were shown to be bacteriocin.

The high incidence of bacteriocin producing LAB in these foods suggests that they may represent abundant source of potentially useful bacteria. A similar observation was made by Hartnett et al. (2002) in raw and malted cereals. LAB is also abundant contaminants in many other foods such as milk, meat and vegetables (Onda et al., 2002; Kalalou et al., 2004). The high incidence of LAB, including bacteriocinogenic strains in cereals has significant implications for the quality, safety and shelf life of these and related foods.

LAB have been reported to out-compete, and so inhibit other bacterial contaminants, resulting in improved quality and extended shelf life of products (Deegan et al., 2006). Many traditional African foods/ beverages including akamu and kunun-zaki are produced by fermention using LAB suggesting that the processes may result in improved quality and shelf life for food which may otherwise be plagued by problems such as inconsistent quality, poor hygiene and early spoilage. It was observed that partially purified bacteriocins produced by LAB isolates in this study inhibited *S. aureus, B. cereus, B. subtilis* and *E. coli.* The activities of the three inhibitory compounds were strongest against *S. aureus* ATCC 12600.

This is consistent with results reported for other bacteriocins (Schillinger and Lucke, 1987; De Martinis et al., 2001; Ogunbanwo et al., 2004). Tagg et al. (1976) suggested that bacteriocins usually have a narrow spectrum of activity inhibiting mostly Gram positive bacteria especially those closely related to the producer organism. The compounds produced by our isolates were also shown to be active against *E. coli* ATCC 11775. This observation is at variance with some earlier works that reported activity against only Gram positive organisms (Tagg et al., 1976; Schillinger and Lucke, 1987; Stevens et al., 1991). However, a few bacteriocins from LAB with activity against Gram-negative bacteria have also been reported. For examples, bacteriocin produced by strains of *Lactobacillus plantarum*, *Lactobacillus pentosus*, *Lactobacillus rhamnosus* and *Lactobacillus paracasei* isolated from boza, a Bulgarian traditional cereal beverage have been reported to be active against *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *E. coli* (Todorov and Dicks, 2006). Taken together, these results suggest that bacteriocins have a broader spectrum of activity than was previously reported and can inhibit even unrelated organisms.

The bacteriocin produced by isolate AS_1 in this study was active against *C. albicans* and *C. krusei*. This is potentially important for the exploitation of this isolate for food application. Although there are many reports on the production of antibacterial compounds by LAB (Megnusson and Schnurer, 2001; Lavermicocca et al., 2001), reports on bacteriocin inhibition of yeasts are comparatively few (Strom et al., 2002). Loss of antimicrobial property was observed after treatment of partially purified bacteriocin with proteolytic enzymes trypsin and pepsin, confirming the proteinacious nature of the active compounds. Similar observation was reported by Hartnett et al. (2002). Treatment of inhibitory compounds with catalase and α-amylase did not alter their activity, indicating that the inhibition recorded was not due to hydrogen peroxide and also that carbohydrate moieties if they exist in bacteriocins were not required for activity. Bacteriocins that do not contain or require carbohydrate for activity are classified into group I and II bacteriocins. Similar results were reported by Parente et al. (1996) and Todorov and Dick (2006). However, some researchers have reported slight inactivation by α-amylase for group IV bacteriocins. Such bacteriocins contain

a carbohydrate or lipid moiety that is necessary for activity (Klaenhammer, 1988; Shehane and Sizomore, 2002).The inhibitory compounds were shown to be stable over a broad pH range (pH 4.0 to 8.0) at room temperature. Similar observations on pH stability of bacteriocin have been reported (Hartnett et al., 2002; Shehane and Sizemore, 2002). The range of pH stability of these compounds is interesting in that, closely approximating the pH of lactic fermented foods, it can be expected to contribute to the safety and keeping quality of such foods. (Kunu-Nzaki and akamu have pH values in the range of 3.5 to 5.5 in this study). The inhibitory compounds were considerably heat stable, surviving treatment at 80°C for 30 min. Thermostability of bacteriocins have earlier been reported and been attributed to their small molecular sizes (Barefoot et al., 1992; Shehane and Sizemore, 2002). The thermostability of bacteriocins is of significance in their application in food systems, particularly if they are to be used together with pasteurisation in a multiple-hurdle approach to food preservation. However, the tolerance of bacteriocins to heat may be dependent on factors such as the level of purification, pH and other protective components (Barefoot et al., 1992) and these must be taken into account in designing applications for them in food. Based on standard microbiological and biochemical tests, the bacteriocinogenic isolates were tentatively identified.

The observed characteristics of the isolates were consistent with Bergey's Manual of Determinative Bacteriology (Holt et al., 1994) descriptions for LAB. The three isolates obtained from the present study showed great potential for exploitation in food preservation. They were identified as *L. plantarum* (AS_1), *L. brevis* (AS_2) and *L. delbruckii* (KN_4). *L. brevis* has been reported to produce Brevicin, a bacteriocin which is active against *Listeria innocuna*, *L. grayi*, *L. monocytogenes*, *Strept. thermophilus* and *Enterococcus faecalis* (Teixeira, 1999). There is also a report on the extension of shelf life of fufu, a cassava based product by *L. brevis* (Ogunbanwo et al., 2004). *L. brevis* is used as starters in the production of many foods including pickled vegetables and rye breads and is now industrially available in freeze-dried form for use as starter culture. It is convenient and quick to use these cultures to make sourdough breads (Teixeira, 1999). *L. plantarum* produces plantaricin, which has been reported to inhibit beer spoilage LAB and is used in the brewing industry to prevent beer spoilage (Vaunghan et al., 2006). *Lactobacillus delbruckii* is utilized in the production of yoghurt. Incorporation of strains of *L. delbruckii* during fermentation increases the thickening properties which are necessary in yoghurt with low or no fat (Teixeira, 1999).

Conclusion

The three bacteriocin producing isolates of lactic acid obtained from the present study show great potential for exploitation in food preservation. It is conceivable that they and similar LAB may find application in African and related fermented foods where long shelf life products may be achieved by selection of appropriate bacteriocinogenic LAB that produce preservatives *in situ* in the fermented products.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENT

OAN is grateful to the Federal Polytechnic, Mubi, Nigeria for scholarship and study leave.

REFERENCES

Adams MR, Moss MO (1997). Food Microbiology. The Royal Society of Chemistry, Cambridge. pp. 254-262.

Batt CA (1999). *Lactobacillus*: In Robsin, P.D. (ed) Encyclopaedia of Food Microbiology Vol. 2. Academic Press, New York. pp. 1134-1151.

Buddle BB, Hornbaeck T, Jacobson T, Barkhott V, Kochi AG (2003). *Leuconostoc carnosum* 4010 has the potential for use as a protective culture for vacuum-packed meats: Culture isolation, bacteriocin identification, and meat application experiments. Int. J. Food Microbiol. 83:171-184.

Cadirci BH, Citak S (2005). A comparison of two methods used for measuring antagonistic activity of lactic acid bacteria. Pakistan J. Nutr. 4(4):237-240.

Choi HJ, Lee HS, Her S, Oh, DH, Yoon SS (1999). Partial characterization and cloning of Leuconocin J, a bacteriocin produced by *Leuconostoc sp*. J_2 isolated from the Korean fermented vegetable kimchi. J. Appl. Microbiol. 86: 175-181.

De Martinis ECP, Publio MRP, Santarosa PR, Freitas FZ (2001). Antilisterial activity of lactic acid bacteria isolated from vacuum-packaged Brazilian meat and meat products. Br. J. Microbiol. 32:1-13.

Deegan LH, Cotter PD, Hill C, Ross P (2006). Bacteriocins: Biological tools for bio-preservation and shelf-life extension. Rev. Int. Dairy J. 16:1058-1071.

Garcia-Graells C, Valckx CA, Michiels CW (2000). Inactivation of *Escherichia coli* and *Listeria innocua* in milk by combined treatment with high hydrostatic pressure and the lactoperoxidase system. Appl. Environ. Microbiol. 66(10):41-4179.

Gould GW (1996). Industry perspectives on the use of natural antimicrobials and inhibitors for food applications. J. Food Prot. (Suppl):82-86.

Hartnett DJ, Vaughan A, van Sinderen D (2002). Anti-microbial – producing lactic acid bacteria isolated from raw Barley and sorghum. J. Inst. Brew. 108(2):169-177.

Holt JG, Krieg NR, Sneath PHA, Stanley JT, Williams ST (eds.) (1994). Bergey's manual of determinative bacteriology. The Williams and Wilkins Company/Baltimore: USA. pp. 566-568.

Kalalou I, Faid M, Ahami AT (2004). Extending shelf life of fresh minced camel meat at ambient temperature by *Lactobacillus delbrueckii* subsp. delbrueckii Electronic J. Biotechnol. 7(3):0717-3458.

Kalchayanand N, Sikes T, Dunne CP, Ray B (1994). Hydrostatic pressure and electroporation have increased bactericidal efficiency in combination with bacteriocins. Appl. Environ. Microbiol. 60(11):4174-4177.

Klaenhammer JR (1988). Bacteriocin of lactic acid bacteria. Biochimie 70:337-349.

Lowe DP, Arendt EK (2004). The use and effects of lactic acid bacteria

in malting and brewing with their relationship to antifungal activity, mycotoxins and gushing: Rev. J. Inst. Brew. 110(3):163-180.
Moreno I, Lerayer ALS, Leitao MFF (1999). Detection and characterization of bacteriocin-producing *Lactococcus lactis* Strains. Rev. Microbiol. 30:130-136.
Nettles CG, Barefoot SF (1993). Biochemical and genetic characteristics of bacteriocins of food-associated lactic acid bacteria. J. Food Prot. 56:338-356.
Ogunbanwo ST, Sanni AI, Onilude AA (2004). Effect of bacteriocinogenic *Lactobacillus spp* on the shelf life of fufu, a traditional fermented cassava product. World J. Microbiol. Biotechnol. 20:57-63.
O'Keeffe T, Hill C (1999). Bacteriocins: Potential in food preservation. In Robinson, R.K., Batt, C.A. and Patel, P.D. (eds) Encyclopaedia of Food Microbiology, Vol. 1, Academic Press, New York. pp. 183-196.
Onda T, Yanagida F, Uchimura T, Tsuji M, Ogino S, Shinohara T, Yokotsuka K (2002). Widespread distribution of the bacteriocin-producing lactic acid cocci in miso-paste products. J. Appl. Microbiol., 92:695-705.
Oxoid (1982). The Oxoid manual of culture media, ingredients and other laboratory services. Turnergraphic Ltd, Basingstoke, England. pp. 209-210.
Savadogo A, Quattara CAT, Bassole HN, Traore AS (2004). Antimicrobial activities of lactic acid bacteria strains isolated from Burkina Faso fermented milk. Pakistan J. Nutr. 3(3):174-179.
Schillinger U, Lucke FK (1987). Identification of lactobacilli from meat and meat products. Food Microbiol. 4:199-208
Shehane SD, Sizemore RK (2002). Isolation and preliminary characterization of bacteriocins produced by *Vibrio vulnificus*. J. Applied Microbiol. 92:322-328.
Soomro AH, Masud T, Anwaar K (2002). Role of lactic acid bacteria (LAB) in food preservation and human health. Review, Pakistan J. Nutr. 1(1):20-24.
Strom K, Sjogren J, Broberg A, Schnurer J (2002). *Lactobacillus plantarum* MiLAB 393 produces the antifungal cyclic dipeptides CYClo (L-Phe-L-Pro) and Cyclo (L-Phe-trans-4-OH-L-Pro) and 3-phenyllactic acid. Appl. Environ. Microbiol. 68(9): 4322-4327.
Tagg JR, Djani AS, Wannamaker LW (1976). Bacteriocins of Gram-positive bacteria. Review, J. Bacteriol. 40: 722-756.
Toora S (1995). Partial purification and characterization of bacteriocin from *Yersinia pestis*. J. Appl. Bacteriol. 78: 224-228.
Vaughan A, Rouse S, Van Sinderen D (2004). Investigating the antimicrobial efficiency of the Lactococcal bacteriocin for the development of Microbiologically stable beer. J. Inst. Brew., 110(3): 181-188.