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Ecology and Antibacterial Potential of Lactic Acid Bacteria Associated with Fermented Cereals and Cassava

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Abstract: The ecology and the antibacterial potential of lactic acid bacteria associated with the fermentation of cereals and cassava, during the production of *ogi* and *fufu* were reported. Whereas the concentration of the Lactic Acid Bacteria (LAB) increased in all the substrates throughout the 96h fermentation period, the total bacteria reduced by about 29.3% in *fufu*, 20.3 and 37.84% in the maize *ogi* and sorghum *ogi* respectively after 72 h fermentation. Thirteen strains of LAB were isolated in all with the species of *Lactobacillus* predominating. The LAB cells constituted between 3.63 to 43.1%, 7 to 48.5% and 3.8 to 62.6% of the total bacteria concentration in *fufu*, maize *ogi* and sorghum *ogi*. The cells of *Leuconostoc lactis, Lactobacillus fermentum*, *L. casei* and *Pediococcus acidilacti* were isolated early during fermentation and therefore regarded as the natural flora of the food substrates. Only nine of the LAB isolates exhibited inhibitory activity against the indicator organisms. The bacteriocins produced by *Lactobacillus brevis* and *L. casei* were very active against *Escherichia coli*, *Salmonella* Group B and *Shigella flexneri*, thus showing great potential as bio-preservative against some food- borne pathogens.

Key words: Bacteriocin, cereals, cassava, ecology, submerged fermentation, *fufu*, lactic acid bacteria, *ogi*

INTRODUCTION

Fermented foods are largely consumed in Africa where they constitute a bulk of the diet. These include *ogi*, a fermented maize or sorghum paste and *fufu*, a product of fermented cassava tuber in Nigeria, *uji* in Kenya, *mahewu* in South Africa and *kenkey* in Ghana. They are desirable because of the advantages of improved flavour, texture, nutritive value and digestibility over the unfermented ones (Aderiye and Adebayo, 1999). However, these food products are plagued with the problem of reduced shelf life due to chance inoculation by spoilage organisms and the humid conditions in the tropics (Olukoya *et al*., 1993; Aderiye *et al*., 2006). Attempts had been made to reduce this effect by the use of starter cultures rather than allow spontaneous fermentation by the natural flora.

Lactic Acid Bacteria (LAB) are the main organisms associated with the fermentation of foods. They had been known to produce antimicrobial substances such as organic acids, diacetyl, hydrogen peroxide and bacteriocin (Mathieu *et al*., 1993; Bonade *et al*., 2001). Many studies tend to isolate LAB that possess antimicrobial activity as starter cultures (Hugas *et al*., 1995; Herero *et al*., 1996). In contrast to many antibiotics, bacteriocins are regarded to be safe additives in foods (Garver and Muriana, 1993). Thus bacteriocin-producing LAB have been studied extensively and used as starter cultures for food preservation (Schillinger *et al*., 1995). Isolation of an array of bacteriocin-producing LAB is a necessary step to extend the shelf life of African fermented foods.

Unlike synthetic chemicals, cells of LAB have no known adverse effects. Hence many consumers prefer their use for bio preservation of food. The use of LAB or their antibacterial compounds to control food-borne pathogens and spoilage organisms has been well documented by Seuk-Hyun and

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Cheol, (2000) and Ogunbanwo *et al*. (2004) to mention a few. Among the numerous bacteriocins produced by the LAB, only nisin from *Lactococcus lactis* has had practical use in food processing to date (Hansen, 1994). There is the need therefore to investigate bacteriocin production from other LAB cells, especially those from indigenous fermented foods in Nigeria. This paper therefore reports on the ecology and the antibacterial potential of the lactic acid bacteria associated with the fermentation of cereals and cassava tubers during the production of *ogi* and *fufu*, respectively.

MATERIALS AND METHODS

Source of Raw Food Materials

Clean and healthy maize (*Zea mays*) and guinea corn (*Sorghum bicolor*) grains used in this study were purchased from a retail market in Ado-Ekiti, Nigeria, while the cassava (*Manihot esculentus*) tubers were obtained from the Department of Agriculture, Federal Polytechnic, Ado-Ekiti.

Fermentation of Cereal and Cassava tuber-production of *ogi* and *fufu*

The submerged fermentation method was employed in the production of *ogi* and *fufu*. For *ogi*, white maize (*Zea mays*) and guinea corn (*Sorghum bicolor*) grains were washed and separately steeped in water and allowed to ferment for four days by the natural flora of the grains and some chance inoculants. After fermentation, the grains were drained, wet-milled into slurry in a grinding mill and sieved through a fine mesh (0.2 mm). The chaff was discarded and the resulting starch paste at the bottom of the container was the *ogi*. The paste obtained from the sorghum grains was designated as the sorghum *ogi* while that from the maize grains was designated the *ogi*.

For *fufu*, the fresh cassava tubers were peeled and cut into pieces. The cassava tissues were also soaked in water to ferment for 4 days at ambient temperature. The soft fermented cassava tissues were mashed manually and later passed through a plastic sieve (1 mm). The fibres were discarded and the thick paste (mash) that settled under the water was the *fufu*.

Chemical Analysis

The fresh and fermented food substrates were separately analysed for ash, crude fibre, pH and protein as described by Pearson *et al.* (1981). The AOAC (1990) method was employed in determining the reducing sugar content and the total titratable acidity of the foods.

Isolation and Enumeration of Lactic Acid Bacteria

Lactic acid bacteria were isolated and enumerated using the De Mann, Rogosa and Sharpe agar [(MRSA-35 g L^{-1}): Peptone (10 g); Yeast extract (5.0 g); K_2HPO_4 (2.0 g); $NaNO_3$ (4.0 g); $MnSO_4$ (50 mg); $MgSO_4$ (2.0 g); $(NH_4)_2 CO_3$ (2.0 g); Tween (0.1 mL); Lab M powder (10.0 g); and distilled water (1L)]. While Nutrient agar [(NA-28 g L^{-1}): Peptone (10.0 g); NaCl (3.0 g); minced beef (5 g); agar (10 g) and DW (1L)] was used to enumerate the total viable bacteria. The media were prepared according to the manufacturers' instruction and sterilized by autoclaving at 121°C and 1.05 kg cm^{-2} for 15 min.

One gram of each food sample was comminuted in 9 mL of sterile 0.1% peptone water, shaken vigorously for a minute and serially diluted. One milliliter of each sample was plated in duplicates using the pour-plate method on MRSA and NA plates. The NA plates were incubated aerobically at 30°C for 24-48 and the MRSA plates anaerobically (Gaspak Jar; BBL, USA) at 37°C for 48 h. Colonial growth on the plates were enumerated and recorded after 24 h while the representative LAB cells were also purified by streaking on MRSA. Strains were characterized using the API 50CH strips and API 50CHL medium (API Systems, Biomerieux Sa, France) Each LAB species was later stored on MRSA slopes at 4°C until when needed.

Source and cultivation of Indicator Prganismsn indicator organisms were used in this study. The microbes include *Bacillus subtilis, Lactobacillus casei, L. plantarum, L. bulgaricus and Staphylococcus aureus* which were isolated from various food sources. *Shigella flexneri* and Salmonella Group B were

obtained from the International Centre for Tropical Diseases, Republic of Bangladesh (ICRDB). *Escherichia coli* and *Pseudomonas aeruginosa* were obtained from the American Type Culture Collection Center (ATCC) and the University College Hospital (UCH) Ibadan respectively.

All the organisms were stored on appropriate agar slopes and whenever required, the LAB strains were cultivated on MRSA, the *Salmonella* Group B cells and *Shigella flexneri* on Salmonella and Shigella agar, *Staph aureus* on Mannitol Salt agar and *B. subtilis*, *P. aeruginosa* and *E. coli* on Nutrient agar.

Preparation of Cell-Free Culture Supernatant (CFCS)

Each LAB isolate was cultured anaerobically (Gaspak Jar, BBL, USA) in MRS broth for 72h at 30°C in a shaking incubator. The culture extracts were obtained by centrifugation at 3,000 g for 15 min. The supernatants were decanted and adjusted to pH 7.0 with NaOH (1 mol L^{-1}), to eliminate any effect of acidity (Mathieu *et al*., 1993). Inhibitory activity from hydrogen peroxide was eliminated by the addition of a catalase (5 mg mL^{-1}, C-100 bovine liver, Sigma) (Daba *et al*., 1993) and filter-sterilized using a 0.2 μm pore size filter (FP 030/3 Schleicher and Schuell, GmbH, Dassel, Germany). The CFCS was stored at 4°C for further experiments.

Screening for Antagonistic Activity

The agar-well diffusion method was employed in the screening of the LAB cells for antagonistic activity. Indicator lawns were prepared with 40 mL of appropriate agar seeded with 100 μL of an overnight culture of known concentration of each indicator organism. Wells were cut into the agar with a sterile 5 mm diameter cork borer and sealed with 2 drops of sterile agar. Fifty microlitre of the Cell Free Culture Supernatant (CFCS) of each potential producer strain was placed into each well.

The plates, prepared in triplicates, were kept at 4°C for 2h (Bonade *et al*., 2001) to prevent pre-diffusion, then incubated at 30°C for 24-48 h after which they were examined for probable clearing of zones. The antimicrobial activity was determined by measuring the diameter of the inhibition zones around the well. Inhibition was described as weak, strong and very strong, respectively when the diameter is < 5 mm, 5-9 mm and > 10 mm.

Statistical Analysis

All the experiments were carried out in triplicates and repeated twice. The results were tested for significant differences by the Duncan's multiple range tests.

RESULTS AND DISCUSSION

The occurrence and growth of the chance inoculants got to the peak after 72h fermentation of the cassava mash (1.33×10^{7} cfu g^{-1}), the maize grain (1.63×10^{7} cfu g^{-1}) and the sorghum grain ($1.85\times^{7}$ cfu g^{-1}), respectively (Table 1). There was a reduction of the Total Aerobic Bacterial Load (TBC) by about 29.3% in *fufu* and 20.3% in the maize *ogi* while 37.84% of the microbes were affected in the sorghum *ogi* by the 96th h fermentation. These observations could be due to an increase in the acidity and the anaerobic condition of t he fermenting medium, and/or the inhibitory effect of antimicrobial products from the Lactic Acid Bacteria (LAB) on the growth of these organisms (Mataragas *et al*., 2002).

Anaerobiosis increased because of the low oxygen tension and the production of carbon (iv) oxide by hetero-fermentative organisms which occurred during the submerged fermentation. And this could only favour the growth of facultative anaerobes and/or aciduric organisms (Brock and Madigan, 2001). Inhibition of the growth of other microorganisms is a common phenomenon during lactic acid fermentation. This is a desirable effect because the growth of these anaerobic bacteria could produce an unpleasant flavour in fermented foods (Aderiye and Adebayo, 1999).

LAB cells have been reported to release toxic substances such as hydrogen peroxide, diacetyl, organic acid and bacteriocins into the medium during food fermentation (Skytta *et al*., 1993;

Herero *et al*., 1996). These antimicrobials have been used to control the growth of pathogens (Hugas *et al*., 1995; Ogunbanwo *et al*., 2004) and spoilage organisms (Olsen *et al*., 1995; Santos *et al*., 1996; Sanni *et al*., 1997) in foods. They adduced the reduction in the population of the non-lactics to the presence of the indigenous organisms which are responsible for fermentation and also play an additional inhibitory role, thus creating an environment that is unfavourable to food-pathogens and spoilage organisms.

The result obtained in this study is in agreement with the reports of Svanberg *et al.* (1992) and Ogunbanwo *et al.* (2004) who observed a reduction in the survival of the enteropathogens in fermented cereal gruels and cassava mash, respectively. Similarly, Olsen *et al.* (1995) reported a reduction in the bacterial load of steeped maize from 10^6 to 10^2 cfu g^{-1} after 24 h fermentation and was attributed to an interaction between the microbial flora of the indigenous fermented foods and the total bacterial counts of the non-lactics.

The concentration of the LAB cells constituted between 3.63 to 43.1%, 7 to 48.5% and 3.8 to 62.6% of the total bacteria count in *fufu*, maize *ogi* and sorghum *ogi* respectively (Table 1). Generally, there was an increase in the growth of the lactic acid bacteria in each of the substrates throughout the fermentation period. This is not surprising as LAB have been reported as the major organisms involved in the fermentation of *fufu* and *ogi* (Akinrele, 1984; Kunene *et al*., 1989; Olasupo *et al*., 1997; Steinkraus, 1996). LAB occur widely as indigenous contaminants during the natural fermentation of African foods (Odunfa, 1987). Similarly their growth is favoured by the low pH of the fermenting medium as observed in this study. LAB generally prefer an acidic medium for growth. These organisms are usually found on plant materials and associated with the fermentation of indigenous foods (Frazier and Westhoff, 2002).

There was no significant difference ($p \leq 0.5$) between the growth rates of the LAB cells found in the foods. Generally, the TBC and the LAB counts are higher in the *ogi* products than in *fufu*. This could be attributed to availability of some easily metabolizable nutritional components of 'the fermenting cereal grains essential for the growth of microbes (Table 2). For example, the values recorded for the ash and the reducing sugar contents of the grains are higher indicating the presence of high mineral nutrients and metabolizable sugar in the grains, while the crude fibre content of the cassava mash was too high to encourage rapid degradation even by other groups of microbes besides the LAB

Table 1: The pH, TTA, total and lactic acid bacteria counts of cassava mash, maize and sorghum grains during fermentation

	Fermentation priod (h)					
Food substrate/ Component	0*	24	48	72	96	Growth rate (cfu h^{-1})
Cassava mash						
LAB	0.04	0.40	1.26	1.62	4.05	0.02
TBC	1.10	2.70	12.00	13.30	9.40	0.17
LAB (%)	3.63	14.80	10.50	12.20	43.10	
pH	6.10	5.40	4.30	4.00	3.80	
TTA (%)	1.10	1.50	2.00	3.20	3.80	
Maize grain						
LAB	0.42	1.00	1.40	3.50	6.30	0.04
TBC	6.00	11.00	12.80	16.30	13.00	0.14
LAB	7.00	9.10	11.00	21.50	48.50	
pH	6.80	6.50	5.30	9.50	4.10	
TTA (%)	0.46	0.74	1.80	1.96	2.13	
Sorghum grain						
LAB	0.30	1.30	2.40	3.10	7.20	0.04
TBC	8.00	10.00	16.60	18.50	11.50	0.25
LAB (%)	3.80	13.00	14.50	16.75	62.60	
pH	6.60	5.10	4.10	3.60	3.40	
TTA (%)	0.62	1.31	2.80	3.92	4.20	

*Readings taken 1 h after soaking the food substrate in water, for fermentation. LAB: Lactic acid bacteria count (x 10^6cfu g^{-1}). TBC: Total aerobic bacteria count (x 10^6 cfu^{-1} g). %LAB: Percentage of LAB cells in the total bacteria count, TTA: Total Titratable acidity

Table 2: Some nutritional components* of fermented cassava mash, maize and sorghum grains

Component (%)	Cassava mash	Maize grains	Sorghum grains
Ash	0.15	0.52	0.73
Crude fibre	1.42	0.03	0.05
Protein	2.21	11.20	10.06
Reducing sugar	0.02	1.58	2.33
Carbohydrate[1]	86.80	21.00	24.20

*Readings taken after 96 h of submerged fermentation. [1]Carbohydrate was determined by difference

cells. Oyewole and Ogundele (2001) also reported an increase in the fibre content of *fufu* as fermentation progressed. Also the inhibitory effect of hydrogen cyanide in the fermenting mash could delay the rapid proliferation of the organisms (Okafor, 1997). The small particle size of the fermenting grains could also encourage easy access and denaturing of the soluble fermentable component than in the bigger cassava pieces. During the study on the optimization of submerged cassava fermentation to process control, Oyewole (1990) reported that the size to which the tubers were cut affected the rate of fermentation.

Table 1 also showed that the % LAB value recorded in the sorghum grain after 96 h fermentation was higher than the values obtained for the maize and cassava samples. This observation could be due to the differences in the concentration and the species of LAB that produced inhibitory substances in the various food substrates (Table 3). Hence, the occurrence of bacteriocinogenic LAB strains was higher in the sorghum *ogi* (Table 4).

In all cases, the pH of the food substrates decreased during fermentation. There also seems to be a corresponding inverse relationship between the growth of the LAB cells and the pH but a parallel relationship exists with the TTA values of the medium during fermentation (Table 1). This is because the organic acids released into the medium caused a lowering of the pH. The production of organic acids during food fermentation has been attributed to the activities of the LAB on the carbohydrates of the food substrates resulting into an increased acidity of the fermenting medium (Aderiye and Adebayo, 1999; Oyewole and Ogundele, 2001). The increase in the number of LAB cells as the pH dropped was not surprising as the LAB had been reported to predominate at low pH (Tahara *et al*., 1996; Soomro *et al*., 2001; Frazier and Westhoff, 2002).

The occurrence and sequence of isolation of the different LAB cells are shown in Table 3. The cells of *Leuconostoc lactis* and *Lactobacillus fermentum* were, respectively isolated from the cassava mash and the maize grains shortly (1 h) after retting the substrates under water. Interestingly, these microbes were frequently isolated from their respective substrates throughout the fermentation period. The fact that these LAB strains were isolated shortly after retting suggests that they were present in the food substrate prior to fermentation. As such, the food substrates were regarded as the natural habitats of these organisms. The predominance of *Leuconostoc* species in the fermenting cassava confirms earlier reports (Okafor, 1997; Onyekwere, 1989; Oyewole and Odunfa, 1990) that these microbes usually predominate during cassava fermentation. Similarly, previous studies had also shown that naturally fermented cereal-based African foods are dominated by *Lactobacillus fermentum* (Oyewole, 1990; Sanni *et al*., 1994).

The occurrence of LAB in the sorghum grains was noticed after 24h fermentation, when six different strains of the LAB cells were isolated. Most of the LAB isolates (84.61%) were obtained from the fermenting cassava, maize and sorghum grains after 48 h. The presence of these organisms in the medium and the substrate throughout the period of fermentation confirms their involvement in the fermentation process. The cells of *Leuconostoc brevis* TRF 2 and *Lactobacillus delbrueckii* TOR 6 were isolated from the cassava mash and sorghum grain, respectively after 72 h fermentation (Table 3). The lateness in the detection of these microbes may be due to either their existence in the medium at low levels, inability to compete with other LAB species or inability to grow at pH values lower than 4.0. Another reason may be that they do not constitute part of the natural flora of the food substrates but were introduced into the medium from the environment with time, since fermentation was by chance inoculation. This however does not preclude that they did not participate in the fermentation process.

Table 3: Source* and sequence of isolation of LAB strains in the fermenting substrates

Code	Identified organism	Isolation period
* Cassava mash (*fufu*)		
TRF 1	*Leuconostoc lactis*	a, b, c, d, e
TRF 2	*Lactobacillus plantarum*	c, d, e
TRF 3	*Lactobacillus fermentum*	c, d, e
TRF 4	*Leuconostoc carnosus*	d, e
* Maize grain (white *ogi*)		
TOW 1	*Lactobacillus fermentum*	a, b, c, d, e
TOW 2	*Leuconostoc mesenteroides*	c, d, e
TOW 3	*Lactobacillus bulgaricus*	c, d, e
*Sorghum grain (> red *ogi*)		
TOR 1	*Lactobacillus casei*	b, c, d, e
TOR 2	*Pediococcus acidilacti*	b, c, d, e
TOR 3	*Lactobacillus plantarum*	c, d, e
TOR 4	*Lactobacillus lactis*	c, d, e
TOR 5	*Lactobacillus brevis*	c, d, e
TOR 6	*Lactobacillus delbrueckii*	d, e

Fermenting substrate; cassava (*fufu*), maize (white *ogi*) and sorghum (red = *ogi*), a, b, c, d, e, Period LAB cells were isolated i.e., 1, 24, 48, 72 and 96 h fermentation, respectively

Table 4: Inhibitory effect* of CFCS of lactic acid bacteria isolates on the indicator organisms

			Lactic acid bacteria isolates						
Indicator organisms	Source	Designated No.	*Leuconostoc lactis* TRF 1	*Leuconostoc plantarum* TRF 2	*Lactobacillus fermentum* TRF 3	*Lactobacillus sarosum* TRF 4	*Leuconostoc fermentum* TOW 1	*Leuconstoc* mesenter oides TOW 2	*Lactobacillus bulgaricus* TOW 3
B. subtilis	Food	MD12	+(9.6)	+(14)	-	+(6.8)	-	-	+(8.0)
S. flexneri	ICDRB	AP23498	+(3.9)	+(3.3)	-	+(5.0)	-	-	+(3.5)
E. coli	ATCC	ATCC25922	+(10)	+(7.3)	-	+(9.0)	-	-	+(10)
Salmonella Group B	ICDRB	AP22096	-	-	-	-	-	-	-
Lactobacillus casei	Food	TOR 1	+(11.2)	+(10)	-	+(12)	-	-	+ (15.2)
L. plantarum	Food	TOR 3	+(2.5)	+(5.8)	-	+(11.1)	-	-	+ (11.5)
L. plantarum	Food	TRF 2	+(5.9)	-	-	-	-	-	+ (8.6)
L. bulgaricus	Food	TOW 3	+(6.0	+(5.7)	-	+(6.0)	-	-	-
S. aureus	Food	MD 20	-	+6.0	-	+(11)	-	-	+ (6.0)
P. aeruginosa	Clinical	UCH 4	-	-	-	-	-	-	-
Total No. of organisms inhibited			7	7	0	7	0	0	7

Table 4: Continued

			Lactic acid bacteria isolates						
Indicator organisms	Source	Designated No.	*Lactobacillus casei* TOR 1	*Pediococcus acidilactici* TOR 2	*Lactobacillus plantarum* TOR 3	*Lactobacillus Lactis* TOR 4	*Lactobacillus brevis* TOR 5	*Lactobacillus delbrueckii* TOR 6	LABI*
B. subtilis	Food	MD12	+(12)	-	+(10)	+(8.9)	+(7.2)	+(9.6)	9
S. flexneri	ICDRB	AP23498	+(4.9)	-	+(5.6)	+(5.0)	+(4.0)	+(3.5)	9
E. coli	ATCC	ATCC25922	+(9.0)	-	+(7.3)	+(7.2)	+(10)	+(9.0)	9
Salmonella Group B	ICDRB	AP22096	+(12)	-	-	-	+(15)	-	2
Lactobacillus casei	Food	TOR 1	-	-	+(10)	+(12)	+(11)	+(12.5)	8
L. plantarum	Food		+(12.1)	-	-	-	+(12.5)	+(11.5)	6
L. plantarum	Food	TRF 2	+(6.7)	-	+(8.0)	+(5.9)	-	+(8.6)	7
L. bulgaricus	Food	TOW 3	+(5.8)	-	+(5.7)	+(5.6)	+(9.0)	+(5.7)	8
S. aureus	Food	MD 20	+(7.0)	-	+(6.2)	+(9.2)	+(11)	+(72)	8
P. aeruginosa	Clinical	UCH 4	-	-	-	-	-	-	-
Total No. of organisms inhibited			8	0	7	7	8	8	

ATCC= American Type Culture Collection. ICDRB = International Centre for Tropical Disease, Republic of Bangladesh. LABI* = The number of LAB isolates that inhibited the growth of the indicator organism, s* = Inhibition zone (mm). - = No inhibition. + = Inhibition

Over 69% of the LAB isolates showed inhibitory activities against 90% of the indicator organisms used (Table 4). Most of the bacteriocin producing isolates inhibited at least 70% of the indicator organisms, thus exhibiting a broad spectrum of inhibitory activity. Seuk-Hyun and Cheol (2000) reported that some bacteriocins produced by a number of LAB strains exhibited a broad spectrum of activity. The percentage of bacteriocinogenic LAB reported in this study was higher than those

observed by previous authors in similar studies. The percentages of the LAB isolates that showed inhibitory activity against at least one of the indicator strains as reported by Mathieu *et al.* (1993), Olukoya *et al.* (1993), Sanni *et al.* (1999) and Ogunbanwo *et al.* (2003) were less than 1, 4, 63 and 65, respectively.

Auto-inhibition was not observed among the isolates. For instance, antibacterial compounds produced by *L. plantarum* TRF 2 isolated from the cassava mash inhibited the growth of *L. plantarum* TOR 3 isolated from the fermented sorghum grain but was not inhibitory to its own cells, a notable characteristic of bacteriocin producing cells (Tagg *et al.*, 1976).

Three of the four Gram-negative indicator organisms investigated in this study were inhibited by the antibacterial compounds obtained from the LAB isolates (These compounds are yet to be fully characterized). Present result is in contrast with the report of Mathieu *et al.* (1993) who observed that Mesenterocin 52 obtained from *Leuconostoc mesenteroides* was non-inhibitory to the entire Gram negative indicator organisms used. Bacteriocins obtained from all the isolates produced inhibitory zones against *Escherichia coli* ATCC 25922 and *Shigella flexneri* AP 23498. Furthermore, the bacteriocins from *L. casei* TOR 1 and *L. brevis* TOR 4 produced strong inhibitory zones of 12 and 15 mm, respectively when charged against Salmonella Group B AP22096. This is interesting as the LAB had been known to inhibit mainly the Gram-positive organisms (Skytta *et al.*, 1993).

The cell walls of Gram-negative bacteria have been reported to contain an extra layer, the lipopolysaccharide that is impermeable to the bacteriocin molecules (≥ 3 kDa) (Klaenhammer, 1993). Kalchayanand *et al.* (1992) however reported that bacteriocins could affect Gram negative organisms when subjected to a sub-lethal injury. Bacteriocin production could have resulted from stimulation by the presence of the competing microorganisms either within their diffusion-linked activity domains or by direct physical contact of the cell surfaces (Earnshaw *et al.*, 1990).

Pseudomonas aeruginosa was not inhibited by any of the antibacterial compounds obtained from the LAB isolates. This microbe was reported to possess an array of exo-enzymes which confer resistance against any attacking/invading organisms (Schlossberg, 1980) and to most antiseptics and drugs (Onile *et al.*, 1985; Adebayo and Famurewa, 2002). The spectrum of activity varied among the different LAB species (Table 4). *L. casei* was the best indicator organism exhibiting the widest inhibitory zone (15.2 mm) when charged with *L. bulgaricus* while *Shigella flexneri* was the least sensitive to most of the LAB isolates. Klaenhammer (1993) reported that different LAB species synthesize bacteriocidal agents that vary in their spectra of activity.

The cells of *L. plantarum* obtained from the fermented sorghum grains and the cassava mash displayed similar inhibitory activities on the indicator organisms; thus indicating that inhibition is not substrate but species - dependent. Incidentally, the cells of *L. fermentum* obtained from the cassava mash and the maize grains do not show inhibitory activity against any of the indicator organisms. This is in line with earlier findings (Sanni *et al.*, 1999). In contrast however, our study showed that *L. delbrueckii* produced some inhibitory effects on the test organisms used.

All the bacteriocinogenic LAB cells obtained from the sorghum grains exhibited strong inhibitory activities (i.e., zones between 5 and < 9 mm) against the indicator organisms. This probably accounted for the high reduction in the concentration of the total bacteria cells after 72 h fermentation and subsequently, a more than 3 fold increase in the % LAB. Ogunbanwo *et al.* (2004) reported that the amount and type of bacteriocins produced during the fermentation process usually influenced the microbial activity in the fermenting foods.

The antibacterial compounds obtained from *L. casei* TOR 1 and *L. brevis* TOR 5 showed great potential as bio-preservative against some food-borne pathogens used as test organisms in this study. In view of this and their broad spectra of activities further assay of the LAB strains could potentiate their use as starter cultures of fermented foods. This is more imperative in the developing countries where fermented food products have short shelf life due to chance inoculation and the activities of spoilage organisms, which are more encouraged by the tropical conditions.

In conclusion this study demonstrated that LAB isolates and the antibacterial compounds produced effectively reduced the growth of aerobic bacteria in the fermenting medium and inhibited the growth of some indicator organisms. Thus suggesting that these LAB cells could be used as starter cultures in fermented foods.

REFERENCES

Adebayo, C.O. and O. Famurewa, 2002. Antimicrobial Effectiveness of in-house-use concentration of disinfectants in a Nigerian Hospital. J. Technosci., 6: 17-20.

Aderiye, B.I. and C.O. Adebayo, 1999. Fermentation of Yam. II. Effect of the use of starter cultures on the physico-chemical properties of yam and its product. J. Technosci., 3: 1-5.

Aderiye, B.I., S.A. Laleye and H.A. Akinduro, 2006. Spoilage of some stored fermented foods in South West Nigeria. J. Biol. Sci., 6: 659-663.

Akinrele, J.A., 1984. Fermentation of Cassava. J. Sci., Food Agric., 15: 589-594.

AOAC, 1990. Official Methods of Analysis. 12th Edn., Association of Official Analytical Chemists, pp: 774-778.

Bonade, J.A., A.J. Dagnan and M.J. Garver, 2001. Production of helveticin from lactobacillus helveticus. Lett. Applied Microbiol., 33: 153-158.

Brock, T.D. and M.T. Madigan, 2001. The Biology of Microorganisms. 7th Edn., Prentice Hall International Inc., London.

Daba, H., C. Lacroix, J. Huang and R.E. Simard, 1993. Influence of growth conditions on production and activity of Mesenterocin 52 by a strain of *Leuconostoc mesenteroides*. Applied Microbiol. Biotechnol., 39: 166-173.

Earnshaw, R.G., J.G. Banks, C. Francotte and D. Pefrise, 1990. Inhibition of *Salmonella typhimurium* and *Escherichia coli* in infant milk formulated by an activated lactoperoxidase system. J. Food Protection, 53: 170-173.

Frazier, W.C. and D.C. Westhoff, 2002. Food Microbiology. 4th Edn., Tata McGraw-Hill Book Co. Ltd., New Delhi, pp: 538.

Garver, K.I. and P.M. Muriana, 1993. Detection, Identification and characterization of bacteriocin-producing lactic acid bacteria from retail food products. Int. J. Food Microbiol., 19: 241-258.

Hansen, J.N., 1994. Nisin as a Model Food Preservative. Criti. Rev. Food Sci. Nutr., 34: 69-93.

Herero, M., B. Mayo, B. Gonzalek and J.E. Suarez, 1996. Evaluation of technologically important traits in Lactic acid bacteria isolated from spontaneous fermentation. J. Applied Bacteriol., 81: 565-570.

Hugas, M., M. Garriga, M.I. Aymerich and J.M. Monfort, 1995. Inhibition of *Listeria* in dry fermented sausages by the bacteriocinogenic *Lactobacillus sakei* CTC 494. J. Applied Bacteriol., 79: 536-540.

Kalchayanand, N., M.B. Hanlin and B. Ray, 1992. Sub-lethal injure makes Gram-negative and Gram-positive bacteria sensitive to the bacteriocins, pediocin AcH and Nisin. Lett. Applied Microbiol., 15: 239-243.

Klaenhammer, T.R., 1993. Genetics of bacteriocins produced by lactic acid bacteria. FEMS Microbiol. Rev., 2: 39-86.

Kunene, N.F., J.W. Hastings and A. VonHoly, 1989. Bacterial Populations associated with a sorghum-based fermented weaning cereal. Int. J. Food Microbiol., 49: 75-83.

Mataragas, M., J. Metoxopoulos and E.H. Drosinos, 2002. Characterization of two bacteriocins produced by Leuconostoc mesenteroides. L124 and *Lactobacillus curvatus* L442, isolated from dry fermented sausages. World J. Microbiol. Biotechnol., 18: 847-856.

Mathieu, F., S. Sudirman, N. Rekhif, J.B. Milliere and G. Lefebvre, 1993. Mesenterocin 52, a bacteriocin produced by *Leuconostoc mesenteroides* subspecies mesenteroides FR 52. J. Applied Bacteriol., 74: 372-379.

Odunfa, S.A., 1987. A review of African fermented foods. J. Food Agric., 1: 179-183.

Ogunbanwo, S.T., A.I. Sanni and A.A. Onilude, 2003. Characterization of bacteriocin produced by *L. plantarum* F1 and *L. brevis* OGI. Afr. J. Biotechnol., 2: 219-227.

Ogunbanwo, S.T., A.I. Sanni and A.A. Onilude, 2004. Effect of bacteriocinogenic *Lactobacillus* sp. on the shelf life of *fufu*, a traditional fermented cassava product. World J. Microbiol. Biotechnol., 20: 57-63.

Okafor, N., 1997. Microorganisms associated with cassava fermentation of *gari* production. J. Applied Bacteriol., 42: 279-283.

Olasupo, N.A., D.K. Olukoya and S.A. Odunfa, 1997. Assessment of a bacteriocin-producing *Lactobacillus* strain in the spoilage of a cereal-based-African fermented food. Folia Microbiologica, 42: 31-34.

Olsen, A., M. Halm and M. Jakobsen, 1995. The antimicrobial activity of lactic acid bacteria from fermented maize (kenkey) and their interactions during fermentation. J. Applied Bacteriol., 79: 506-512.

Olukoya, D.K., P.S. Tichazek, A. Butsch, R.F. Vogel and W.P. Hammes, 1993. Characterization of the bacteriocins produced by *Lactobacillus pentosus* DK 8 isolated from *Ogi* and *L. plantarum* DK9 isolated from *fufu*. Chemie Microbiologie Technologie der Lebensmittel., 15: 65-68.

Onile, B.A., T.O. Odugbemi and C. Nwafor, 1995. Antibiotic susceptibility of bacterial agents of septicemia in Ilorin. Nigeria Medical Practitioner, 9: 14-18.

Onyekwere, J.A., 1989. Indigenous fermented foods involving an acid fermentation In: Industrialization of Indigenous Fermented Foods. Steinkraus, K.H. (Ed.), pp: 390-405.

Oyewole, O.B., 1990. Optimization of cassava fermentation for *fufu* production: Effect of single starter cultures. J. Applied Bacteriol., 68: 49-54.

Oyewole, O.B. and S.A. Odunfa, 1990. Characterization and distribution of lactic acid bacteria in cassava retting during *fufu* production. J. Applied Bacteriol., 68: 145-152.

Oyewole, O.B. and S.L. Ogundele, 2001. Effect of length of fermentation on the functional characteristics of fermented cassava *fufu*. J. Food Technol. Africa, 6: 38-40.

Pearson, D., H. Egan, R.S. Kirst and R.C. Suryer, 1981. Pearson's Chemical Analysis of Foods. 8th Edn., Churchill, Edinburgh.

Sanni, A.I., A.A. Onilude, S.T. Ogunbanwo and S.I. Smith, 1999. Antagonistic activity of bacteriocin produced by *Lactobacillus* species from *ogi*, an indigenous fermented food. J. Basic Microbiol., 3: 189-195.

Santos, J.A., T. Lopez-Diaz, M.C. Garcia-Fernandez, M.L. Garcia-Lopez and O. Otero, 1996. Effect of lactic culture on the growth and protease activity of *Aeromonas hydrophila*. J. Applied Bacteriol., 80: 13-18.

Schillinger, U., M. Kaya and F.K. Lucke, 1991. Purification, partial characterization and plasmid-linkage of pediocin SJ01, a bacteriocin produced by Pediococcus acidilactici. J. Applied Bacteriol., 74: 67-77.

Schlossberg, D., 1980. Multiple nodules as a manifestation of *Pseudomonas aeruginosa* septicemia. Arch. Dermatol., 116: 446-447.

Seuk-Hyun, K. and A. Cheol, 2000. Bacteriocin production by *Lactococcus lactis* KCA 2386 isolated from White Kimchi. Food Sci. Biotechnol., 9: 263-269.

Skytta, E., A. Haikara and T. Mattila, 1993. Production and characterization of antibacterial compounds produced by *Pediococcus damnosus* and *Pediococcus pentosaceus*. J. Applied Bacteriol., 74: 18-25.

Soomro, A.H., T. Masud and K. Anwaar, 2002. Role of lactic acid bacteria in food preservation and human health: A review. Pak. J. Nutr., 1: 20-24.

Steinkraus, K.H., 1996. Indigenous Handbook of Fermented Foods. 2nd Edn., Marcel Dekker, New York.

Svanberg, V., E. Sjogren, W. Lorri, A.M. Suennerholm and B. Kaijser, 1992. Inhibited growth of common enteropathogenic bacteria in lactic-fermented cereal gruels. World J. Microbiol. Biotechnol., 8: 601-606.

Tahara, T., M. Oshimura and K. Kanatani, 1996. Mode of action of acidocin 8912, a bacteriocin produced by *Lactobacillus acidophilus* TK8912. Lett. Applied Microbiol., 23: 192-198.

Tagg, J.R., A.S. Dajani and L.W. Wannamaker, 1976. Bacteriocins of Gram-positive bacteria. Bacteriological Rev., 40: 722-756.