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Full Length Research Paper

In vitro antimicrobial characteristics of bacteriocin-producing *Lactobacillus* strains from Nigerian indigenous fermented foods

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A total of 50 bacteriocin-producing *Lactobacillus* strains isolated from some Nigerian indigenous fermented foods and beverages (*ogi*, *fufu*, *garri* and *nono*) and characterized as *L. acidophilus*, *L. casei*, *L. fermentum*, *L. lactis* and *L. plantarum* were screened for their inhibitory potentials against food-borne pathogenic indicator bacterial isolates; *Acinetobater* sp., *Alkaligenes* sp., *Enterobacter aerogenes*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Shigella flexneri*, from the same or similar fermented food sources, and against clinical indicator bacterial isolates and type cultures- *Bacillus subtilis* NCTC8236, K12 *Escherichia coli* V157, NCTC11560, *Vibrio* INABA *B. cereus* CIS25, CIS32, *B. licheniformis* CIS26, *Pseudomonas aeruginosa* CIS23, *Klebsiella aerogenes* CIS24, *Kleb. pneumoniae* CIS29V and *Kleb. aerogenes* CIS55. It was observed that each fermented food had its own microbial interaction with minimal *in vitro* inhibitory activity (1.5 – 10.0%) by the bacteriocin-producing *Lactobacillus* strains against the indicator bacterial isolates from the fermented foods and beverages, indicating narrow to moderate antimicrobial spectrum; while the inhibitory profiles against the clinical bacterial isolates and the type cultures by the putative strains were between 75.0 – 100.0%. The effect of different pH on the antimirobial potentials of the *Lactobacillus* strains indicates highest inhibitory activities between 5.5 and 7.5. The survival rates of the pathogenic indicator bacteria in the fermented food sources were between 8 and 14 days while the clinical isolates survived in simulated fermented food samples between 5 and 9 days.

Key words: Bacteriocin, fermented foods, food-borne pathogens, indicator isolates, *Lactobacillus*.

INTRODUCTION

Bacteriocins are antimicrobial peptides that are produced by some microbes such as lactic acid bacteria and that have great potential as biopreservatives for food (Gravesen et al., 2004). The bacteriocin–producing strains may be used as protective cultures to improve the microbial safety of foods (Buckenhuskes, 1993; Brinkten et al., 1994; Gonzalez et al., 1994, Olasupo et al., 1997), and they also play an important role in the preservation of fermented foods, which is usually achieved by inhibition of contaminating spoilage bacteria such as *Pseudomonas* and pathogens such as *Staphylococcus aureus*, *Salmonella spp*., and *Listeria monocytogenes* (Reinheimer et al., 1988, El- Gazzar and Marth, 1992, Vignolo et

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Table 1. Recovery rates of total and faecal coliforms from the analyzed indigenous fermented foods.

Fermented food sample	No of sample	Total coliform (37^{0}C)	Faecal coliform (45^{0}C)	Mean coliform counts / cfu ml^{-1} (log cfu ml^{-1})
Fufu	56	55 (98.2%)	46 (82.1%)	3.5 x 10^4 – 6.1 x 10^4 (4.54-4.79)
Garri	60	50 (83.3%)	42 (70.0%)	1.6 x10^4 – 4.3 X 10^4 (4.20-4.63)
Nono	50	42 (84.0%)	38 (76.0%)	3.1 x 10^4 - 5.4 x 10^4 (4.49- 4.73)
Ogi	62	58 (93.5 %)	53 (85.5 %)	5.4 x 10^4- 7.1 x 10^4 (4.73- 4.85)
Total	228	205 (89.9%)	179 (78.5%)	

al., 1993, Chiang et al., 2000).

There is a growing consumer demand for processed food products containing lower levels or no chemical presservatives, leading to indigenous research studies in the field of screening bacteriocin as food preservatives. However, most of these indigenous research studies have always attempted to access the effectiveness of bacteriocin-producing lactic acid bacteria strains that were isolated from certain African fermented foods or beverages in the microbial control of spoilage organisms in some other fermented foods, or the (inappropriate) usage of clinical pathogens as the indicator organisms in accessing the effectiveness of the bacteriocin-producing lactic acid bacteria strains. The aim of the current study therefore, is to compare and confirm the inhibitory properties of bacteriocin-producing *Lactobacillus* strains from food sources against food-borne spoilage strains from same or similar fermented food sources; and pathogens of clinical significance.

MATERIALS AND METHODS

Sampling

Two hundred and twenty eight indigenous fermented food samples were obtained from different sellers in three major state capitals in Nigeria-Lagos, Abeokuta and Ibadan between May 2002 and October 2004.

Bacterial strains

A total 50 bacteriocin-producing *Lactobacillus* strains were isolated from *fufu*, *garri*, *nono* and *ogi* - African fermented food products from cassava, cow milk and maize, respectively. The selective isolation of the bacteriocin-producing *Lactobacillus* strains were according to the methods of Tagg et al. (1976), Hachure et al. (1990) and the modified method of Ogunshe (2004). The indicator isolates were from similar spoilt fermented foods and clinical isolates from Department of Medical Microbiology and Parasitology, University College Hospital, Ibadan, Nigeria. Identification studies were according to the conventional taxonomic methods while the confirmatory identities were according to Sharpe (1962, 1979), Buchanan and Gibbons (1974), Kandler and Weiss (1986) and Sneath et al. (1986).

Determination of antimicrobial activities

Sterile assay media were seeded with the indicator organisms by streaking the entire surface of the culture plates- brain-heart infusion agar, Mueller-Hinton agar, nutrient agar, tryptone soy infusion agar (LAB M, Topley House, Wash Lane, Lancashire, Bury, UK), MacConkey agar (Oxoid, Basingstoke, Hampshire, England), and incubating the culture plates at ambient temperature for 3 h. Holes 6 mm in diameter were aseptically punched out of the agar plates, and then, 250 – 1000 µl modified MRS broth cultures (containing 2.5g^{-1} bacteriological agar powder) of the putative organisms were separately introduced into the holes or spotted (modified method of Ogunshe, 2004) onto the surfaces of pre-poured culture plates and incubated aerobically and anaerobically at 35°C for 24 h. After overnight incubation, inhibitions observed by clear zones extending laterally from the border of the putative isolates were noted and recorded in mm diameter (Tagg and McGiven, 1971, Tagg et al., 1976, Elaine et al., 1994).

Effect of different pH on inhibitory activity

The effect of different pH (5 - 9) on the inhibitory potential of the *Lactobacillus* strains was determined. The *Lactobacillus* strains were cultured in the fermented food pastes adjusted with 1 N HCl and 1 N NaOH and antimicrobial productions were recorded as zones of inhibition in mm.

RESULTS

The mean pH of the retail fermented foods were 6.8 (*ogi*), 6.2 (*nono*), 6.2 (*fufu*), and 7.0 (*garri*) respectively while the mean pH for the fermented food samples under controlled fermentation in the laboratory were 5.4 for *ogi*, 5.3 for and *garri* and 5.2 for both *nono* and *fufu*. The recovery rates of coliforms from the fermented foods are as shown in Table 1.

The indicator spoilage/pathogens isolated from the coliform-positive indigenous fermented foods were characterized as *Escherichia coli* (63.8%), *Alkaligenes* sp. (10.6%), *Proteus mirabilis* (8.5%), *Enterobacter aerogenes* (8.5%) *Pseudomonas aeruginosa* (4.3%), *Acinetobater* sp. (2.1%), *Shigella flexneri* (2.1%) and *Klebsiella pneumoniae* (0.2%). *Enterobacter aerogenes*, *Escherichia coli* and *Pseudomonas aeruginosa* were isolated from *fufu*; *Alkaligenes* sp., *Proteus mirabilis* and *Escherichia coli* were isolated from *ogi*; *Acinetobater* sp., *Escherichia coli* and *Shigella flexneri were* isolated from

Table 2. Survival rates of the indicator bacterial isolate from microbially analyzed indigenous fermented foods.

Recovery rates (Days)	TPA 1 2 3 4 5 6	TPB 1 2 3 4 5 6	TPC 1 2 3 4 5 6	Food sample
1 – 5*	+ + + + + +	+ + + + + +	+ + + + + +	F, N, O, G
6 – 7*	+ + - + + +	+ + + + + +	+ + + + + +	N, O, F
8 – 12*	+ + - + - -	+ - - + + -	++ + + + +	N, O
13 - 14*	+ + - - - +	+ - - + - -	++ - + + +	N. O.

Keys: TPA= room temperature (uncontrolled), TPB= room temperature (controlled), TPC = refrigeration temperature (4^0C).
*Presence of moulds.
1 = *Escherichia coli*, 2 = *Proteus aerogenes*, 3 = *Alkaligenes* sp. /*Acinetobacter*, 4 = *Klebsiella pneumoniae*, 5 = *Pseudomonas aeruginosa*, 6 = *Enterobacter* sp.
F = *fufu*, N = *nono*, O = *ogi*, G = *garri*.

Table 3. Percentage *in vitro* phenotypic antibiotic resistance profiles of the indicator bacterial isolates.

Antibiotics	Antibiotics Conc. (µg)	(%) Antibiotic resistance of bacterial isolates				
		Clinical		Food-borne		
		Field (n=14)	Hospital (n=12)	*Fufu* (n=16)	*Ogi* (n=22)	*Nono* (n=10)
Ampicillin	25	100.0	52.9	69.8	86.3	90.0
Cotrimoxazole	25	100.0	58.6	12.6	50.0	30.0
Gentamicin	10	90.0	29.4	12.5	36.3	10.0
Nalidixic acid	30	100.0	70.6	75.0	54.5	60.0
Nitrofurantoin	300	100.0	47.1	50.9	59.0	30.0
Tetracycline	30	100.0	47.1	50.9	55.5	60.0

n = number of strains tested; values are means of duplicates.

Table 4. *In vitro* inhibitory profiles of bacteriocin-producing *Lactobacillus* strains against food-borne bacterial pathogens.

Sources of *Lactobacillus* strains	Food-borne pathogens inhibited			
	Ogi	*Garri*	*Nono*	*Fufu*
Ogi	1/66 (1.5)	1/66 (1.5)	5/132 (3.8)	6/88 (6.8)
Garri	0/66 (0.0)	0/60 (0.0)	0/120 (0.0)	0/80 (0.0)
Nono	3/30 (10.0)	0/30 (0.0)	4/6 (6.7)	3/40 (7.5)
Fufu	1/48 (2.1)	1/48 (2.1)	5/96 (5.2)	1/64 (1.6)

Values in parenthesis are %.

nono while only *Escherichia coli* and *Klebsiella pneumoniae* were isolated from *garri*. The survival rates of the indicator pathogens from fermented foods and beverages with time were as presented in Table 2.

The Gram-negative pathogenic field isolates exhibited 100.0% resistance towards ampicillin, cotrimoxazole, nalidixic acid, nitrofurantoin, and tetracycline while the overall resistance profiles among the Gram-negative clinical (hospital) isolates towards the test antibiotics were between 29.4-70.6%. The antibiotic resistance profiles among the Gram-negative indicator strains isolated from the fermented foods and beverages were between 2.5 and 90.0% (Table 3).

The *Lactobacillus* species isolated in this study were strains characterized as *Lactobacillus acidophilus* from *ogi, fufu, nono* and *garri*; *L. casei* from *ogi* and *fufu*; *L. fermentum* from *ogi and fufu*; *L. lactis* from *nono* and *ogi* and *L. plantarum* from, *nono, ogi* and *fufu*. All the bacteriocin-producing *Lactobacillus* strains had between low (12.0 - 15.0 mm) and moderate (16.0 - 28.0 mm) inhibittory zones towards the food-borne pathogens from the same food source(s) as compared to the mean inhibitory

Table 5. Inhibition zones (cm) of bacteriocin-producing *Lactobacillus* strains against food-borne bacterial pathogens in mm diameter.

Bacteriocin–producing *Lactobacillus* strains	Food-borne pathogens inhibited			
	Fufu	***Nono***	***Ogi***	***Garri***
Fufu				
L plantarum FM5	-	1 (20.0)	-	-
L. acidophilus FM22	-	1 (20.0)	1 (20.0) –	-
L. casei SFM32	-	2 (20.0, 20.0)	-	-
L. fermentum SFM2	2 (20.0, 24.0)	1 (23.0)	-	-
Ogi				
L. plantarum OG2	1 (14.0)	-	-	-
L. lactis OG3	1 (22.0)	-	-	-
L. acidophilus OG4	-	1 (15.0, 17.0)	1 (20.0)	1 (10.0)
L. casei OG8	1 (14.0)	-	-	-
L. casei OG12	1 (22.0)	-	-	-
L. fermentum OW2	1 (10.0)	-	-	-
L. acidophilus SOW31	1 (15.0)	1 (20.0)	-	-
Nono				
L. lactis N3	-	1 (23.0)	1 (19.0)	-
L. plantarum N11	1 (23.0)	-	-	-
L. acidophilus SN1	-	-	-	1 (20.0)
L. acidophilus SN21	-	1 (15.0, 22.0)	-	-
L. acidophilus SN22	-	-	1 (23.0)	-
L. lactis SN3	1 (25.0)	-	-	-
L. plantarum SN42	1 (25.0)	-	1 (28.0)	-

< 10.0 – 15.0 mm in diameter = low susceptibility; 16.0 - 22.0 mm = moderate susceptibility; 23.0 mm >= high susceptibility.
*The values indicate the diameter of inhibition zones in mm using 500, 750 and 1000 µl of the lactobacilli culture in agar spot and agar well–diffusion on nutrient agar, tryptone soy agar, MacConkey agar, and Mueller-Hinton agar. Zones are means of triplicates.

activities of between moderate and high (16.0 -28.0 mm and 29.0 - 38.0 mm) in diameter inhibitory zones towards the clinical indicator organisms (Table 4–7).

The effect of pH on inhibitory effects by the bacteriocin-producing *Lactobacillus* strains using modified-MRS broth indicate that the highest antimicrobial productions were recorded mostly between pH 5.5 and 7.5 although there was no growth/survival of the *Lactobacillus* strains at pH 3 after 24 h incubation. The rate of antimicrobial production was indicated by increase in zones of inhibition (in diameter) from pH 5.5 to pH 7.5 followed by gradual decrease between pH 8.0 and 9.0.

DISCUSSION

Microbial food safety is an increasing public health concern worldwide (Zhao et al., 2001) and many Gram-negative bacteria such as *Escherichia coli*, *Salmonella* serovars; *Campylobacter* spp., *Enterobacter* spp. *Klesiella spp*. etc. have been implicated in food borne diseases (Mead et al., 1999). Similar report of Ogunshe et al. (2005) also isolated such Gram-negative bacteria of clinical importance from a fermented food condiment. It was also observed that most of the fermented foods microbially analysed in this study harbour multiple antibiotic resistant food indicator isolates as shown in (Table 3). Considering the fact that resistance to antimicrobial agents (AMR) has resulted in morbidity and mortality from treatment failures, and increased health care costs leading to public health risk (Lalitha, 2004), there is little doubt that emergent antibiotic resistance in food-borne isolates can become a serious global problem.

The identification carried out for representative *Lactobacillus* strains from the fermented food products demonstrated the dominance of *Lactobacillus acidophilus*, *L. casei L. fermentum L. lactis* and *L. plantarum*. These identified *Lactobacillus* species were in accordance with those earlier identified from similar fermented food products by Oyewole and Odunfa (1990), Halm et al. (1993), Hounhouigan et al. (1993, 1994), Johansson et al. (1995) and Teniola and Odunfa (1995).

In the study of Olasupo et al. (1995, 1997) assessed bacteriocin-producing *Lactobacillus* strains from fermented foods is active against enterotoxigenic *E. coli* of non-

Table 6. Inhibition zones of bacteriocin- producing *Lactobacillus* strains against Type cultures inmm diameter.

Bacteriocin-producing *Lactobacillus* strains	Type cultures				
	B. subtilis **NCTC**		***E. coli*** **NCTC**		***Vibrio* INABA**
	K12	**8236**	**V157**	**11560**	
Fufu					
L plantarum FM5	12.0	21.0	-	16.0	-
L. acidophilus FM22	18.0	-	18.0	22.0	28.0
L. casei SFM32	18.0	24.0	24.0	20.0	22.0
L. fermentum SFM2	-	18.0	22.0	18.0	-
Ogi					
L. plantarum OG2	21.0	24.0	28.0	24.0	-
L. lactis OG3	23.0	20.0	28.0	24.0	22.0
L. acidophilus OG4	20.0	26.0	24.0	25.0	-
L. casei OG8	-	28.0	22.0	18.0	25.0
L. casei OG12	18.0	26.0	24.0	-	26.0
L. fermentum OW2	18.0	-	26.0	30.0	24.0
L. acidophilus SOW31	18.0	35.0	24.0	**-**	22.0
Nono					
L. lactis N3	24.0	22.0	20.0	24.0	20.0
L. plantarum N11	-	24.0	-	22.0	–
L. acidophilus SN1	32.0	22.0	24.0	24.0	20.0
L. acidophilus SN21	28.0	18.0	-	23.0	22.0
L. acidophilus SN22	24.0	-	24.0	21.0	–
L. lactis SN3	18.0	18.0	28.0	20.0	24.0
L. plantarum SN42	18.0	24.0	28.0	24.0	28.0

< 10.0 – 15.0 mm in diameter = low susceptibility; 16.0 - 22.0 mm = moderate susceptibility; 23.0 mm >= high susceptibility.
*The values indicate the diameter of inhibition zones in mm using 500, 750 and 1000 µl of the lactobacilli culture in agar spot and agar well–diffusion on nutrient agar, tryptone soy agar, MacConkey agar, and Mueller-Hinton agar.
Zones are means of triplicates.

fermented food origin as well as clinical isolates of enterotoxigenic *E. coli*, enterohaemmorhagic *E coli*, *Aeromonas sobria*, *Aeromonas cavice*, *Staphylococcus aureus*, *Pleisiomonas shigelloides*, *Vibrio cholerae*, *Salmonella typhimirium*, *Serratia* and *Pseudomonas* sp. According to Sanni et al. (1999), the food spoilage and pathogenic bacteria also screened for antagonistic activity against bacteriocin produced by *Lactobacillus* species from *ogi* were obtained from the culture collection of Medical Microbiology, University College Hospital, Ibadan, Nigeria. Microbial interaction observed in such studies would definitely be quite different from those bioassay studies in which both the bacteriocin-producing *Lactobacillus* strains and the indicator organisms are from same or similar food source(s) as observed in this present study.

The inhibition zones of between 0.5–13.0 mm in diameter by the bacteriocin-producing *Lactobacillus* strains against the indicator organisms as reported by Sanni et al. (1999) however can only be classified as being between non-inhibition and moderate inhibition, indicating a relatively none or narrow antimicrobial spectrum. This finding may be supported by that of Piard and Desmazeaud, (1992) who stated that bacteriocins produced by *L. plantarum* and the heterofermentative lactic acid bacteria generally have a narrower antimicrobial spectrum than the bacteriocins from pediococci. This may also account for the high recovery rates of the indicator pathogens from the fermented foods even after ten weeks of storage.

Survival rates of the fermented food-borne indicator organisms inoculated into laboratory-prepared *ogi* preseeded with the bacteriocin-producing *Lactobacillus* strains showed inhibition of the various food-borne indicator isolates between 8 and 14 days (and above 14 days in some isolates), while the viability of the type cultures and clinical bacteria, *Pseudomonas aeruginosa* CIS23S, *Klebsiella aerogenes* CIS24S, *Bacillus cereus* CIS25S, *Bacillus licheniformis* CIS26S, *Klebsiella pneumoniae* CIS29V, *Bacillus cereus* CIS32S, *Citrobacter sp.* CIS55S, and four reference strains, *Bacillus subtilis* NCTC8236, *Escherichia coli* NCTC11560, *Escherichia coli* V157, *Vib-*

Table 7. Inhibition zones of bacteriocin- producing *Lactobacillus* strains against clinical bacterial pathogens in mm diameter.

Bacteriocin–producing *Lactobacillus* strains	*B. cereus*		*B. lich.*	*Ps. aer.*	*Kleb. aer.*	*Kleb. pn.*	*Citr.*
	25S	32S	26S	23S	24S	29V	55S
Fufu							
L plantarum FM5	16.0	25.0	22.0	20.0	22.0	25.0	32.0
L. acidophilus FM22	16.0	18.0	24.0	26.0	20.0	23.0	28.0
L. casei SFM32	22.0	-	28.0	24.0	28.0	22.0	26.0
L. fermentum SFM2	23.0	22.0	26.0	28.0	28.0	20.0	–
Ogi							
L. plantarum OG2	32.0	24.0	-	28.0	18.0	18.0	26.0
L. lactis OG3	28.0	-	35.0	28.0	32.0	22.0	22.0
L. acidophilus OG4	29.0	24.0	2 8.0	28.0	22.0	28.0	26.0
L. casei OG8	25.0	28.0	22.0	18.0	25.0	32.0	–
L. casei OG12	25.0	28.0	24.0	28.0	26.0	34.0	22.0
L. fermentum OW2	28.0	20.0	20.0	20.0	28.0	26.0	26.0
L. acidophilus SOW 31	-	18.0	20.0	28.0	32.0	29.0	22.0
Nono							
L. lactis N3	26.0	26.0	22.0	28.0	26.0	20.0	20.0
L. plantarum N11	22.0	22.0	26.0	26.0	22.0	26.0	22.0
L. acidophilus SN1	26.0	28.0	28.0	24.0	-	29.0	23.0
L. acidophilus SN21	20.0	23.0	30.0	33.0	22.0	-	21.0
L. acidophilus SN22	20.0	25.0	22.0	24.0	21.0	32.0	20.0
L. lactis SN3	26.0	24.0	28.0	26.0	24.0	28.0	24.0
L. plantarum SN42	-	28.0	28.0	29.0	28.0	29.0	22.0

< 10.0 – 15.0 mm in diameter = low susceptibility; 16.0 - 22.0 mm = moderate susceptibility; 23.0 mm >= high susceptibility.
*The values indicate the diameter of inhibition zones in mm using 500, 750 and 1000 µl of the lactobacilli culture in agar spot and agar well–diffusion on nutrient agar, tryptone soy agar, MacConkey agar, and Mueller-Hinton agar.
Zones are means of triplicates.

rio INABA inoculated into *ogi* and *nono* pre-seeded with the bacteriocin-producing *Lactobacillus* strains redu- ced significantly between 5 and 9 days of storage. The observed differences in the survival rates of the inocu-lated indicator organisms may be strongly determined by the environmental conditions of the fermented food samples, having pH of 5.2 - 6.8 which is more acidic as compared with the human physiological pH usually around neutral, which is the environmental condition of the clinical isolates.

A modified Charteris et al. (1998) *in vitro* simulated method of exposing the *Lactobacillus* strains to different pH, using HCl and HCl-modified MRS broth also showed that the inhibitory activities of the bacteriocin-producing *Lactobacillus* strains were more prominent between pH 5.5–7.5. This observation may be explained by the resea-rch findings which indicate that bacteriocins have been found to display antagonistic activity towards many food spoilage and food-borne pathogenic microorganisms including *Bacillus cereus*, *Clostridium botulinum*, *Cl. perfringes, Listeria monocytogens*, *Staphylococcus aur-eus*, *Shigella*, *Escherichia coli*, *Salmonella*, etc. (Park et al., 1973, Dicks et al., 1992, Pouwels et al., 1998, Jacob-sen et al., 1999, Chang et al., 2001, Walter et al., 2003, van der Kaaj et al., 2004). However, in few studies, strains of *Shigella flexneri* (Mensah et al., 1988, Nout et al., 1989), enterotoxigenic *Escherichia coli* (Mensah et al., 1991), *Salmonella sp*. (Nout et al., 1989; Foster and Hall, 1991) and *Staphylococcus aureus* (Anderson, 1986, Lewus et al., 1991) have been observed to resist or adapt to the growth inhibitory conditions in lactic-fermented products with a pH of 4.0 which may be due to strain or species variability.

In the study of kingamkono et al. (1995), a starter culture that was recycled to initiate the lactic fermentation process for inhibiting 28 strains of enteropathogens was found to accelerate the decrease and the production of organic acids as earlier reported by Daeschel et al. (1991), Nout et al. (1989), Anderson et al. (1990). The decrease in pH was to a critical level of 4.0 for the successful inhibition of the enteropathogens. This result of kingamkono et al. (1995) was similar to that of Nout et al. (1989) in terms of the growth inhibition of strains of *Salmonella*, *Shigella*. However, the rate of inhibition was faster in the earlier study because the pathogens were then inoculated into the fermented gruel when it was al-

ready at pH < 4. Therefore, the fact that indicator isolates were inhibited in studies carried out by previous workers like Olasupo et al. (1997) and Sanni et al. (1999) does not necessarily mean that the bacteriocin-producing *Lactobacillus* strains exclusively inhibited the food borne pathogens but that the pH of the food samples that were as low as 3.0 - 5.0 were probably detrimental to the cell membranes of the indicator isolates, which in any case were from clinical (different physiological environment with different pH) sources.

Although there is paucity of information describing the physiological mechanisms underlying the environmental–induced effect on the survival of human clinical pathogens in food environment, this can result in structural and physiological injury to the bacterial cells, resulting in substantial loss of viability. The investigation of Olsen et al. (1995) of a natural ecosystem has also shown that microbial developments are controlled by complex interactions, which are assumed to explain the microbial stability and safety of fermented maize, and similar traditional fermented foods in Africa and other parts of the world. The present study thus suggests the selection of bacteriocin-producing *Lactobacillus* strains as starter cultures for controlled fermentations from candidates targeted as indicator organisms from same or similar food source(s) instead of using clinical or exclusively type or reference cultures, since otherwise, may not fulfill the requirements for food safety and quality as also demonstrated by the lack of success in attempt to use lactic acid bacteria to control the growth of *Listeria monocytogenes* in foods by Mathieu et al. (1994).

Relative abilities of the indicator organisms to survive lethal acid conditions of the fermented food products through certain mechanisms may occur. The indicator organisms can acquire enhanced resistance to certain environmental conditions probably due to pre-exposure of the bacterial cells to such or similar food environmental conditions. Such resistance mechanisms play important roles by contributing to the survival against a variety of challenges imposed by the adverse conditions. Repetitive assays carried out on the food borne/spoilage pathogens have demonstrated that they possess certain inherent ability to adapt to unfavourable environmental conditions such as the presence of bacteriocin(s) in a food based environment probably by the induction of certain general or specific responses. The trend thus obtained in these results is in agreement with the report of Olsen et al. (1995) that, inhibitory effects varied for putative isolates belonging to same species or closely related group of *Lactobacillus* species, since varied inhibitory effects were obtained among the food borne pathogens and the clinical pathogens.

In the study of Olukoya et al. (1993) the selected *Lactobacillus* species were chosen as potential antagonistic strains to some diarrhogenic bacterial species based on their inhibitory effects on diarrhogenic bacterial isolates while the study of Ogunshe (2004) also selected the *Lactobacillus* species as potential probiotics based on their *in vitro* inhibitory effects on gasrtoenteritic bacterial isolates from paediatric sources.

Using the antibiotic susceptibility profiles of the food-borne and clinical indicator organisms as another criterion for bacteriocin assay, it was observed that among the Gram-negative indicator strains isolated from the fermented foods and beverages, there were more susceptible strains than those from clinical sources. This phenotypic characteristic indicates that the two sets of indicator organisms (food-borne and clinical) have different inherent genotypic characteristics that determine their inhibitory activities. It is confirmed by the result findings of this study that investigations into the bio-preservative potentials of bacteriocins in food samples must make use of food-borne indicator pathogens from same or similar food sources in bioassays of food related studies. Further studies to screen for bacteriocin-producing *Lactobacillus* species as selective starter cultures for the indigenous fermented foods are currently going on in our laboratories.

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REFERENCES

Anderson RE (1986). Inhibition of *Staphylococcus aureus* and spireroplast of Gram-negative bacteria by a strain of *lactobacillus plantarum.* Int. J. Food Microbiol. 3: 149-160.

Anderson RE, Erickson CE, Salomosson AC, Theander HM (1990). Lactic acid fermentation of fresh and stored carrot: chem. Microb. Sensory evaluation of products.

Brinkten B, Minekns M, Vossen MV, Leer RJ, Huis in't Veld JHJ (1994). Antimicrobial activity of lactobacilli. J. Appl. Bacteriol. 77: 140-148.

Buckenhuskes HJ (1993). Selection criteria for lactic acid bacteria to be used as starter cultures for various food commodities. FEMS Microbiol. Rev. 12: 253–272.

Buchnan RE, Gibbons NE (1974). Bergey's Manual of Determinative Bacteriology*,* 8th edition. Williams and Wilkins Co. Baltimore, USA.

Chiang BL, Sheih YB, Wang LH, Lino CK, Gill HS (2000). Enhancing immunity by dietary consumption of a probiotic lactic acid bacterium (*Bifidobacterium lactis* HN019) optimization and definition of cellular immune responses. Eur. J. Clin. Nutr. 54: 849-855.

Chang Y, Kim J, Kim H, Kim W, Kim Y, Park Y, Chang YH, Kim JK, Kim HJ, Kim WY, Kim YB, Park YH (2001). Selection of a potential probiotic *Lactobacillus* strain and subsequent *in vitro* studies. Ant. Leeuw. 80: 193-199.

Charteris WP, Kelly PM, Morelli L, Collins JK (1998). Antibiotic susceptibility of potentially probiotic Lactobacillus species. J. Food Protect. 61: 1636-1643.

Daeschel MA, McCKenney MC, McDonald IC (1991). Bactericidal activity of *Lactobacillus plantarum* C-11. Food Microbiol. 7: 91–98.

Dicks LMT, van Jaarsveld DE, van Vuuren HJJ (1992). Casein LHS, a broad-spectrum bacteriocin produced by *Lactobacillus casei.* Abstra-

cts and Books 7th Biennial Cong. S. Afr. Soc. Microbiol. p. 214.
Elaine E, Vaughan EC, Looney R, O'Rourke N, Coveney H, Daly C, Fitzgerald GF (1994). Isolation from food sources of lactic acid bacteria that produced antimicrobials. J. Appl. Bacteriol. 76: 118-123.
El-Gazzar RE, Marth EH (1992). Food borne-disease investigating procedures and economic assessment. J. Environ. Health. 55:24–27.
Foster JW, Hall HK (1991). Induced pH: Homeostasis and the acid tolerance response of *Salmonella typhimurium*. J. Bacteriol. 173: 5129-5135.
Gonzalez B Area, D Mayo B, Suarez JE (1994). Detection purification and partial characterization of plantarum c, a bacteriocin produced by a strain of *Lactobacillus plantarum* of diary origin. Appl. Environ Microbiol. 60: 2158 –2163.
Gravesen A, Kallipolitis B, HolmstrǾm K, HǾiby PE, Ramnath M, KnǾchel S (2004). *Pbp2229*-Mediated nisin resistance mechanism in *Listeria monocytogenes* confers cross-protection to class IIa bacteriocins and affects virulence gene expression. Appl. Environ. Microbiol. 70: 1669-1679.
Halm M, Lillie A, Srensen AK, Jakobsen M (1993). Microbiological and aromatic characteristics of fermented maize dough for *kenkey* production in Ghana. Int. J. Food Microbiol. 19: 135–143.
Hounhonigan DJ, Nout MJR, Nago CM, Houben JH, Rombouts FM (1993). Characterization and frequency distribution of species of lactic acid bacteria involved in the processing of *mawe*, a fermented maize dough from Benin. Int. J. Food Microbiol. 18: 115-125.
Hounhouigan DJ (1994). Fermentation of maize (*Zea mays* L.) meal for *mawe* production in Benin: physical, chemical and microbiological aspects. Ph.D. Thesis. Agric. Univ. Wageningen.
Jacobsen CN, Nielsen VR, Hayford AE, Moller PL, Michaelsen KF, Paerregaard A, Sandstrom B, Tvede M, Jakobsen M (1999). Screening of probiotic activities of forty-seven strains of *Lactobacillus spp.* by *in vitro* techniques and evaluation of the colonization ability of five selected strains in humans. Appl. Environ. Microbiol. 65: 4949-4956.
Johansson ML, Sanni AI, Lonner C, Mollin G (1995). Phenotypically based taxonomy using AP150CH of lactobacilli from Nigerian *ogi* and the occurrence of starch fermenting strains. Int. J. Food Microbiol. 25: 159-168.
Kandler O, Weiss N (1986). The Genus: *Lactobacillus*. P. 1208-1260. In Bergey's Manual of Systematic Bacteriology, vol.2, PHA. Sneath, NS Mair, ME Sharpe, JG Holt (eds.), Williams and Wilkins, Baltimore, MD. pp. 1209-1234.
Kingamkono R, Sjogren E, Svanberg U, Kajser B (1995). Inhibition of different strains of enteropathogens in lactic-fermenting cereal gruel. W. J. Microbiol Biotech. pp. 299-303.
Lalitha MK, Manayani DJ, Priya L, Jesudason MV, Thomas K, Steinhoff MC (1997). E test as an alternative to conventional MIC determination for surveillance of drug resistant *S. pneumoniae*. Indian J. Med Res.106: 500-503.
Lewus CB, Kaiser A, Montville TJ (1991). Inhibition of food borne bacterial pathogens by bacteriocins from lactic acid bacteria isolated from meat. Appl. Environ. Microbiol. 57: 1683-1688.
Mathieu F, Michel M, Lebrihl A, Lefebvre G (1994). Effect of the bacteriocin carnoich CP5 and of the producing strain Carnobacterium pisciola CP5 on the vialibility of *Listeria monocytogenes* ATCC 15313 in salt solution, broth and Skimmed milk at various incubation temperatures. Int. J. Food Microbiol. 22: 155–172.
Mead PS, Slutsker L, Dietz V, McCaige LF, Bresee JS, Shapiro C, Griffin PM, Tauxe RV (1999). Food related illness and death in the United States. Emerg. Inf. Dis. 5: 607–625.
Mensah PPA, Tomkins AM, Drasar BS, Harrison TJ (1988). Effect of fermentation of Ghanaian maize dough on the survival and proliferation of 4 strains of *Shigella flexneri*. Trans Roy. Soc. Trop. Med. Hyg. 82: 635-636.
Mensah P, Thomkins AM, Prasar BS, Harrisons TJ (1991). Antimicrobial effect of fermented Ghanian maize dough. J. Appl. Bacteriol. 70: 203–210.
Nout MJR, Rombouts FM, Havelaar A (1989). Effect of accelerated natural lactic fermentation of infant food ingredients on some pathogenic microorganisms. Int. J. Food Microbiol. 8: 351-361.
Ogunshe AAO (2004). Characterization and selection of *Lactobacillus* species as probiotics for the control of infantile bacterial gastroenteritis. PhD Microbiology, University of Ibadan, IBADAN, Nigeria.
Ogunshe AAO, Ayodele AE, Okonko O (2005). Microbial studies on *aisa*- a potential indigenous laboratory fermented food condiment from *Albiziz saman* (Jacq.) F. Mull Pak. J Nutr. 5(1): 51-58.
Ogunshe AAO, Olasugba OK, Adekoya BA (2005). Submitted
Olasupo NA, Olukoya DK, Odunfa SA (1995). Studies on bacteriocinogenic *Lactobacillus* isolates from selected Nigerian fermented foods. J. Bacteriol. Microbiol. 39: 181-186.
Olasupo NA, Olukoya DK, Odunfa SA (1997). Assessment of a bacteririocin-producing *Lactobacillus* strain in the control of spoilage of a cereal-based African fermented food. Folia microbiol. 42(1):31-34.
Olsen A, Halm M, Jackobsen M (1995). The antimicrobial activity of lactic acid bacteria from fermented maize (kenkey) and their interactions during fermentation. J. Appl. Bacteriol. 79:506–512.
Olukoya DK, Tichaczek PS, Butsch A, Vogel RF, Hammes WP (1993). Characterization of the bacteriocins produced by *Lactobacillus pentosus Lactobacillus plantarum* DK9 from *fufu*. Chem. *Mikrobial. Technol. Lebensm.* 15: 65 – 68.
Olukoya DK, Ebiwei SI, Olasupo NA, Ogunjimi AA (1994). Production of *DogikK* an improved *ogi* (Nigerian fermented weaning food) with potentials for use in diarrhoeal control. J. Trop. Paed. 40, 108-113.
Oyewole OB, Odunfa SA (1990). Microbiological studies on cassava fermentation for *lafun* production. Food Microbiol. 5: 125-133.
Piard JC, Desmazeaud M (1991). Inhibiting factors produced by lactic acid bacteria. 1. Oxygen metabolites and catabolism end products. Lait 71: 525-541.
Park HS, Marth EH, Olson NF (1973). Fate of enteropathogenic strains of *Escherichia coli* during manufacture and ripening of Camembert cheese. J. Milk Food Tech. 36: 543-546.
Piard JC, Desmazeaud M (1992). Inhibiting factors produced by lactic acid bacteria. 2. Bacteriocin and other antibacterial substances. Lait 72: 113–142.
Pouwels PH, Leer RL, Shaw M, den Bak-Glashouwer MJH, Tielen FD, Smith E, Martinez B, Jore J, Conway PL (1998). Lactic acid bacteria as antigen delivery vehicles for oral immunization purposes. Int. J. Food Microbiol. 41: 155-167.
Reinheimer JA, Candioti MC, Zalazar CA, Demkow MR (1988). Inhibizione died batteri coliformi con colturecommerciali di *Streptococcus thermophilus* e *Lactobacillus bulgaricus*. *Scienza e Tecnia Lattiero-Casearia* 39: 349-366.
Rogosa M, Wiseman RF, Mitchell JA Disraely MN, Beaman AJ (1953). Species differentiation of oral lactobacilli from man including descriptions of *Lactobacillus salivarus* nov. spec. and *L. cellobiosus* nov. spec. J. Bacteriol. 65: 681-699.
Sanni AI, Onilude AA, Ogunbanwo ST, Smith SI (1999). Antagonistic activity of bacteriocin produced by *Lactobacillus* species from ogi an indigenous fermented food. J. Basic Microbio. 39: 189–195.
Sharpe ME (1955). A serological classification of lactobacilli. J. Gen. Microbiol. 12: 107-122.
Sharpe ME (1962). Lactobacilli in meat products. Food Man. 37: 582-589.
Sharpe ME (1979). Identification of the lactic acid bacteria. In: Identificative methods for microbiologists. Skinner PA, Lovelock DW (eds),. Academic Press, London. pp. 233–259.
Sneath PHA, Mair NS, Sharpe ME, Hotts JG (1986). Bergeys Manual of Systematic Bacteriology, vol. 2. Baltimore, MD, Williams and Wilkins.
Tagg JR, McGiven AR (1971). Assay system for bacteriocins. Appl. Microbiol. 21: 943.
Tagg JR, Dajani AS, Wannamaker LW (1976). Bacteriocins of Gram-positive bacteria. Bacteriol Rev. 40: 722-756.
Teniola OD, Odunfa SA (1995). Indigenous microflora of *ogi* and their role as spoilage microorganisms. The third Biennial Seminar of African fermented foods, Ghana, pp. 71-76.
Van der Kaaij, H Desiere, F Mollet B, Germond J (2004). L-alanine aux-

otrophy of *Lactobacillus johnsonii* as demonstrated by physiological, genomic and gene complementation approaches. Appl. Environ. Microbiol. 70: 1869-1873.

Vignolo GM, Suriani F, de Ruiz Holgado AP, Oliver G (1993). Antibacterial activity of *Lactobacillus* strains isolated from fermented sausages. J. Appl. Bacteriol. 75: 344-349.

Walter J, Heng NCK, Hammes WP, Loach DM, Tannock GW, Hertel C (2003). Identification of *Lactobacillus reuteri* genes specifically induced in the mouse gastrointestinal tract. Appl. Environ. Microbiol. 69: 2044-2051.

Zhao C, Beilei G, Vllena J, Sudler R, Yeh E, Zhao S, White DG, Wagner D, Meng A (2000). Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella* serovars in retail chicken, turkey, pork and beef from the greater Washington D.C. area. Appl. Environ. Microbiol. 66: 5431 – 5436.