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Assessment of the antimicrobial activity of lactic acid bacteria isolated from two fermented maize products - *ogi* and *kunnu-zaki*

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ABSTRACT

The lactic acid bacteria (LAB) used in this study were isolated from two traditional fermented maize products- *ogi* and *kunnu-zaki.* The antimicrobial activities of the isolated LAB against some indicator organisms (*Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Proteus mirabilis and Bacillus cereus*) was investigated. The LAB population in the *ogi* samples ranged from 5.39 log cfu/g to 6.40 log cfu/g while that of *kunnu-zaki* ranged from 6.11 log cfu/g to 6.70 log cfu/g. The lactic acid bacteria isolated were identified as *Lactobacillus plantarum*, *L. fermentum*, *L. lactis*, *L. acidophilus, L. mesenteroides*, *L. casei*, and *Streptococcus thermophilus.* Cell free supernatant of the LAB was able to inhibit the growth of the indicator organism used. The largest zone of inhibition was 11 mm produced by *L. plantarum* against *Klebsiella pnuemoniae* while the least zone of inhibition was 3 mm produced by *L. acidophilus* against *E. coli.* The LAB tested also produced various antimicrobial compounds such as lactic acid, diacetyl and hydrogen peroxide and quantitative estimation of these antimicrobial compounds was carried out. The highest yield of lactic acid was 2.18 g/L produced by *S. thermophilus,* highest yield of diacetyl was 2.5 g/L produced by *L. plantarum* while the highest yield of hydrogen peroxide was 0.006 g/L produced by *L. plantarum.*

Keywords: antimicrobial, diacetyl, hydrogen peroxide, lactic acid bacteria and lactic acid

INTRODUCTION

Lactic acid bacteria (LAB) are widely distributed in nature, they are typically involved in a large number of the spontaneous food fermentation, and they have been extensively studied for their involvement in the production of many indigenous fermented foods (Holzapfel *et al.*, 1995). Some members of LAB produce bacteriocins and bacteriocins-like substances which may inhibit growth of spoilage and pathogenic microorganisms (Klaenhammer, 1988). Lactic acid fermentation of cereal-based foods is a traditional technology in Africa and has long been used in the processing of different foods (Mensah, 1997; Oyewole, 1997). Maize and sorghum are often used for preparation of most of these foods. Some serve as weaning or staple foods, while some are fermented alcoholic beverages (Umoh and Fields, 1981; Akobundu and Hoskins, 1982).

In Nigeria, *ogi* and *Kunnu-zaki* are two of the most common lactic acid fermented products popular with consumers. Consumption of these fermented foods has many advantages including enhanced nutritional value, digestibility, therapeutic benefits, and safety against pathogens (Oranusi *et al.*, 2003).

The traditional methods of preparation of *ogi* and *Kunnu-zaki* have been described and there is general agreement on the dominance and beneficial effects of lactic acid bacteria (LAB) in the fermentation processes of these foods (Odunfa, 1985; Teniola and Odunfa, 1995; Omemu *et al*., 2007).

The LAB contribute to the enhancement of the organoleptic attributes of these foods, as well as to their preservation and microbial safety (Hounhouigan *et al.,* 1993; Olsen, *et al.*, 1995; Caplice and Fitzgerald, 1999; Calderon *et al.*, 2001).

The primary antimicrobial effect exerted by LAB is the production of lactic acid and reduction of pH (Daeschel, 1989). In addition, LAB produce various antimicrobial compounds, which can be classified as low-molecular-mass (LMM) compounds such as hydrogen peroxide (H_2O_2), carbon dioxide (CO_2), diacetyl (2,3-butanedione), uncharacterized compounds, and high-molecular-mass (HMM) compounds like bacteriocins (Jay, 1982; Piard and Desmazeaud, 1992). All of which can antagonize the growth of some spoilage and pathogenic bacteria in foods. The antimicrobial-producing LAB may be used as protective cultures to improve the microbial safety of foods 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* (Buckenhuskes, 1993; Brinkten *et al.*, 1994; Olasupo *et al*., 1995).

The aim of the present study is to assess antimicrobial activity of some lactic acid bacteria strains isolated from traditional fermented maize products against some pathogens of clinical significance. Preliminary quantitative estimation of the different antimicrobial

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substances produced by each of the LAB species isolated from *ogi* and *kunnu-zaki* were also determined.

MATERIALS AND METHODS

Collection of samples and bacterial strains

Traditionally prepared samples of fermented *ogi* and *kunnu-zaki* were randomly obtained from five different markets in Abeokuta, South-west Nigeria. Samples were transported immediately to the laboratory for microbiological analysis.

The indicator isolates used were obtained from Department of Medical Microbiology and Parasitology, University College Hospital, Ibadan, Nigeria.

Isolation of lactic acid bacteria

Each sample (25 mL) was mixed with 225 mL of buffered peptone water (BPW, Oxoid) to obtain a 1: 10 dilution. Serial dilutions of the samples were prepared in 0.1 % peptone water. MRS agar was supplemented with 0.01 % sodium azide to inhibit the growth of Gram-negative bacteria. The diluted sample was streaked on the MRS agar plates and then incubated anaerobically at 30 °C for 48 h. Colonies were isolated, sub-cultured and purified by repeated streaking.

The morphological, physiological and biochemical examination of the isolates were determined by the standard procedure of Gram staining, catalase test and gas production test. Colonies of catalase negative, Gram-positive rods or cocci were presumed to be LAB. API 50 CHL stripes were used to identify all isolates to species level. Working cultures were prepared as slants on MRS agar for LAB or nutrient agar supplement for the indicators, and stored at 4 °C.

Determination of the antimicrobial activities of the LAB isolates

Cell-free culture supernatants for antibacterial assay was prepared by growing the LAB isolates in MRS broth at 37 °C and centrifuged at 12, 000 x *g* for 10 min. The antimicrobial activity of the cell-free culture supernatants of isolated LAB against the indicator organisms was determined by the agar well diffusion assay according to the method by Elaine *et al.*, (1994). Aliquots of supernatants (100 µL) were placed in wells (6 mm diameter) cut in cooled soft nutrient agar plates previously seeded (1 % v/v) with the appropriate indicator strains. The plates were incubated under optimal conditions for growth of the target microorganisms after which they were examined for clear zones around the wells. The diameters of the growth inhibition zones were measured and recorded in millimetre (mm). The indicator strains used in this study are *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis and Bacillus cereus.*

Determination of Lactic acid, hydrogen peroxide and diacetyl production by the LAB isolates

The test organisms were grown of MRS broth for 72 h and samples were taken at 12 h interval for Lactic acid, hydrogen peroxide and diacetyl production.

Hydrogen peroxide

Diluted sulphuric acid (25 mL) was added to 25 mL of the broth culture of the test organism. Titration was carried out with 0.1 N potassium permanganate each mL of 0.1 N potassium permanganate is equivalent to 1.070 mg of hydrogen peroxide. A disclourization of the sample was regarded as end point (AOAC, 1990).

Lactic acid

NaOH (0.1 N) was titrated against 25 mL broth culture of the organism using 3 drops of phenolphthalein as indicator. The NaOH was added until the colour changes to pink. Each millilitre of NaOH is equivalent to 90.08 mg of lactic acid (AOAC, 1990).

Diacetyl

Twenty five mL of broth cultures were transfered into conical flasks and 7.5 mL of hydroxylamine solution were used for the residual titration. Titration was done with 0.1 N HCl to a greenish end point using bromophenol blue as indicator. The equivalent factor of HCl to diacetyl is 21.5 mg (AOAC, 1990).

Statistical analysis

The data obtained were subjected to analysis of variance, mean differences determined by the least square difference (LSD) test and bivariate correlations determined using SPSS 10.0 for windows software.

RESULTS

Result presented in Table 1 shows the pH and LAB population in the *ogi* and *kunnu zaki* samples evaluated from the different markets. The pH of the *ogi* samples ranged from 2.87 to 3.67 while the pH of the *kunnu-zaki* samples ranged from 3.87 to 4.10. The LAB population in the *ogi* samples ranged from 5.39 log cfu/g to 6.40 log cfu/g while that of *kunnu-zaki* ranged from 6.11 log cfu/g to 6.70 log cfu/g.

The LAB species isolated from *ogi* samples were *Lactobacillus plantarum*, *L. lactis*, *L. casei*, *L. mesenteroides* and *L. acidophilus* while *Lactobacillus plantarum*, *L. fermentum*, *L. fermentum*, *L. lactis*, *L. mesenteroides* and *Streptococcus thermophillus* were obtained from *kunnu-zaki* samples (Table 2).

The indicator spoilage/ pathogens used in this study were *Staphylococcus aureus*, *E. coli*, *K. pneumonia*, *P. mirabilis* and *B. cereus.*

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he LAB species isolated from the fermented maize products were able to inhibit the tested indicator organisms with varied zone of inhibition. With the exception of *E. coli*, *L. plantarum* produced significantly (p<0.05) higher zone of inhibition against all the pathogens tested as compared to the other LAB tested. The largest zone of inhibition of 11 mm was produced by *L. plantarum* against *K. pneumonia* and this was significantly (p<0.05) higher than the least zone of inhibition of 3 mm produced by *L. acidophilus* against *E. coli. Lactobacillus acidophilus* and *L. fermentum* were not able to inhibit *Proteus* sp. since no zone of inhibition was observed.

Table 1: Mean pH and LAB population in *ogi* and *kunnu-zaki* samples.

Product	Market code	No. of samples	Mean pH	LAB population (log cfu/g)
OGI	MK1	10	3.02	5.39
	MK2	10	3.42	6.40
	MK3	14	3.25	6.23
	MK4	13	2.87	6.43
	MK5	9	3.67	6.36
KUNNU	MK1	11	4.07	6.15
	MK2	9	4.05	6.70
	MK3	8	3.91	6.53
	MK4	10	4.10	6.12
	MK5	9	3.87	6.11

Table 2: Lactic acid bacteria isolated from fermented *ogi* and *Kunnu-zaki*.

Product	Associated lactic acid bacteria
Ogi	*Lactobacillus plantarum*
	L. fermentum
	L. lactis
	L. mesenteroides
	L. casei
	L. acidophilus
Kunnu-zaki	*Lactobacillus plantarum*
	L. fermentum
	L. lactis
	L. mesenteroide
	Streptococcus thermophillus

Preliminary quantitative estimation of different antimicrobial substances produced by each of the LAB species isolated from *ogi* and *kunnu-zaki* was determined. The highest yield of lactic acid (2.18 g/L) was produced by *S. thermophillus* at 36 h while the lowest yield of 0.73 g/L was produced by *L. plantarum* at 12 h. *L. plantarum* produced the highest amount of hydrogen peroxide (0.006 g/L) at 12 h while *L. acidophilus* produced the lowest amount (0.0009 g/L) at 12 h. For diacetyl, the highest (2.5 g/L) was produced by *L. plantarum* at 24 h while the least (0.1 g/L) was produced by *L. lactis* at 60 h.

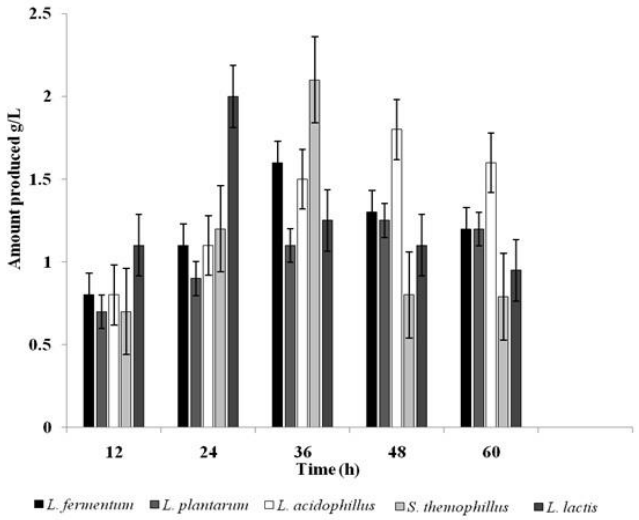

Figure 1: Amount of lactic acid produced by LAB isolates with time. Error bars represent standard errors of mean of triplicate determinations.

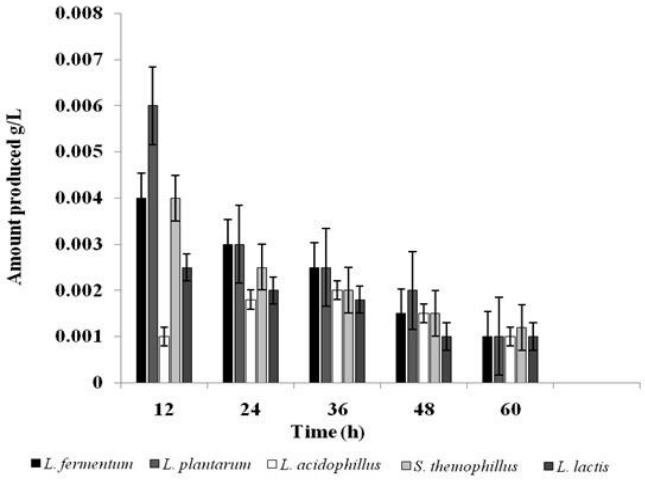

Figure 2: Amount of hydrogen peroxide produced by LAB isolates with time. Error bars represent standard errors of mean of triplicate determinations.

DISCUSSION

The identification carried out for representative *Lactobacillus* strains from the fermented food products demonstrated the dominance of *L. 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 (Halm *et al.*, 1993; Wakil *et al.*, 2004).

Microbial food safety is an increasing public health concern worldwide and many Gram negative bacteria such as *E. coli*, *Klebsiella* sp. together with Gram positive

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Table 3: Zone of inhibitions (mm) of pathogens by lactic acid bacteria isolated from *ogi* and *kunnu-zaki*

LAB isolates	*B. cereus*	*S. aureus*	*P. mirabilis*	*K. pneumoniae*	*E. coli*
L. plantarum	+ 8	+ 8	+ 6	+ 11	NZ
L. lactis	+ 7	+ 7	+ 4	+ 7	+ 7
L. acidophilus	+ 7	+ 5	NZ	+ 6	+ 3
L. casei	+ 7	+ 6	+ 7	+ 5	+ 4
L. fermentum	+ 7	+ 5	NZ	+ 6	+ 5
L. mesenteroides	+ 6	+ 6	+ 4	+ 8	+ 5
Streptococcus thermophillus	+ 7	+ 4	+ 10	+ 7	+ 5

NZ: no zone of inhibition, indicating resistance of the organism to the antibiotics

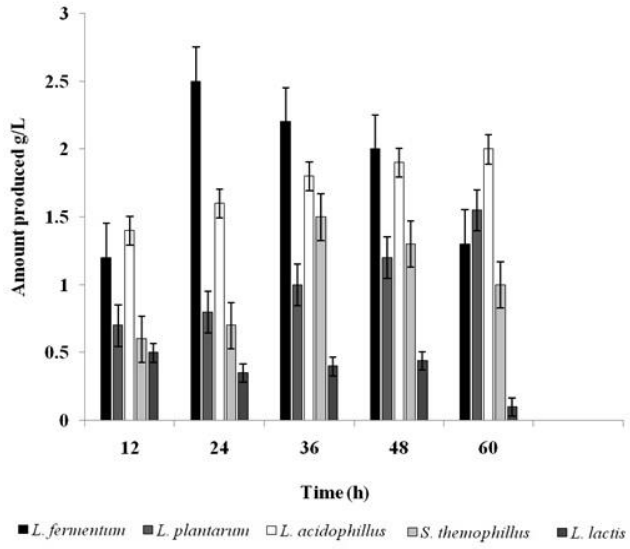

Figure 3: Amount of diacetyl produced by LAB isolates with time. Error bars represent standard errors of mean of triplicate determinations.

bacteria such as *S. aureus* have been implicated in food borne diseases (Mead *et al.*, 1999). Ogunshe *et al.* (2005) also isolated some Gram negative bacteria of clinical importance from a fermented food condiment.

Several studies have shown that pathogens such as enterotoxigenic *E. coli*, *Shigella flexneri*, *Salmonella typhimurium* and *B. cereus* are adversely affected when present in traditional fermented foods (Kingamkono *et al.*, 1995; Kunene *et al.*, 2000; Obadina *et al.*, 2006). Some of the antimicrobial properties exhibited by these fermenetd foods may be as a result of the low pH of the food as well as metabolites produced by microoragnisms such as LAB involved in the fermentation. The pathogens used in this study were sensitive to the LAB metabolites. Brooks *et al.*, 1998 reported rapid development of resistance by *Staphylococcus* sp. to antimicrobial agents. However, the *Staphylococcus aureus* used in this study was sensitive to the entire LAB used against it. Lactic acid bacteria are known to produce antimicrobial substances mainly in the form of organic acids and metabolites (Obadina *et al.*, 2006). Antimicrobial activity caused by growth of lactic acid bacteria may be due to decrease in pH, depletion of nutrients and production of antimicrobial compounds (Olsen *et al.*, 1995), including bacteriocins (Parente and Ricciardi, 1999), and various organic acids such as lactic acids, acetic acid.

The LAB isolated from fermented *ogi* and *kunnu-zaki* in this study produced different antimicrobial compounds the quantity of which varies with time. The increase in the production of lactic acid with time has been attributed to lowered pH which permit the growth of LAB. The inhibitory effect of hydrogen peroxide from LAB agrees with the report of Collins *et al.,* (1983) who noted the inhibition of *Psuedomonas fragi* and *S. aureus* by hydrogen peroxide by some LAB strains which contribute to their inhibitory activity against other microorganisms.

The antibacterial compounds produced by LAB obtained from femented food may be used to combat the growth of pathogenic microorganisms in the food industry. The use of bacteriocinogenic starter/protective cultures could improve the quality and increase safety by inhibiting the food-borne pathogens and spoilage microorganisms.

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