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Antimicrobial activity of lactic acid bacteria isolated from fermented milk products

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The bacteriocins produced by lactic acid bacteria (LAB) which acted as antimicrobial substances or preservative component on fermented food product (yoghurt, kunu and fufu) was investigated against selected indicator food spoilage causing bacteria and antibiotics. The agar-well diffusion assay was employed to investigate the antagonistic activity against indicator organisms (*Staphylococcus aureus*, *Shigella* spp., *Salmonella typhi* and *Escherichia coli*). *Lactobacillus plantarum*, *Lactobacillus bulgaricus*, *Lactobacillus delbrueckii sub bulgaricus*, *Lactobacillus amylophilus*, *Lactococcus lactis* and *Leuconostoc mesenteroides* were isolated from yoghurt, fufu and kunu. *L. lactis* showed the largest inhibition zone against *S. typhi* and *S. aureus* as 19 and 14 mm, respectively, while *L. amylophilus* showed antimicrobial activity against all the indicator organisms. Isolates from fufu generally showed more inhibition on *S. aureus*, *S. typhi* and *E. coli*, while *L. plantarum* showed no inhibitory zone against the indicator organisms. All the isolates were susceptible to ciprofloxacin, but resistant to ampiclox, zinnacef and amoxicillin, while variable susceptibility/resistance to pefloxacin, gentamycin rocephin, streptomycin, septrin and erythromycin was observed. LAB showed inhibitory properties against the food spoilage and pathogenic organisms tested, and have been reported to produce various antimicrobial compounds including organic acid, hydrogen peroxide, di-acetyl and bacteriocin. This study is important in risk assessment related to fermented food products, as well as the potential use of these LAB isolates in pharmaceutical industries.

Key words: Lactic acid bacteria, fermented foods, yoghurt, 'kunu', 'fufu'.

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 use in the production of indigenous fermented foods (Holzapfel et al., 1995). Members of LAB produce bacteriocins and bacteriocin-like substances which may inhibit growth of spoilage and

pathogenic microorganisms (Klaenhammer, 1988). Lactic acid fermentation of cereal-based foods and mammary glands derivatives (milk) is a traditional technology in Africa and has long been used in the processing of different foods (Mensah, 1997; Oyewole, 1997). Furthermore, LAB contributes to the enhancement of the organoleptic attributes of foods, as well as to their

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preservation and microbial safety (Caplice and Fitzgerald, 1999; Calderon et al., 2001).

The preservative action of starter culture in food and beverage systems is due to a range of antimicrobial metabolites produced during the fermentation process, which include many organic acids such as lactic, acetic and propionic acids which provide an acidic environment unfavorable for the growth of many pathogenic and spoilage microorganisms (Caplice and Fitzgerald, 1999; Rattanachaiakunsopon and Phumkhachorn, 2010). These acids compounds generally exert their antimicrobial effect by interfering with the maintenance of cell membrane potential, inhibiting active transport, reducing intracellular pH and inhibiting a variety of metabolic functions (Ross et al., 2002). Others are low-molecular-mass (LMM) compounds such as hydrogen peroxide, carbon dioxide, diacetyl (2,3-butanedione), uncharacterized compounds, and high-molecular-mass compounds like bacteriocins (Jay, 1982; Piard and Desmazeaud, 1992). The antimicrobial-producing LAB may be used as protective cultures to improve the microbial safety of foods and 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 foodborne pathogens such as *Staphylococcus aureus*, *Salmonella* spp. and *Listeria monocytogenes* (Buckenhusk, 1993; Brinkten et al., 1994; Olasupo et al., 1995). LAB have the ability to trap mycotoxins, some might be used to reduce the availability and toxicity of toxins in the gastrointestinal tract of humans and animals, and might therefore be used as a probiotic agent (Dalié et al., 2010).

In Nigeria, yoghurt, fufu and Kunu-zaki are part of 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). Yoghurt is a smooth, fermented milk product that evolved empirically some centuries ago through the growth of thermophilic LAB such as *Streptococcus thermophilus* and *Lactobacillus bulgaricus* which ferment the lactose to produce lactic acid. It has a characteristic acidic taste possessing 0.95-1.5% and pH ranging from 3.7-4.2 with viable and abundant fermenting microorganisms (Adolfsson et al., 2004). Fufu is a fermented white paste made from cassava, it is ranked next to gari as an indigenous food of most Nigerians in the South. Fufu is made by steeping whole or cut peeled cassava roots in water to ferment for maximum of three days, during the steeping, fermentation decrease the pH, softens the roots and help to reduces the potentially toxic cyanogenic compound (Agbor-Egbe and Lape Mbome, 2006, Egwim et al., 2013).

Kunu is a product obtained from cereal grains such as sorghum (*Sorghum vulgare*), maize (*Zea mays*) and millet (*Pennisetum typhoideum*). It is a Nigerian drink

made of germinated grain, mostly millet. These grains can be used singly or in combination. Sorghum and millet are the most common combinations used in the ratio of 1:2 (w/w). It is a non-alcoholic cereal beverage commonly consumed by the people of the northern Nigeria, though it is becoming popular in other parts of the country (Gaffer et al., 2002).

In Nigeria, cassava is processed into garri and fufu, pellets for compounding animal feeds, kpokpo garri and also into instant aromatized (fermented) flour (Oyewole, 1997). Fufu (variants of the name include foofoo, foufou, fufuo) is a staple food of the Asante, the Akyem, Bono and Fante peoples of the Akan ethnic group of Ghana and is eaten in Guinea (Guinean cuisine). It is made by boiling starchy food crops like cassava, yams or plantains and then pounding them into a dough-like consistency. In this context, the aim of this work was to identify the bacterial isolates from fermented sorghum (kunu), cassava (fufu) and milk product (yoghurt), and to determine the antimicrobial properties of the isolates against foodborne pathogens.

MATERIALS AND METHODS

Collection of samples

Three samples of yoghurt, fufu and kunu-zaki were bought from Akungba market in Akungba Akoko. The samples were carefully labeled. They were brought to the laboratory and processed within 6 h of collection.

Foodborne pathogens used

The food and human pathogenic organisms used include *Staphylococcus aureus*, *Shigella* spp., *Escherichia coli* and *Salmonella typhi*. They were obtained from Adekunle Ajasin University Microbiology Laboratory.

Analysis of samples

The pour plate method was used to isolate microorganisms producing antimicrobial activity from samples of fufu, kunu and yoghurt. Each sample was serially diluted using sterile distilled water as diluents (Speck, 1976). Nine milliliters of distilled water were put into test tubes. One milliliter of each of yoghurt, fufu and kunu-zaki sample were added into the first test tube and thoroughly mixed. Using a different sterile pipette, 1 ml from the first test tube was pipetted into the second test tube already containing 9 ml of distilled water. This procedure continued until the last dilution. Using the pour plate assay, 1 ml of the last dilution of each sample was pipetted into Petril dishes, which were subsequently filled with MRS agar. The plates were mixed and after solidification, they were incubated anaerobically at 37°C for 48 h.

After incubation, the representative colonies on the plates were sub-cultured on fresh MRS agar to obtain pure cultures of the isolates. The pure cultures were then transferred into MRS agar slants for biochemical identification.

Microbiological analysis

Isolation of bacteria

After incubation time, the different culture plates were examined for

microbial growth; subcultures were made to get discrete colonies, which were then stored at 40°C for further biochemical investigations in order to identify microorganisms. Bacterial isolates were characterized to generic level and were possible to the species level on the basis of their cultural features (shape, colour, edge, elevation) and morphological features such as motility, gram staining, cell arrangement and shape, and biochemical features.

Preparation of cell-free supernatant

The cell-free supernatants were prepared based on methods by Schillinger and Lucke (1989). The culture extract of the producer strain were obtained from 72 h culture grown on MRS broth. The cultures were then centrifuged at 4.3008 kg force for 15 min. The supernatant was used immediately.

Antibiotic susceptibility tests

A modified method of Bauer-Kirby disk diffusion assay was used for the study. The antibiotics discs were tested on sterile Muller Hinton Agar, which was put into sterile Petri dishes and waited for solidification. A suspension of the isolated organisms was spread over the agar plates, and incubated at 37°C for 1 h. A forceps was used to transfer each antibiotic disc on the plate and incubated for 24 h at 37°C. Ten antibiotics including Pefloxacin, Gentamycin, Ampiclox, Zinnacef, Amoxicillin, Rocephin, Ciprofloxacin, Streptomycin, Septrin and Erythromycin were tested. Each disc was firmly pressed to ensure complete contact with the agar surface. The discs were evenly distributed so that they were not too close to one another. In an inverted position, the plates were incubated at 37°C for 24 h. The results were expressed as inhibition zones around the disc. These were measured, each antibiotic disc was grouped as susceptible or resistance by comparing the measured diameter with the standard given in the manufacturer's instruction.

In vitro inhibition test with indicator organisms

The antimicrobial activity of the isolated LAB (cell free filtrate) against *E. coli*, *Shigella* sp., *Salmonella* sp. and *S. aureus* was performed using the well diffusion assay. Elimination of inhibitory substances like hydrogen peroxide, organic acids and bacteriophages to ensure that the inhibition was caused by only bacteriocin was performed. This was carried out by using MRS containing 0.2% glucose and preparation of cell free supernatant at pH 6.5. The pathogenic indicator bacteria were incubated in Muller Hinton broth at appropriate temperature for 24 h.

Petri dishes containing 20 ml of Muller Hinton agar were prepared previously, another broth was prepared and incubated for 4 h and inoculated with 0.1 ml of 4 h broth culture of pathogenic bacteria. Once solidified, the dishes were stored for 2 h in a refrigerator. Three wells were made and filled with 2 ml of cell-free filtrate. Incubation of the Petri dishes was done at 37°C for 24 h. The antimicrobial activity was determined by measuring the zone inhibited around the wells (Zhennai, 2000).

Identification of bacterial isolates

Identification of isolates was based on cultural, morphological and biochemical characteristics following standard methods.

Gram staining

The method described by Carpenter (1977) and Thomas (1973)

were used. Smears of the isolates were prepared and heat fixed on clean grease free slides. The smears were stained for one minute with crystal violet. This was washed out with a gentle running tap water. The slides were flooded with dilute Gram's iodine solution. This was washed off with water and the smears were decolorized with 95% alcohol until the blue colour no more dripped out (about 30 s). The smears were then counter stained with saffranin solution for about 10 s. Finally, the slides were washed with tap water; air dried and observed under oil immersion objectives.

Biochemical tests

Catalase test

This test was performed to demonstrate which of the isolates could produce the enzyme catalase that release oxygen from hydrogen peroxide. This test is usually used as an aid to differentiate *Staphylococci* from *Streptococci* and to differentiate other catalase positive organism from catalase negative. The method employed here was that described by Speck (1976). The catalase production was indicated by the prompt effervescence of oxygen due to the fact that the enzyme aids in the conversion of hydrogen peroxide into water and oxygen bubbles (in the form of effervescence). Effervescence of gas as a white froth indicates a catalase positive reaction while the absence of the effervescence showed negative reaction (AOAC, 2000).

Motility test

To demonstrate the ability of isolates to be motile, this indicating the possession of movement structures (flagellum), was investigated by using semi-solid medium. This medium was prepared and the test performed. The isolates were used to sub-inoculate the medium to a depth of about two-third of the medium and incubated overnight at 37°C. Motility of an organism was indicated by growth beyond the inoculation line, while growth confined to the line of inoculation was regarded as being non-motile.

Sugar fermentation

Each of the isolates was tested for their ability to ferment a given sugar with the production of acid and gas or acid only. Since most bacteria especially Gram-negative bacteria utilize different sugars as source of carbon and energy with the production of both acid and gas, or acid only the test is used as an aid in their differentiation. The growth medium used was peptone water and the method used was that described by Kirk et al. (1975). Peptone water was prepared in a conical flask and the indicators bromocresol purple was added. The mixture was dispensed into test tubes containing Durhams tubes. The tubes with their content were sterilized by autoclaving at 121°C for 15 min. One percent solution of the sugar was prepared and sterilized separately at 115°C for 10 min. This was then aseptically dispensed in 5 ml aliquot volume into the tubes containing the peptone water and indicator. The tubes were inoculated with young culture of the isolates and incubated at 37°C. Acid and gas production or acid only were observed after about 24 h incubation. Acid production was indicated by the change of the medium from light green to yellow color, while gas production was indicated by the presence of gas in the Durham's tubes.

Coagulase test

Slide and tube method was used (Carpenter 1977). In slide test, a

Table 1. Lactic acid bacteria isolated from different food samples.

Fermented food	LAB
Yoghurt	<i>Lactobacillus plantarum</i>
	<i>Lactobacillus bulgaricus</i>
	<i>Lactococcus lactis</i>
Fufu	<i>Lactobacillus delbrueckii</i> sub <i>bulgaricus</i>
	<i>Lactobacillus amylophilus</i>
	<i>Lactococcus lactis</i>
Kunu-Zaki	<i>Lactobacillus plantarum</i>
	<i>Lactococcus lactis</i>
	<i>Leuconostoc mesenteroides</i>

loop full of the isolate was mixed with human plasma and allowed to stand for some minutes. Particles indicating agglutination was used as indication of coagulase reaction. In the tube method, plasma was added unto a culture of the isolate in peptone water in bijou bottles. The bottles were incubated at 37°C for 24 h. A clumping/agglutination of the plasma were used to indicate presence of coagulase.

Citrate utilization

In actual practice, Koser's citrate medium containing 'citric acid' serves as the exclusive carbon source. It detects the ability of an organism to use citrate as the sole source of carbon and nitrogen. Evidently, the ability as well as the efficacy for the 'citrate utilization' (the prevailing substrate) is adequately indicated by the production of measurable turbidity in the medium. In carrying out this test, the isolate was stabbed into test tube containing the citrate medium from a 24 h culture. Simmon Citrate Agar was prepared and dispensed into each clean test tube, sterilized, and allowed to solidify in a slanted position. An inoculum from the broth culture was picked with an inoculating loop and incubated unto the surface of the slanted Citrate Agar prepared in each test tube. It was then incubated at 37°C for 5 days and examined. Those that changed color from green (original color of prepared medium) to blue or yellow were considered positive, while those that retained the green color were negative.

Starch hydrolysis test

A medium containing starch was used. After inoculation and overnight incubation, iodine reagent was added to detect the presence of starch. Iodine reagent complexes with starch to form a blue-black color in the culture medium. An inoculation from a pure culture is streaked on a sterile plate of starch agar. The inoculated plates were incubated at 35-37°C for 24 h. Iodine reagent was then added to flood the growth. Clear halos surrounding colonies is indicative of their ability to digest the starch in the medium due to the presence of alpha-amylase.

Production of hydrogen sulphide (H₂S)

There are several sulphur containing amino acids like cystein, methionine that may decompose certain organisms to yield

hydrogen sulphide gas among the products of microbial degradation. Lead acetate was duly incorporated into the culture media which eventually turned into either black or brown due to the formation of lead sulphide (AOAC, 2000).

Spore stain

The malachite green staining method was used. The staining was carried out as described by Carpenter (1977). Smears of the pure isolates were made on grease-free glass slide and heat fixed. The slides were flooded with 5% v/v malachite green solution. The slides were flamed in such a way that the stain steamed but did not boil. The slides were then allowed to stand for 5 min. The stain was then washed out in running tap water. The smears were counter-stained with safranin for 30 s. It was stained with safranin, blotted dried and examined under the oil immersion objective. The spores stained green while vegetative cells stained red.

RESULTS

The results show the LAB isolated from different fermented dairy products: yoghurt and the fermented foods (fufu and kunu). Six LAB were isolated from the samples, *Lactobacillus plantarum*, *L. bulgaricus* and *Lactobacillus lactis* were obtained from yoghurt, *Lactobacillus delbrueckii* sub *bulgaricus*, *Lactobacillus amylophilus* and *L. lactis* were obtained from fufu, while *L. plantarum*, *L. lactis* and *Leuconostoc mesenteroides* were isolated from kunu (Table 1). *L. plantarum* was obtained from yoghurt and kunu, while yoghurt, fufu and kunu showed presence of *L. lactis*.

The morphological and biochemical characteristics of the bacterial isolates are shown in Table 2. The antibiotic resistance patterns of the isolates are presented in Table 3. All the isolates were susceptible to ciprofloxacin, but resistant to ampiclox, zinnacef and amoxicillin, while variable susceptibility/resistance to pefloxacin, gentamycin rocephin, streptomycin, septrin and erythromycin was observed. Table 4 shows inhibitory properties of the metabolites of the selected LAB against the target foodborne pathogenic organism (*S. aureus*, *Shigella* spp., *Salmonella typhi* and *E. coli*). The metabolites possess antagonistic activities against all the organisms used in this work.

L. plantarum isolates obtained from yoghurt and kunu was observed to be inactive against any of the indicator organisms. *L. lactis* showed the largest inhibition zone against *S. typhi* and *S. aureus* as 19 and 14 mm respectively, while *L. amylophilus* showed antimicrobial activity against all the indicator organisms. Isolates from fufu generally showed more inhibition on *S. aureus*, *S. typhi* and *E. coli*.

DISCUSSION

In this study, six LAB were isolated from traditionally fermented and diary food product: yoghurt, fufu and kunu obtain from Akungba. The results of the present

Table 2. Morphological and biochemical characteristics of the bacterial isolates.

Shape	Pigmentation	Surface	Margin	Elevation	Gram stain	Cell morphology	Motility	Catalase	Starch	Manitol	Fructose	Glucose	Sucrose	Lactose	Identified organisms
Circular	Creamy	Rough	Entire	Convex	+	Rod	-	-	-	+	+	+	+	+	<i>Lactobacillus plantarum</i>
Circular	Whitish	Shiny	Entire	Convex	+	Long rod	-	-	-	+	+	+	-	-	<i>Lactobacillus bulgaricus</i>
Circular	Creamy	Rough	Entire	Flat	+	Cocci	-	-	-	+	+	+	-	-	<i>Lactococcus lactis</i>
Circular	Whitish	Shiny	Entire	Flat	+	Rod	-	-	-	+	+	+	-	+	<i>Lactobacillus delbrueckii sub bulgaricus</i>
Circular	Creamy	Smooth	Entire	Flat	+	Short rod	-	-	-	-	+	+	-	-	<i>Lactobacillus amylophilus</i>
Circular	Creamy	Smooth	Entire	Convex	+	Cocci	-	-	-	-	+	+	-	-	<i>Lactococcus lactis</i>
Circular	Creamy	Rough	Entire	Flat	+	Rod	-	-	-	+	+	+	-	-	<i>Lactobacillus plantarum</i>
Circular	Creamy	Smooth	Entire	Flat	+	Cocci	-	-	-	-	+	+	+	-	<i>Lactococcus lactis</i>
Circular	Whitish	Shiny	Entire	Flat	+	Cocci	-	-	-	+	+	+	+	+	<i>Leuconostoc mesenteroides</i>

-: Negative, +: positive.

Table 3. Antibiotic susceptibility discs test

Lactic acid bacteria	Inhibition zone (mm)									
	PEF	GEN	APX	ZNF	AMX	RCP	CPX	STP	SEP	ERT
<i>Lactobacillus bulgaricus</i>	S	R	R	R	R	R	S	R	R	R
<i>Lactobacillus plantarum</i>	R	S	R	R	R	S	S	R	S	R
<i>Lactococcus lactis</i>	S	R	R	R	R	S	S	S	R	R
<i>Lactobacillus delbrueckii sub bulgaricus</i>	R	R	R	R	R	R	S	R	R	R
<i>Lactobacillus amylophilus</i>	S	R	R	R	R	R	S	R	S	R
<i>Lactococcus lactis</i>	R	R	R	R	R	S	S	S	S	R
<i>Lactobacillus plantarum</i>	S	S	R	R	R	S	S	R	R	R
<i>Lactococcus lactis</i>	S	R	R	R	R	R	S	S	R	S
<i>Leuconostoc mesenteroides</i>	R	R	R	R	R	R	S	R	S	R

R- Resistance (no value), S- Susceptible (value ranges from 4-32 mm); PEF- Pefloxacin, GEN- Gentamycin, APX- Ampiclox, ZNF- Zinnacef, AMX- Amoxicillin, RCP- Rocephin, CPX- Ciprofloxacin, STP- Streptomycin, SEP- Septrin, ERT- Erythromycin.

study is in accordance with the report of Odunfa and Adeyele (1985), who revealed that members

of LAB could be detected in a variety of habitats including fermented foods. Alli et al. (2010)

reported the presence of *L. acidophilus*, *L. bulgaricus*, *Lactobacillus cremoris*, *L. fermentum*,

Table 4. Inhibition of indicator organisms by LAB.

Sample	Bacteria isolated	Test organisms (mm)			
		<i>Staphylococcus aureus</i>	<i>Shigella spp</i>	<i>Salmonella typhi</i>	<i>Escherichia coli</i>
Yoghurt	<i>Lactobacillus bulgaricus</i>	NI	NI	9	10
	<i>Lactobacillus plantarum</i>	NI	NI	NI	NI
	<i>Lactococcus lactis</i>	8	6	7	NI
Fufu	<i>Lactobacillus delbrueckii sub bulgaricus</i>	8	NI	12	10
	<i>Lactobacillus amylophilus</i>	8	4	8	7
	<i>Lactococcus lactis</i>	14	NI	19	11
Kunu-Zaki	<i>Lactobacillus plantarum</i>	NI	NI	NI	NI
	<i>Lactococcus lactis</i>	9	7	NI	6
	<i>Leuconostoc mesenteroides</i>	8	NI	8	NI

Value shows the mean number of triplicate well zone (mm); NI- No zone of inhibition.

L. lactis, *Lactococcus mesenteroides*, *Micrococcus acidophilus*, *Rhodospirium spp.*, *Saccharomyces bayanus*, *Saccharomyces cerevisiae*, *Streptococcus lactis* and *Streptococcus thermophilus* in yoghurt samples, out of which only *L. bulgaricus*, *L. plantarum* and *L. lactis* were obtained in this study.

The identification carried out for representative LAB strains from the fermented food products demonstrated the dominance of *L. amylophilus*, *L. bulgaricus*, *L. delbrueckii*, *L. lactis* and *L. mesenterioide*. These identified *Lactobacillus* species were in accordance with those earlier identified from similar fermented food products (Halm et al., 1993; Wakil et al., 2004).

Lactobacilli grow best in highly nutritive substrates. They use the nutrients in the substrate for their own metabolism and cell growth and multiply in food (from one million per millilitre to one billion per millilitre). They are present in the fermented food not only as viable cells and non-colony forming units, but also with the primary and secondary metabolites produced during the fermentation process (Robinson, 1991). The MRS medium used was selective for the isolation of *Lactobacillus* species since they are extremely fastidious. Lindquist (1998) reported that a medium that would support their growth must contain a fermentable carbohydrate and many growth factors. Gilliland and Speck (1977) had earlier reported that *Lactobacilli* showed stronger antibacterial properties against Gram-positive bacteria (*S. aureus* and *Clostridium perfringens*) than Gram-negative bacteria (*E. coli* and *Salmonella typhimurium*). *L. lactis* from yoghurt, fufu and kunu inhibited *S. aureus* in the order of 8, 14 and 9 mm with respect to diameter of inhibition zone. *L. bulgaricus*, *L. lactis* and *L. amylophilus* also inhibited *E. coli* in order of 10, 7 and 6 mm. Among the lactobacilli, there has been great interest in *L. plantarum*, due to the potential applications of the microorganisms as a starter bacterium for a variety of fermented foods. The bacteriocin produced from *L. plantarum* has been found to be

inhibitory towards closely related LAB, particularly the mesophilic and thermophilic lactobacilli. In this study, *L. plantarum* produced no inhibition zone against the tested microorganisms.

Several studies have shown that pathogens such as enterotoxigenic *E. coli*, *Shigella flexneri*, *S. 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 fermented foods may be as a result of the low pH of the food as well as metabolites produced by microorganisms 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 *S. aureus* used in this study was sensitive to six LAB used against it.

Antimicrobial activity of LAB 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. 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 bacteria such as *S. aureus* have been implicated in food borne diseases (Mead et al., 1999). The LAB isolated from yoghurt, fufu and kunu-zaki in this study probably produced different antimicrobial compounds, the quantity of which might vary with time. Collins et al. (1983) also noted the inhibition of *Psuedomonas fragi* and *S. aureus* against other microorganisms by hydrogen peroxide by some LAB strains which contribute to their inhibitory activity. Liasi et al. (2009) emphasized that research on antimicrobial substances produced by LAB, had led to their potential use as natural preservatives, which may be used to combat the growth of pathogenic microorganisms in the food industry.

Conclusion

The promising results of bacteriocins produced by LAB from fermented food underline its important role in improving food quality and increasing safety, by using it as starter/protective cultures to combat the growth of food-borne pathogens and spoilage microorganisms.

Conflict of Interests

The author(s) did not declare any conflict of interests.

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