Original Research Article

ANTIBIOTIC SUSCEPTIBILITY AND INTESTINAL EPITHELIAL CELL ADHESION PATTERN OF HOMOFERMENTINGLACTIC ACID BACTERIA (LAB) ISOLATED FROM KUNU-ZAKI, A SPONTANEOUSLY FERMENTING NIGERIAN CEREAL BEVERAGE

ABSTRACT

Aim: -To determine theantibiotic reactionand adhesion pattern ofantimicrobial homo-fermenting LAB strains in the fermenting slurries of kunu zaki.**Methodology**: -Kunu-zaki drinks were produced using spontaneously fermenting cereal grains of *Digitaria exilis (*acha), *Sorghum bicolor (*sorghum*)* and *Pennisetum americanaum* (millet) in composite and non-composite proportions.LAB isolates were obtained on MRS agar. Homo-fermenting isolates were identified to species level using the API50CHL test kit. Antibiotic sensitivity testing on the identified isolates followed the modified standard Kirby-Bauer procedure on MRS agar (pH 7.4) using the disc diffusion technique with selected antibiotics.For quality control of the antibiotics, sensitive reference strains *S.aureus* ATCC 25923 and *E.coli* ATCC 25922 obtained from the Nigeria Institute of Medical Research were used. Adhesion and antimicrobial properties were determined using standard method.**Results**: -Antimicrobial substances produced by the eight LAB isolates inhibited the growth of four selected human pathogens *in vitro*.All eight LAB isolates were resistant to amoxicillin, gentamycin and ciprofloxacin. *L.plantarum1*[26], *L.paracasei subsp paracasei3*[39] *and Pediococcus damnosus*[32] were resistant to erythromycin whilst all others were susceptible.*L.plantarum*1[26]and *L.paracasei subsp paracase*i3[39] was resistant to all antibiotics tested. All LAB isolates demonstrated high *in-vitro*intestinal epithelial cell adhesion potential. Lactic acid bacteria are present in ready to drink kunu-zaki as viable strains and information regarding their antibiotic reaction pattern is scanty. **Conclusion**: -LAB antimicrobial activitymay not be affected if kunu zaki is consumed simultaneously withthese antibiotic therapies. However, if these LAB strains are intended for use as kunu-zaki starter cultures, it is important that they should be furthercarefully examined for inability to transfer antibiotic resistance genes to food pathogens.

Keywords: lactic acid bacteria,antimicrobial, antibiotics, susceptibility, resistance

Introduction

The lactic acid bacteria (LAB) are easily identified as Gram positive bacteria, non-respiring, non-spore forming, cocci or rods. Lactic acid is the major end product of the fermentation of carbohydrates, though some fermentationproduces other substances in addition to lactic acid.Fermentation of sour dough bread, sorghum beer, milks, cassava (to produce *gari* and *fufu*), *ogi*, *kenkey,injera,obiolor* are all examples of African fermented foods involving lactic acid bacteria. The major genera of lactic acid bacteria include the*Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus* species. A few more have been identified, but they play a minor role in lactic fermentations(Carr et al [1].Lactic acid bacteria are usually found in decomposing plants and lactic products. Lactic acid is the major metabolic end product of the carbohydrate fermentation. LAB are a large group of fermentative, anaerobe aero-tolerant microorganisms which are usually present in the gut of humans and other animals, raw vegetables, meat and meat products, and cereals Carr et al. [1]. In animals, their numbers may vary with the species, the age of the host, or the location within the gut De Vries et al. [2]. In the food industry, lactic acid bacterial strains are widely employed either as starter cultures or as non-starter lactic acid bacteria. Furthermore, owing to their probiotic properties, several LAB strains have found use as adjunctive cultures in foods and feed.Leroy and Vuyst,[3].Increasing commercial interest for functional food containing probiotics, has increased scientific interests in these products. Lately, the researches in the food biotechnologies have centered on careful isolation and selection of strains of *Lactobacillus*having antimicrobial propertiesthat might both ensure the microbiological safety of the food as well as bring benefits to the consumer`s health,Bomba *et al*.[4]. One of the anxieties which has evolved over the use of LAB as starter cultures,however, is resistance to antibiotics. Antibiotics are one of the great discoveries against bacterial infections. Unfortunately bacteria can fight back by being resistant towards antibiotics. . From a human perspective, resistance to antibiotics is an un-desirable ability of microorganism.Antibiotic resistance is the ability of a microorganism to remain unaffected by an antibiotic. When a microorganism becomes antibiotic resistant, it is difficult to eliminate the infectioncaused by these microorganisms if and when it happens. Microorganisms can become resistant to antibiotics either by inactivating the drug, by altering the target site, by altering the metabolic pathway, or by reducing drug accumulation, Cauragh and Collins [5]. In recent times, antibiotic resistance in bacteria has become a publicconcern issue. This is because a patient could develop antibiotic resistance by contacting a resistant microorganism or the emergence of a microorganism in the

patient's body when treatment with antibiotic begins. Catalouk and Gogebakan[6]. Even though a health beneficial microorganism such as LAB with antibiotic resistance may have the advantage of not being affected by the antibiotic when a consumer takes it together with the antibiotic, however, such an organism with antibiotic resistance could eventually become an opportunistic pathogen. This could be highly detrimental,Perreten *etal*.[7], to the infected host and the transfer of antibiotic resistant genes from the health beneficial food LAB to food pathogens may occur. Some species of LAB commonly used in the food industry or naturally occurring in raw food materials have been found to be resistant to glycopeptides antibiotics, Tynkkynen *et al*.[8]. Genes conferring resistance to several antimicrobials (including chloramphenicol, erythromycin, streptomycin, tetracycline, and vancomycin) located on transferable genetic elements (plasmids and transposons) have already been characterized in lactococci, Perreten *et al*. [7], and lactobacilli, Gfeller *et al*. [9], from foods.Frequency of spontaneous mutation to kanamycin and streptomycin was reported to be very high for an appreciable number of lactobacilli, Curragh and Collins [5]. Currently there are lots of data on the prevalence of antibiotic resistance and the mechanisms implicated in clinical bacteria, Gfeller et al. [9]. It is therefore important that strains intended for use in the food systems should also be carefully examined for antimicrobial susceptibility/resistance.Kunu-zaki is a traditional beverage made from spontaneously fermenting cereals. The beverage originated from Northern Nigeria but has found wide acceptability in all parts of the country. Cereals used for kunu-zakiinclude sorghum, millet or maize in non-composite proportions. Regardless of the cereal used, kunu-zakiwild fermentation is largely dominated by Lactic acid,Efuevwere *et al*. [10], Elmahmood and Douglari [11]. The spontaneous fermentation production process however usually results in kunu zaki of inconsistent quality from batch to batch of each production. The use of starter cultures, which would produce a uniform fermented kunu-zaki product quality remains yet unexplored. Yet this drink has become very popular in a country referred to as the most populous African nation.The hypothesis behind this present work therefore is that it would be possible to select strains with antimicrobial properties which can be used as starter cultures for kunu-zaki. The aim of this current study was to evaluate theantibiotic reaction pattern and the adhesion ability ofantimicrobial homo-fermentingLAB strains present in fermenting slurries ofkunu zaki. This would be helpful in determining if the LAB would be affected if a kunu-zaki drinker is on an

antibiotic therapy. The information obtained from this study could contribute to the potential use of these LAB isolates in the food and pharmaceutical industries

MATERIAL AND METHODS

Laboratory Production of Kunu-zaki

Sorghum (*Sorghum bicolor)*, millet,(*Pennisetumamericanum)* and hungry rice (locally known as Fonio or Acha) *Digitariaexilis* grains were obtained from the Nigeria Cereal Research Institute in Ibadan, Oyo State, Nigeria . The grains were cleaned, weighed and washed before steeping in distilled water.200 grams of cereal grains were used for the kunu-zaki production. A control experiment was set up with distilled water without the grains. For the kunu-zaki made from composite grains, an equal weight of grains was used for each part. The laboratory production method was done according tothe traditional process of kunu-zaki fermentation reported by Adeyemi and Umar[12] with slight modifications.

Sorghum grains Millet grains

↓

Washing

↓

Soaking in water (**1st Fermentation)(48hhrs)**

↓

Wet milling

↓

Sieving of the slurry

↓

Cooking of 2/3 parts filtrate to gelatinization

↓

(Cooling to 35°C)

↓

Addition of uncooked 1/3 part(‘back-slopping”)

↓

2nd fermentation (24hrs)

↓

Sweetening

↓

Bottling

↓

Storage (Refrigeration temperature)

Figure 1: Flow diagram for the traditional processing of kunu-zaki from composite grains

Isolation of the Lactic acid bacteria

The isolation of the lactic acid bacteria was done following the pour plate technique.
Samples were obtained directly from the 72 hours fermenting mash of the kunu-zaki made from eachcereal type. The samples were collected in sterile carriers and stored on ice until delivery to the laboratory. Once delivered to the laboratory,they were kept under refrigeration temperature until ready for analysis.
The Pour plate technique was used to isolate theorganisms. The kunu-zaki samples were used directly, diluted to 10^{-1}, 10^{-2} and 10^{-3} using sterile peptone water. 1 ml aliquot of the samples and dilutions were plated into MRS agar (pH 6.2 and pH5.5) .The plates were incubated at 37 °C for 3 days under anaerobic conditions (in an anaerobic jar using Oxoid anaerogen compact). The use of the medium was to isolate and enumerate the lactobacilli present in the sample. After incubation, individual colonies were selected and transferred into sterile broth mediums. The streak plate technique was used to obtain pure colonies.

Morphological, physiological and biochemical examination of LAB

The pure colonies isolates were examined according to their colony morphology, catalase reaction and gram reaction. The Gram positive and catalase negative cocci and bacilli colonies were stored in glycerol as lactic acid bacteria. Morphological, physiological and biochemical examination of the isolates were determined by the standard procedure of gram staining, catalase test and test for CO2 gas production according to methods offered by Bulut,[13].

Catalase Test

Lactic acid bacteria do not contain the catalase enzyme and are designated catalase negative. The catalase test was performed on the pure culture isolates in order to confirm their catalase reaction.

Overnight cultures of the pure culture isolates were grown in MRS broth at 37^0C under anaerobic conditions. After 24 hours, 3% hydrogen peroxide solution was dropped into 1 ml of the overnight culture. The isolates, which did not give gas bubbles, were chosen as catalase negative,Bulut,[13].

Gram Staining

The gram reaction of the isolates was determined by light microscopy after gram staining. LAB are gram positive and appear purple-blue color under the microscope after gram staining..

Homo/Hetero-fermentative property

The catalase negative, gram positive isolates were tested for CO_2 production from glucose to determine which of the identified isolates were either homo fermentative or hetero fermentative. Citrate lacking MRS broths with inserted inverted Durham tubes were prepared and inoculated with 1% overnight fresh cultures. Then the test tubes were incubated at 37^0C for 5 days. Gas production in the Durham tubes evidenced by displacement of the broth was observed during the 5 day incubation period. Evidence of gas production was recorded as positive indicating hetero-fermentation,Bulut,[13].

Temperature and NaCl tolerance test

To determine tolerance to different temperatures, MRS containing bromo-cresol purple indicator was prepared and 5mls transferred into tubes. Fifty µl of overnight cultures was inoculated into the tubes and incubated for 7 days at temperatures 10 °C, 15°C, 45°C. For each incubation temperature, the cell growth was observed by the color change of the indicator purple to yellow and recorded as positive (+). For the NaCl tolerance test, two different NaCl concentrations (4.5% and 6.0%) were added to MRS broth containing the indicator .The tubes were inoculated with 1% overnight cultures and incubated at 37^oC for 7 days. Cell growth was indicated by color change and recorded as positive(+), Bulut,[13].

Identification with API 50CHL

The means of identification used was the API 50 CHL medium intended for the identification of the genus Lactobacillus and related genera.A suspension was made in the medium with the

microorganism to be identified and each tube of the strip was inoculated with the suspension. During incubation, the carbohydrates fermented to acids which produced a decrease in the pH detected by the change in color of the indicator. The results made up the biochemical profile which was used by the apiweb^{TM} identification software BioMériux (Ref.40011) to identify the strain. Strains were identified to species level with specified accuracy percentages.

Determination of antibiotic susceptibility

Standardised culture suspensions (0.5 McFarland standard, equivalent to cell density of 10^8 cfu/ml) was spread evenly on the surface of the MRS agar plate (Oxoid) using a sterile cotton swab.. The inoculated plate was allowed to dry before placing the diffusion discs containing antibiotics onto the surface of the inoculated agar plates.Susceptibility of the eight isolates to 5 types of antibiotics was performed by the Kirby Baeur disc diffusion method as used by Rojo-Bezares et al. [27]. Commercially available antibiotics discs (Oxoid) containing amoxicillin (25µg), erythromycin (10µg), ciprofloxacin (10µg), cotrimoxazole (25µg) and gentamycin (10µg) were used. Precaution was taken to ensure uniform contact between the antibiotic disc and agar plate. After 36 hours incubation at 30-32^{0}C under anaerobic conditions, inhibition zone diameters were measured.Inhibition zone diameters were measured inclusive of the diameter of the discs. Results were expressed as sensitive S, intermediate, I, and resistant, R, respectively according to the cut-off points given by the manufacturer NCCLS [21b].For quality control of the antibiotics used during the study, sensitive reference strains *Staphylococcus aureus* ATCC 25923 and *E. coli* ATCC 25922 obtained from the Nigerian Institute of Medical Research (NIMER), Lagos Nigeria were used.

Adhesion assay

This test was done according to the method of Mardh and Westron [14] with slight modifications as reported by Otero and Nader-Macias [15].Apparently healthy guinea pigs were obtained from the National Food and Drug Administration and Control (NAFDAC), Lagos, NigeriaIntestinal epithelial cells were collected by scrapping the intestinal wall of the guinea pigs with a brush and suspending at pH 7.0 in MEM (Eagles Minimal Essential Media).The suspension was kept under refrigeration until the adhesion assay. For the adhesion assay, bacterial suspension was standardised by harvesting the LAB isolate from a 12 hour pure culture and washing twice with saline (0.8% NaCl) solution, once with MEM (pH 7.0) and re-suspension in MEM to obtain a

final concentration of 10^7 cfu/ml (OD_{540}=0.15). The bacterial suspension (500µl) was then added to 500µl of guinea pig intestinal cell suspension and incubated at 37^0C for 1 hour under low agitated conditions (35 rpm). The control was prepared by substituting the bacterial suspension with only MEM. In order to remove non adherent bacteria, tubes were centrifuged for 10 minutes at 800 rpm and the supernatant discarded. The pellets were re-suspended in 1ml MEM and washed three times under the same conditions. Bacterial binding to the epithelial cells was examined by light and phase contrast microscopy using differential staining,Otero and Nader Macias. [15].

The percentage adhesion was expressed as:-

$$\%\ adhesion = \frac{No\ of\ intestinal\ epithelial\ cells\ with\ bacteria\ adhered}{Total\ No\ of\ intestinal\ epithelial\ cells}\ x\ 100$$

The adhesion index was expressed as:-

$$Adhesion\ index = \frac{Total\ No\ of\ bacteria\ attached\ to\ epithelial\ cells}{No\ of\ cells\ with\ adhered\ bcteria}$$

Antimicrobial activity

Antimicrobial activity was assessed using the method of Rojo-Bezares *et al*. [16a]After 18 hours incubation, active cultures were spotted on the surface of MRS agar plates. The plates were incubated to grow the cultures for 24 hours at 37^0C under anaerobic conditions. Overnight indicator reference pathogen inoculated to soft agar containing 0.7% agar was overlaid on the MRS plates.Indicator organisms used were *S.aureus* ATCC 25923, *E.faecalis* ATCC 29212, *P.aeuruginosa* ATCC27853, *and E.coli* ATCC 25922. Indicator organisms were obtained from the Nigeria Institute of Medical Research (NIMER), Lagos, Nigeria. The plates were incubated according to the appropriate condition for the indicator organism. At the end of incubation, the inhibition zone around the spotted isolates was measured. Isolates which gave an inhibition zone bigger than 1mm was determined to have antimicrobial activity. Data on the zone of inhibition obtained against the different bacterial pathogens was statistically analysed and significance evaluated at 5% level.

RESULTSAND DISCUSSION

Table 1: Laboratory production of kunu-zaki

Kunu-zaki type	Appearance of beverage	Final pH
Sorghum-Millet	Light chocolate brown	5.12
Acha-Sorghum	Milky brown	4.04
Sorghum	Deep brown	5.08
Millet	Milky white	5.41

Table 2: Characteristics of the LABisolates

Code	Cultural characteristics	Cell mMorphology	Gram reaction	Catalase reaction	CO_2 production	API web™ software iIdentification	% accuracy of iIdentity
32	Pin point, creamwhite, entireedge, and smooth, raised.	Cocci	+	-	-	*Pediococcus damnosus*	99.8
33	Pin point, creamwhite, entireedge, smooth, raised	Rods	+	-	-	*Lactobacillus plantarum1*	99.9
6	Pin point, creamwhite, entireedge, smooth, raised	Rods	+	-	-	*Lactobacillus paracasei subsp paracasei1*	99.7
29	Pin point, creamwhite, entireedge, smooth, raised	Rods	+	-	-	*Lactobacillus paracasei subsp paracasei2*	99.3
26	Pin point, creamwhite, entireedge, smooth, raised	Rods	+	-	-	*Lactobacillus plantarum1*	99.9
39	Pin point, creamwhite, entireedge, smooth, raised	Rods	+	-	-	*Lactobacillus paracasei subsp paracasei3*	99.8
27	Pin point, creamwhite, entireedge, smooth, raised	Cocci	+	-	-	*Pediococcus pentosaceus 2*	99.9

Table 3: Physicochemical properties of identified LAB

Name of LAB isolate	Homo / Hetero fermenter	Growth at 10^0C	Growth at 15^0C	Growth at 45^0C	Growth in 4% NaCl	Growth in 6.5% NaCl
Lactobacillus plantarum1[33]	HM	+	+	-	+	_
Pediococcuspentosaceus2[27]	HM	+	+	+	+	+
Pediococcus damnosus2	HM	+	+	_	_	_
Lactobacillus paracasei subsp para1[6]	HM	+	+	_	+	+
Lactobacillus plantarum1[26]	HM	+	+	+	+	_
Lactobacillus paracaseisubsp para2[29]	HM	+	+	_	+	+
Lactobacillus paracasei subsp paracasei3[39]	HM	+	+	_	+	+
Lactobacillus plantarum1[12]	HM	+	+	_	+	_

+ = Positive; - = Negative; HM = Homo-fermenter

Table 4: Adhesion assay of LAB to intestinal epithelial cells of guinea pigs

Name of LAB[CODE]	Adhesion (%)	Adhesion index	Kunu-zaki fermentingcereal
P.damnosus2 [32]	80	15	Sorghum-Millet
L.plantarum1 [33]	65	18.4	Sorghum-millet
L.paracasei subsp paracasei1 [6]	100	22	Sorghum-Millet
L.paracasei subsp paracasei2[29]	55	14.5	Acha-sorghum
L.plantarum1[26]	80	15	Sorghum
L.paracasei subsp paracasei3[39]	75	6.8	Sorghum-Millet
P.pentosaceus2 [27]	60	12	Millet
L.plantarum1[12]	70	24	Sorghum-Millet

Table 5: Inhibition zones (mm) ofhomo-fermenting LAB against selected human pathogens

	Zones of inhibition (mm) Reference pathogens			
LAB significant taxa	***E. coli***	***S. aureus***	***P. aeruginosa***	***E. faecalis***
L. plantarum1 [33]	21.00±1.00$^{\text{def DEF}}$	24.17±0.76^{gDEFGH}	23.17±1.26^{efghiDEFGH}	17.83^{C}±0.76^{defg}
P. pentosaceus2 [27]	28.00±1.00$^{\text{jk GHIJ}}$	22.17±0.289$^{\text{f EFG}}$	22.33±1.15^{cdefgCDEFG}	17.83^{E}±0.29^{defg}
P. damnosus [32]*p*	19.67±1.53$^{\text{de BC}}$	20.67±0.58$^{\text{de BCDEF}}$	18.67±0.71^{abcAB}	0.00^{A}±0.00^{a}
L. paracasei subsp para2 [29]	11.00±1.00$^{\text{b B}}$	22.50±0.5$^{\text{f EFGH}}$	23.83±0.76^{cdefEFGH}	12.83^{C}±0.29^{b}
L. plantarum1 [26]	22.83±1.76$^{\text{gh DEFGHI}}$	26.27±1.00^{hiGHI}	21.07±1.10^{ghiBCDEF}	24.17^{HIJ}±0.29^{b}
L. paracasei subsp para1 [6]	28.00±1.00$^{\text{jk IJ}}$	24.00±0.76^{gFGH}	25.00 ±1.32^{ijkGH}	14.17^{C}±0.29^{i}
L. paracasei subsp para3 [39]	23.33±2.8^{hiH}	20.00±1.00^{dBCDEF}	29.00±.00^{bcdeHI}	14.00^{C}±0.50^{bcd}
L. plantarum1 [12]	15.00±1.0^{cBC}	20.50±0.89^{deBCDEF}	20.17±0.28^{bcdefBCD}	18.00^{CD}±0.52^{efg}

Values are means of duplicate readings.

Values with the same superscripts across columns in small letters and down rows in capital letters are not significantly different

Table6: Antibiotic reaction of viable kunu-zakihomo-fermenting LAB

NAME OF LAB /CODE	Amoxicillin 25µg	Erythromycin 10µg	Ciprofloxacin 10µg	Cotrimoxazole 25µg	Gentamycin 10µg
L.plantarum1[33]	R	13±0.7	R	12±1.1	R
L. paracasei subsp para1 [6]	R	15±1.4	R	R	R
L.paracasei subsp para2[29]	R	7±1.1	R	R	R
L. plantarum1 [26]	R	R	R	R	R
L.paracasei subsp para3[39]	R	R	R	R	R
P. pentosaceus2 [27]	R	13±1.1	R	9±2	R
L. plantarum1 [12]	R	10±1.1	R	R	R
P. damnosus[32]	R	R	R	14±1	R
S. aureus	R	R	20±2	17±1	R
E. coli	8±1.1	R[NIT]	22±1	R	12±0.3

Values (mm) represent growthinhibition zones
Values aremeans of duplicate readings with standard deviations.
R= Resistant; NIT= Nitrofurantoin;

The interpretive criteria used according to the manufacturer's instructions were:-

Inhibition Zone diameter (mm)
Susceptible : ≥ 10mm
Intermediate: 6-9mm
Resistant: ≤5mm
Resistant breakpoint = 5mm
Susceptible breakpoint= 10mm

Table1explains the appearance of the resulting kunu- zaki beverages from the different cereals and their final pH. The appearance of kunu-zaki, along with its acid taste is one of the reasons why the drink has remained attractive to consumers. The low pH in fermented foods is as a result of the production of lactic acid, the end product of the metabolism of the principal fermenting organisms. Because of their low pH, fermented foods are judged to be safe. The pH of the kunu-zaki types produced in this present study ranged from 4.04-5.41. This concurs with values that have been obtained by other previous research work Amusa and Ashaye[16b]. Table 2 which

describes the cultural characteristics and identification of the LAB isolated from the different kunu zaki types gives the identification accuracy(99.3%-99.9%). The fermentation strips were read with the API web^{TM} BioMerieux(France) software which gave confidence as the percentage accuracy indicated a 'very good" identification to "excellent" identification. Table 3 displays the physicochemical properties of these LAB. The findings of this research indicate that *Pediococcus pentosaceus* 2[27] was able to grow at all conditions of temperature and NaCl concentrations tested. Majority of African spontaneously fermented foods take place at room temperatures and kunu-zaki is no exception. Halting the fermentation process requires the use of high temperatures after which cooling takes place before "back-slopping"(Fig 1)."Back slopping" is the use of a portion of freshly fermenting product to start or continue a new fermentation batch of the same product. Most traditional African fermentation processes use 'back-slopping' to ensurea continuous fermentation success. The scientific principle is that in an actively fermenting product, the population of actively fermenting organisms is quite high. The new fermentation process will therefore proceed with a less likelihood of failure if a portion of the actively fermenting product is added to it. The disadvantage of "back-slopping" however is that even though the fermentation proceeds without failure, products of inconsistent quality are often obtained from batch to batch. Kunu zaki production is heavily dependent on the method of "back-slopping" (Fig 1). The development of starter cultures for kunu-zaki is therefore an idea whose time has come.It is important to study the ability of homo-fermenting LAB to grow at different temperatures Odunfa [17]. From this study, *Pediococcus pentosaceus* 2[27] and *L.plantarum1*[26] were both able to grow at 45^{0}C. This would make both LAB isolates likely candidates as starter cultures for the large scale production of kunu-zaki under controlled fermentation conditions. All LAB isolates with the exception of *Pediococcus damnosus* were able to grow at 4.5% NaCl concentrations. The introduction of NaCl is also sometimes used in African fermentations to halt fermentation processes,Odunfa[17]. In the event of choice of starter cultures for use in controlled fermentations, ability to withstand NaCl concentrations becomes important Leroy and DeVuyst[3].Table 4shows the adhesion percentage of the various LAB isolates to intestinal cells *in vitro*. The highest adhesion in this study was demonstrated by *L.paracasei subsp paracasei1*[6].Adhesion to the GI tract has been widely used as a criterion for the selection of health benefitting lactobacilliCollins and Sullivan[18].Many bacteria use their ability to adhere as a strategy to maintain stability in their numbers. Some studies have shown

that the high capability of adhesion of a certain microorganism indicates that nutrients can be competed for with a higher efficiency than non-adherent bacteria and in this way adherent bacteria can out-number the non-adherent ones.The adhesion to epithelial surfaces is a critical step in the colonization of *Lactobacillus* in the intestineand one of the suggestedmechanisms by which they could protect the intestines from the colonization of pathogens is by their competition for the receptor sites for adhesion. The entire homo-fermenting LAB investigated in this study gave high adhesion capabilities*in vitro*. This agreed with the work of Otero and Nader-Macias [15]. Table 5 shows that all the homo fermenting LAB isolates were able to inhibit indicator pathogens with inhibition zones (11-28mm). *P.pentosaceus2* was however unable to inhibit *E.faecalis*. These results agree also with the findings reported byAshenafi *et al*. [19] andOdugbemi *et al*.[20]where LAB isolates from various fermented foods displayed antimicrobial activity towards *S.aureus* and *E.coli* respectively. The findings of this study suggest that thesehomo-fermenting LAB isolates from kunu-zaki would be beneficial to the gastrointestinal tract of humans when kunu-zakiisconsumed. This conclusion also tallies with the observations from the work ofDe Vries *et al*.[2].The introduction of health benefitting products into the market, and the use of these specific organisms as starter cultures,has however constantly raised the question of antibiotic resistance andwhat possible interactions may occur if a consumer is taking such a product simultaneously with antibiotic therapy,Mathur and Singh[21]. Plasmids are genetic elements independent of the cell chromosome. Over time,some plasmids have developed genes that make them resistant to selected antibiotics. Plasmids are easily transferred between cells, and this allows the efficient spread between bacteria of resistance to an antibioticPerreten *et al*.[7]. The number of antibiotics now available and their widespread use in prescription drugs and for general disinfection purposes seems to have increased microbial potential for the development of antibiotic resistance. Table 6 shows the antibiotic reaction pattern of the homo-fermenting LAB isolates to five selected clinically common antibiotics used to treat infections in Nigeria. In this study,all the homo-fermenting LAB isolates were resistant to amoxicillin, gentamycin and ciprofloxacin. However, these homo-fermenters were susceptible to erythromycin with the exception of*L.plantarum 1*[26], *L paracasei subspparacasei*3[39] and.*P. damnosus 2*[32]. Homo-fermenters that were susceptible to cotrimoxazole were *P.pentosaceus*2[27],*L.plantarum*1[33] and *P.damnosus*. The finding of this research is significant. From the result of the adhesion study, the implication is that a

regulardrinkerof kunu-zaki(using LAB either singly or in combination as starter culture)may likely have a high colonisation of adherent homo-fermenting starter culture LAB within his/her intestine. Furthermore,these homo-fermenters would also likely inhibit the specific pathogens tested in this study. If the consumer is on any of these antibiotic therapies, the antimicrobial activity of the LABwill likely remain unaffected by the antibiotic.However, resistance to commonly used antibiotics would raise concerns of the possibility kunu-zaki containing antibiotic-resistant LAB bacteria passing antimicrobial resistance genes or genes that encode for virulence factors ontoresident bacteriaLei and Jacobsen [22].The findings of this research contradict the findings reported byKetema *et al*. [23]that reported LAB isolated from *wakalim*, a traditional fermented beef sausagesusceptible to all antibiotics tested. Strains intended for use in the food systems as starter should be carefully examined for antimicrobial susceptibilityTeuber *et al*. [24]. Lactobacilli are known to possess naturally a wide range of antibiotic resistance but in most cases the antibiotic resistance is not of the transmissible type as suggested byLei and Jacobsen [22]. Of the antibiotics tested by Olukoya *et al*. [25],indigenous lactobacilli were resistant to cloxacillin, penicillin and streptomycin. This is comparative to the pattern revealed in our study. Also, Abdulkadir *et al*. [26] investigated the antimicrobial pattern of LAB isolated from fermented milk in Ethiopia. Their work noticed resistance to methicillin by all strains of LAB tested but they concluded that the LAB could not consist of reservoirs for transmissible genes of erythromycin or streptomycin resistance based on their susceptibility. Homo-fermenters are usually preferred to hetero fermenters as choice starter cultures for fermented foods since the end product of their metabolism is lactic acid only. The result of this studydocuments findings on the antibiotic resistance pattern of these eight homo-fermentingLAB present in ready to drink kunu-zaki. If these homo-fermenting strains are to be used in kunu-zaki as starter cultures, it is important that they should be further carefully examined for inability to transfer antibiotic resistance genes to food pathogens.

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