



Full Length Research Paper

***In vitro* screening for human probiotic potentials of *Lactobacillus tuccei*  
CECT 5920 and *Lactobacillus mindensis* TMW isolated from Nigerian  
Fermented foods**

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**ABSTRACT**

*In vitro* Probiotic potentials of Lactic acid bacteria (LAB) isolated from traditional fermented foods namely: *ugba*, *ogi*, fermenting cassava and *kunu-zaki* were studied. Twenty five (25) samples each of the four types of fermented foods were serially diluted in sterile peptone water 0.1ml aliquots of appropriate dilution was streaked on De Man Rogosa Sharpe (MRS) agar containing 50mg of nystatin for the isolation of LAB. 48 LAB isolates were recovered from the samples these were screened for bacteriocin production by the Agar Well Diffusion assay and two best bacteriocin producers characterized by molecular method as *Lactobacillus tuccei* CECT 5920 and *Lactobacillus mindensis* TMW were tested for their human probiotic potentials. Typed cultures of *Staphylococcus aureus* NCTC 8325 and *Escherichia coli* 0157:H7 were used as test pathogens. *L. tuccei* CECT 5920 and *L. mindensis* TMW had the same level of bacteriocin production and antimicrobial activity ( $P \leq 0.05$ ). The LAB isolates resisted the pH range of 2-8 for 24 hrs while higher bile salt assimilation was shown by *L. tuccei* CECT 5920. Both LAB strains tolerated pepsin enzyme after 72 hrs. Cholesterol assimilation was better with *L mindensis* TMW. Both LAB strains did not show any haemolytic effect. *L. tuccei* CECT 5920 was sensitive to Cotrimoxazole while *L mindensis* was resistant to all the antibiotics tested. *L. tuccei* CECT 5920 gave better results as a LAB isolate with better probiotic potentials than *L mindensis* TMW.

**Keywords:** Bacteriocin, fermented food, *in vitro*, LAB, probiotic.

## INTRODUCTION

Traditional food fermentation is a food preservation method intended to extend shelf-life, improve palatability, digestibility and the nutritive value of food by the activities of naturally occurring microorganisms especially Lactic acid bacteria (LAB). Fermentation of various foods by LAB is one of the oldest forms of bio-preservation practiced by mankind. Bacterial antagonism has been recognized for over a century but in recent years this phenomenon has received more scientific attention, particularly in the use of various strains of lactic acid bacteria. One important attribute of LAB is their ability to produce anti-microbial compounds called bacteriocins (Lindgren and Dobrogosz, 1990).

An increasing interest exists for fermented food containing specific species of LAB (probiotics) with potential health-improving properties in human and animal intestinal tract (Holzapfel *et al.*, 1998). Probiotics are emerging as an important new therapy for prevention and treatment of infectious diseases mainly gastrointestinal infections, deconjugation of bile salt and reduction in the assimilation of cholesterol in the body. This work aimed at evaluating the in vitro probiotic potentials of lactic acid bacteria isolated from traditional fermented food.

## MATERIALS AND METHODS

### *Sample collection and analyses*

Twenty five samples of each traditional fermented foods: *ugba* (fermented oil bean seed), *ogi* (pap) and *kunu-zaki* (a non-alcoholic beverage produced from millet and spices) were purchased from the retailers at Umuahia main market while fermented cassava samples were collected from the local producers using sterile spatula into universal sterile bottles. The samples were packaged inside a cooler containing ice cubes and quickly transported to the laboratory for analyses. One gram each of the solid food samples was homogenized in 0.1% peptone water while 1 ml of *kunu-zaki* was serially diluted. 0.1ml aliquots of appropriate dilutions were inoculated onto De Man Rogosa and Sharpe (MRS, Oxoid, England) agar medium fortified with 50mg of nystatin (Roissart and Luguët, 1994) for the isolation of lactic acid bacteria (LAB). The plates prepared in triplicates were incubated at 35°C for 48 hrs anaerobically (using anaerobic gas packs) for isolation of mesophilic LAB. The mixed isolates were sub-cultured on MRS agar plates and the pure cultures were stored on MRS agar slants at 4°C. All the isolates recovered were maintained by by-weekly sub-culturing on MRS agar for 48hrs (Cheesbrough, 2004)

### *Culture identification*

The cultures were identified by observing the colonial morphologies, microscopy, biochemical, sugar fermentation tests (Cheesbrough, 2004; Bergey, 1957) and by (GTG)5-PCR and rDNA sequencing for molecular identification using two primers: RIBOS 1 with sequence: GGACTACAGGGTATCTAAT and RIBOS 2 with sequence: AGAGTTTGATCCTGG.

***Test bacterial pathogens***

Typed Cultures of *Escherichia coli* 0157:H7 and *Staphylococcus aureus* NCTC 8325 procured from the Veterinary Department of National Root Crops Research Institute, Umudike, Abia state were used as test isolates.

***Screening for bacteriocin production and partial purification***

Forty-eight LAB recovered from the fermented food samples were screened down to 10 species after gram staining, biochemical, sugar fermentation tests and molecular characterization. They were screened for bacteriocin production (Lasta *et al.*, 2008) by inoculating 10 ml of each of the LAB isolates into 500 ml of MRS broth in a 1000 ml glass beaker previously sterilized by autoclaving at 121 °C for 15 min. The broth was incubated at 37 °C for 48 h for bacteriocin production. Two LAB isolates identified as *Lactobacillus tuccei* CECT 5920 and *Lactobacillus mindensis* TMW were picked as the best bacteriocin producers and used for further studies in this work. After the incubation, the bacteriocin was partially purified by aseptically collecting and centrifuging 100 ml of the culture broth at 10,000 rpm for 15 min so as to separate the microbial cells. The pH of the cell free supernatant (CFS) thus obtained was adjusted to 6.5-7.0 with 1N NaOH to neutralize the organic acids while the inhibitory activity of hydrogen peroxide was eliminated by the addition of 5 mg/ml catalase (c-100 bovine liver) (Daba *et al.*, 1991).

***Studies on probiotic potentials LAB******Determination of antimicrobial activity of partially purified bacteriocin***

The test bacteria were inoculated in 100 ml of peptone water in a 200 ml glass beaker and incubated for 18 h at 37 °C. The test bacteria broth was then matched against 0.5 Mcfarland Standard to obtain a concentration of  $1.0 \times 10^6$  CFU/ml and were then seeded aseptically on the surface of Mueller-Hinton Agar (Oxoid, England) previously prepared using sterile swab sticks. Three mm deep wells were made on the Mueller-Hinton agar using sterile cork borer (4 mm wide) and the 40 µl of the Lactic acid bacteria's CFS was aseptically placed into each agar well using sterile micropipette. The inoculated plates were kept at room temperature for 2 h and then incubated with the test pathogens at 37 °C for 24 h. The ability of the Lactic acid bacteria isolates to inhibit growth of the test bacteria was indicated by a zone of inhibition along its growth line (Joshi *et al.*, 2006).

***Acid tolerance by LAB isolates***

By applying the method proposed by Pereira *et al.* (2003) with slight modifications, homogenized samples of lactic acid bacteria isolates were inoculated (1 %, v/v) into acidified MRS broth previously adjusted to pH of 3, 4, 5, 6, 7 and 8 using 1N HCl and incubated at 35 °C for 72 h. After incubation, 1 ml of the inoculated broth was serially diluted in peptone water (0.1 % w/v) and pour plated (Dave and Shah, 1997). The plates were incubated at 35 °C for 24 h to ascertain the viability of the lactic acid bacteria. The cultures were designated positive (+) for growth and negative (-) for no growth. The control had the lactic acid bacteria isolates

incubated in MRS broth without acidification (Md *et al.*, 2007).

### ***Bile salt assimilation from culture medium***

This was determined by inoculating 18 h old LAB cultures into MRS broth (1 % v/v) containing 0.2, 0.3, 0.5 and 1 % (w/v) concentrations of bile salt (Sodium taurocholate, Koch-Light Laboratories Ltd, England) and incubated at 35 °C for 12 h. The control was prepared without bile salt (Dora *et al.*, 2002). After incubation, the cultures were centrifuged and unutilized bile salt in the supernatant was estimated (Zlatkis *et al.*, 1953). The bile salt tolerance was determined after using the formular:

$$\% \text{ bile salt assimilated} = \frac{(a - b)}{a} \times 100 \%$$

a = initial concentration of bile salt in the medium.

b = final concentration of bile salt left in the medium after 12 hours of incubation

### ***Determination of pepsin tolerance by LAB isolates***

#### ***Effect of pepsin***

The pH of 20 ml of Lactic acid bacteria's CFS was adjusted with 1N NaOH to 6.5-7.0 so as to neutralize the organic acids while the inhibitory activity of hydrogen peroxide was eliminated by the addition of 5 mg/ml catalase (c-100 bovine liver). A 5-ml aliquot of the pH adjusted CFS was aseptically

transferred into test tubes and mixed with protease enzyme at 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 mg/ml concentrations respectively. The test tubes with and without the enzyme (control) were incubated for 2 h at 37 °C (Rajaram *et al.*, 2010). Both the control and the samples were assayed for antimicrobial activity by using well diffusion method as described earlier. The result was reported as either sensitive or resistant.

### ***Cholesterol assimilation from culture medium by LAB***

MRS broth supplemented with 10, 20, 30, 40 and 50 % concentrations (w/v) of cholesterol (BDH Laboratory Supplies, England) was inoculated with 1 % of 18 h old bacteriocin-producing lactic acid bacteria broth and incubated at 35 °C for 20 h (Liong and Shah, 2005) to determine the removal of cholesterol from culture medium. After incubation, the cultures were centrifuged and unutilized cholesterol in the supernatant was estimated (Zlatkis *et al.*, 1953). The result was determined using the formular:

$$\% \text{ cholesterol assimilated} = \frac{(a - b)}{a} \times 100$$

a = initial concentration of cholesterol in the medium.

b = final concentration of cholesterol left in the medium after 20 h of incubation.

### ***Determination of haemolytic activity of LAB***

Haemolytic activity of the lactic acid bacteria isolates was evaluated on Blood agar base

plates (Oxoid). Each bacterial suspension was streaked on the blood agar plates and incubated for 24 h at 37 °C. After the incubation, the plates were examined for signs of  $\beta$ -haemolysis (clear zones around the colonies),  $\alpha$ -haemolysis (a green-hued zone around the colonies) or  $\gamma$ -haemolysis (no halo around the colonies) (Maragkoudakis *et al.*, 2009).

#### ***Antibiotic susceptibility of LAB***

Bacteriocin-producing Lactic acid bacteria isolates were inoculated into MRS broth (Hi-Media, India), individually and incubated for 18 h and was matched with a 0.5 McFarland standard to give a concentration of  $1.0 \times 10^6$  CFU/ml. The pH of the broth was then adjusted to 6.5-7 using 1N NaOH to neutralize the organic acids and the inhibitory activity of hydrogen peroxide was stopped by the addition of 5 mg/ml catalase. The broth was evenly streaked with sterile swab sticks on the surface of Muller Hinton agar plates that was previously prepared and seeded with culture broth of lactic acid bacteria isolates. Antibiotic discs that contained eight antibiotics namely Cotrimoxazole, Cloxacilin, Erythromycin, Gentamycin, Augmentin, Streptomycin, Tetracycline and Chloramphenicol were aseptically placed upside down on the agar using sterile forceps, firmly pressed and kept for 15 min with the lid slightly opened. The plates were then incubated at 37 °C for 24 h and the zones of inhibition measured (Vijai *et al.*, 2004).

#### **STATISTICAL ANALYSIS**

Mean separation was done using Duncan Multiple range test using Statistical Package for Social Sciences (SPSS) version 20.

Differences in statistical significance were considered at  $P \leq 0.05$  and  $n=3$ .

## **RESULTS**

### ***Studies on cultural characteristics LAB isolates***

#### ***Phenotypic characterization of LAB isolates from fermented foods.***

Table 1 shows the LAB isolated from the food samples were distributed into five genera namely *Leuconostoc*, *Lactobacillus*, *Lactococcus*, *Pseudochrobactrum* and *Streptococcus* and ten species. The four bacteriocin-producing LAB were identified as *Pseudochrobactrum asaccharolyticum*, *P. saccharolyticum*, *L. tucetii* cect 5920 and *L. mindensis* TMW

#### ***Diversities of LAB isolates***

In Table 2, the diversity of the ten LAB isolates is presented with the highest number of LAB isolates (7) from *ugba* while the lowest number of isolates (3) was from fermenting cassava. *L. mindensis* TMW was the most isolated LAB (38 %) while *L. plantarum* occurred least (6.4 %) in the four food samples.

### ***Studies on probiotic potentials LAB isolates***

#### ***Antimicrobial activity of partially purified bacteriocin extract (mm).***

Table 3 shows that the antimicrobial activity of partially purified bacteriocin extract of *L. tucetii* CECT 5920 statistically had same level of inhibitory activity against both test bacteria. *L. mindensis* TMW also had equal

level of antimicrobial activity against the two test pathogens.

***Acid tolerance of lactic acid bacteria isolates***

In Table 4, result shows that the two LAB tolerated the pH of the medium of 3-8 and grew in it for 10 h of incubation.

***Determination of bile salt resistance by lactic acid bacteria isolates (%)***

Figure 1 shows the bile salt resistance of the two LAB in broths containing 0.2-1.0 % Ox-bile salt. *L. tuccei* CECT 5920 showed 54.06 % resistance at 0.35 % concentration while *L. mindensis* TMW showed less than 54.06 % resistance at 0.35 % concentration.

***Pepsin tolerance by LAB isolates (mm)***

Table 5 shows that when the two LAB were cultured in the presence of pepsin enzyme at 0.1-1.0 mg/ml concentrations, they showed moderate growth after 24 hrs and heavy

growth after 72 hrs. Pepsin enzyme did not stop the growth of the LAB isolates.

***Cholesterol assimilation from culture broths by Lactic acid bacteria isolates***

In Figure 2, *L. mindensis* TMW absorbed 15.91 mg/ml from the growth medium while *L. tuccei* CECT 5920 absorbed 6.21 mg/ml both at 30 % concentration.

***Haemolytic activity of lactic acid bacteria isolates***

Table 6 shows that none of the two LAB showed any haemolytic activity on blood agar.

***Antibiotic susceptibility pattern of lactic acid bacteria isolates***

Table 7 shows that *L. tuccei* CECT 5920 was sensitive to cotrimoxazole out of the eight antibiotics while *L. mindensis* TMW was resistant to the eight antibiotics.

**Table 1: Phenotypic and biochemical identification of lactic acid bacteria isolates**

S/N	Colonial Morphology	Gram reaction	Catalase	Motility	Coagulase	Growth at 37°C	Growth at 45°C	4% NaCl	6.5% NaCl	Glucose	Sucrose	Maltose	Fructose	Lactose	Mannitol	Galactose	Saccharose	Oxidase	Methyl red	Gas production	V. P	Isolate
1	Flat, creamy Mucoid	+rods	-	-	-	+	-	+	-	+	+	+	+	+	+	+	-	-	+	-	-	<i>Lactobacillus casei</i>
2	Circular, milky, Mucoid	+rods	-	-	-	+	+	+	-	+	+	+	+	+	+	+	-	-	+	-	-	<i>Pseudochrobactrum asaccharolyticum</i>
3	Creamy, Mucoid	+cocci	-	-	-	+	-	-	-	+	+	+	+	+	+	+	-	-	+	-	-	<i>Pseudochrobactrum saccharolyticum</i>
4	Circular, milky, slimy	+cocci	-	-	-	+	-	-	+	+	+	+	+	+	+	+	-	-	+	-	-	<i>Leuconostoc mesenteroides</i>
5	Circular, creamy, convex	+rod	-	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	<i>Lactococcus plantarum</i>
6	Circular, milky, slimy	+cocci	-	-	-	+	+	-	-	+	+	+	-	+	+	+	-	-	+	-	-	<i>Streptococcus thermophilus</i>
7	Circular, milky, Mucoid	+rod	-	-	-	+	+	-	-	+	-	+	+	+	+	+	-	-	+	-	-	<i>Lactobacillus acidophilus</i>
8	Flat, milky, slimy	+rod	-	-	-	+	+	+	+	+	-	+	-	+	+	+	-	-	+	-	-	<i>Lactobacillus helveticus</i>
9	Circular, creamy, convex	+rod	-	-	-	+	+	-	-	+	+	+	+	+	+	+	-	-	+	-	-	<i>Lactobacillus tuccei</i> CECT 5920
10	Flat, membranous	+rod	-	-	-	+	+	-	-	+	+	-	+	+	+	+	-	-	+	-	-	<i>Lactobacillus mindensis</i> TMW

Positive reaction (+), Negative reaction (-)

**Table 2: Microbial diversity in fermented foods and bacteriocin activity**

S/No	Fermented food	Lactic acid bacteria isolate	Number of species/percentage	Bacteriocin activity
1	Cassava	<i>Pseudochrobactrum saccharolyticum</i>	10(4.0)	+
		<i>Lactobacillus acidophilus</i>	5(2.0)	-
		<i>Lactobacillus helveticus</i>	8(3.2)	-
2	Ugba	<i>Lactobacillus casei</i>	18(7.2)	-
		<i>Pseudochrobactrum asaccharolyticum</i>	17(6.8)	+
		<i>Leuconostoc mesenteroides</i>	16(6.4)	-
		<i>Lactococcus plantarum</i>	8(3.2)	-
		<i>Lactobacillus acidophilus</i>	10(4)	-
		<i>Lactobacillus tuccei</i> CECT 5920	12(4.8)	+++
		<i>Lactobacillus mindensis</i> TMW	20(8.0)	+++
3	Ogi	<i>Pseudochrobactrum asaccharolyticum</i>	17(6.8)	+
		<i>Lactococcus plantarum</i>	8(3.2)	-
		<i>Streptococcus thermophilus</i>	9(3.6)	-
		<i>Lactobacillus acidophilus</i>	10(4.0)	-
		<i>Lactobacillus tuccei</i> CECT 5920	10(4.0)	+++
		<i>Lactobacillus mindensis</i> TMW	18(7.2)	+++
		<i>Pseudochrobactrum saccharolyticum</i>	10(4.0)	+
4	Kunu-zaki	<i>Leuconostoc mesenteroides</i>	16(6.4)	-
		<i>Streptococcus thermophilus</i>	9(3.6)	-
		<i>Lactobacillus helveticus</i>	20(8.0)	-
		Key: (-) No inhibition; (+) Low inhibition; (+++) High inhibition.		

**Table 3: Diameter of clearance zone of partially purified bacteriocin (mm)**

<i>Lactobacillus tuccei</i> CECT 5920		<i>Lactobacillus mindensis</i> TMW	
<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>
19±0.08 <sup>a</sup>	18±0.08 <sup>a</sup>	18±0.08 <sup>a</sup>	17±0.07 <sup>a</sup>
*Negative Control	0	0	0

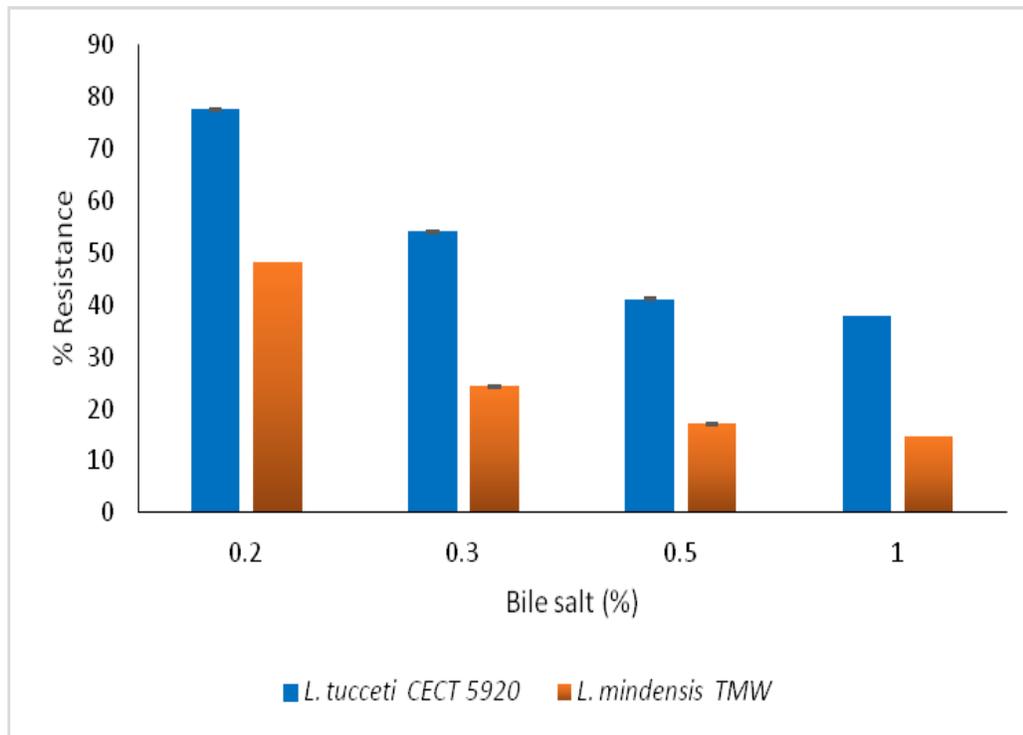
<sup>a</sup>Data values with the same letters are not significantly different (P≤0.05).

\*Negative Control: MRS broths without LAB isolates

**Table 4: Acid tolerance of LAB isolates**

pH	<i>L. tuccei</i> CECT 5920					<i>L. mindensis</i> TMW				
	Time (h)									
	2	4	6	8	10	2	4	6	8	10
3.0	+	+	+	+	+	+	+	+	+	+
4.0	+	+	+	+	+	+	+	+	+	+
5.0	+	+	+	+	+	+	+	+	+	+
6.0	+	+	+	+	+	+	+	+	+	+
7.0	+	+	+	+	+	+	+	+	+	+
8.0	+	+	+	+	+	+	+	+	+	+

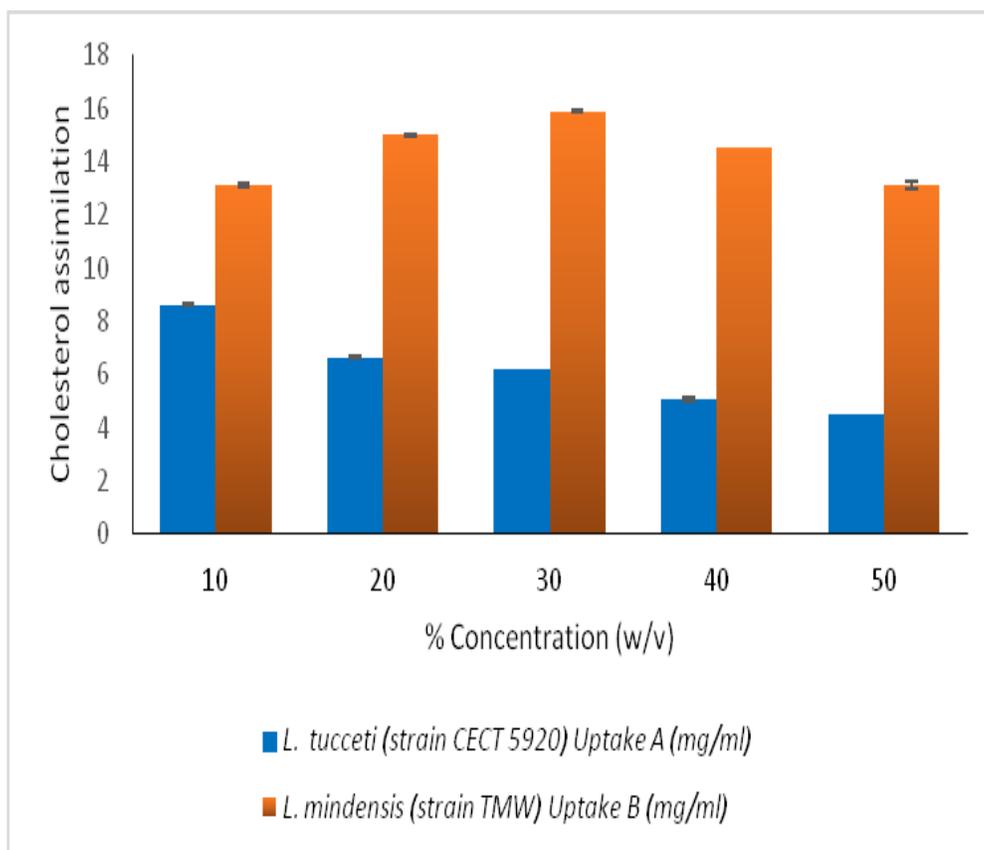
Key: + means growth.



**Fig. 1: Determination of bile salt resistance by LAB isolates (%)**

**Table 5: Pepsin tolerance by LAB isolates**

ISOLATES	VIABILITY OF ISOLATES					
	WITH ENZYME			CONTROL (NO ENZYME)		
	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
<i>L. tuceti</i> (strain CECT 5920)	++	+++	+++	+++	+++	+++
<i>L. mindensis</i> (strain TMW)	++	++	+++	+++	+++	+++



**Fig. 2: Cholesterol uptake by LAB isolates (mg/ml)**

**Table 6: Haemolytic effect of LAB isolates**

Isolate	Reaction (Haemolysis)
<i>L. tucseti</i> (strain CECT 5920)	$\gamma$ -haemolysis (no halo around colonies)
<i>L. mindensis</i> (strain TMW)	$\gamma$ -haemolysis (no halo around colonies)

**Table 7: Antibiotic susceptibility pattern of LAB isolates (mm)**

	Cotrimoxazole (25 $\mu$ g)	Cloxacilin (30 $\mu$ g)	Erythromycin (10 $\mu$ g)	Gentamycin (10 $\mu$ g)	Augmentin (30 $\mu$ g)	Streptomycin (10 $\mu$ g)	Tetracycline (30 $\mu$ g)	Chloramphenicol (30 $\mu$ g)
<i>L. tucseti</i> CECT 5920	18.06 $\pm 0.08$	12.02 $\pm 0.02$	8.10 $\pm 0.14$	8.02 $\pm 0.02$	8.06 $\pm 0.08$	8.07 $\pm 0.09$	8.02 $\pm 0.03$	10.06 $\pm 0.08$
<i>L. mindensis</i> TMW	8.05 $\pm 0.07$	8.06 $\pm 0.09$	9.06 $\pm 0.09$	9.02 $\pm 0.03$	8.07 $\pm 0.09$	8.06 $\pm 0.08$	8.01 $\pm 0.01$	8.02 $\pm 0.02$

Values are means of three replicates  $\pm$  standard deviation (SD).

## DISCUSSION

The present investigation was aimed at determining the *in vitro* probiotic potentials of lactic acid bacteria isolated from traditional fermented food samples. Results showed that 48 isolates were recovered from the 100 samples of traditional fermented food

collected. The preliminary identification of isolated lactic acid bacteria were in agreement with almost each and every study on lactic acid bacteria preliminary identification (Sudi *et al.*, 2008). Non-production of gas by the isolates suggests that they are homo-fermentative lactic acid

bacteria producing only organic acids without gas in growth medium.

*Lactobacillus*, *Leuconostoc*, *Lactococcus* and *Streptococcus* have been isolated from traditional fermented foods in the past (Oyedemi *et al.*, 2013; Oyewole and Isah, 2012) by morphological and biochemical characterization. However, *Lactobacillus tuccei* CECT 5920, *L. mindensis* TMW, *Pseudochrobactrum asaccharolyticum* and *P. saccharolyticum* have not been reported before now as lactic acid bacteria isolated either from Nigerian traditional fermented foods or fermented foods from any other part of the world. This could be due to the type of fermented foods used or the environment of the study.

The highest in diversity of isolates shown by *L. mindensis* TMW in all the traditional fermented food samples indicates that this isolate has become ecologically and physiologically adapted to the fermentation of these food samples. *Ugba* having the highest diversity of lactic acid bacteria could be due to its high proteinous content which supported the greatest number of lactic acid bacteria compared with the other traditional fermented foods sampled. However, contrary to this finding, Muller (2000) showed that *Pediococcus acidilactici*, *L. plantarum*, *L. fermentum*, *L. lactis* and *Leuconostoc mesenteroides* as the most divers lactic acid bacteria isolated from traditionally fermented maize gruels from five different western states of Nigeria respectively

Some factors might have influenced the low number of isolations of bacteriocin-producing lactic acid bacteria such as the

culture medium, incubation conditions and genetic make-up of the isolate, or the sensitivity methods used in determining the antimicrobial activity of the isolates. Wang *et al.* (2010) reported that cell aggregation and medium composition can affect bacteriocin production by LAB.

Bacteriocin production is considered an important probiotic attribute of lactic acid bacteria in the present search for human probiotic LAB. Bacteriocins produced by these isolates have broad spectrum activity against gram positive and gram negative bacteria (*S. aureus* and *E. coli* respectively). Similar results were recorded by Adesokan, *et al.*, (2009) against *S. aureus*, *E. coli* and *Pseudomonas aeruginosa*. Their antagonistic property is attributed to the low pH, the un-dissociated acid and production of other primary and secondary antimicrobial metabolites (Ten Brink *et al.*, 1994). Lactic acid bacteria have potentials to inhibit the growth of pathogenic and spoilage bacteria and the possibilities exist for their use as probiotic LAB.

The tolerance of the isolates to low pH proves they can be used as human probiotics as they can tolerate the acidic nature of the stomach during movement to the intestine. Naoual *et al.*, (2011) reported that the strains of lactic acid bacteria they tested tolerated three hours of acid exposure with pH 2.0 and pH 3.0 and this is in agreement with the findings from this research. This finding is also in agreement with the reports of Charteris *et al.*, (1998), Petros *et al.*, (2006) and Burns *et al.*, (2008). Low acidity is known as the most negative factor that effects the growth and viability of lactobacilli during their passage

through the stomach (Charteris *et al.*, 1998). Because, the pH in human stomach ranges from 1.5 to 4.5 depending on the intervals of feeding or the food variability, and from the duration of food digestion which can take up to 3 hr, some authors proposed that strains intended for probiotic purposes should be screened according to their tolerance to pH 2.5 in an HCl-acidified culture medium during four hours (Pennacchia *et al.*, 2004; Klingberg *et al.*, 2006).

The bile salt lowering effect recorded by the LAB isolates in this work is an indication of their probiotic potential. According to Gilliland *et al.*, (1984) the concentration 0.3% bile salts is considered as critical for resistant strains screening and the same level is critical for the human probiotic's selection. Because of their similarity, a value of 0.3% Oxgall (Ox-bile) solution is the most used to substitute human bile salts (Brashears *et al.*, 2003). Bile salt assimilation is an important probiotic potential of LAB in view of literature support that has accredited bile salt assimilation to some human probiotic bacteria. The bile salt-lowering effect of *Lactobacillus* spp. is by several means through bile salt hydrolase (BSH) activity (Begley *et al.*, 2006). BSH is the enzyme responsible for bile salt de-conjugation during entero-hepatic circulation (Pereira *et al.*, 2003; Moser and Savage, 2001). Sasithorn (2010) evaluated the probiotic and bile salt-lowering property of *Lactobacillus* spp. isolated from non-human origin. Four strains tolerated 0.30% (w/v) bile salt during the 2 hrs of growth and this result is in agreement with the findings here. Naoual *et al.*, (2011) found that ten strains of lactic acid bacteria out of the lot they worked on showed

resistance to 0.3% of Ox-bile (percentage of resistance  $\geq 50\%$ ) and this report too is in agreement with the performance of *L. tuccei* CECT 5920 from this work.

The tolerance showed by the LAB isolates to pepsin enzyme indicates too that the two isolates possess another important potential required of suitable probiotic LAB for use in human body.

The LAB isolates were able to take up cholesterol from the medium. Sasithorn, (2010) reported a reduction in cholesterol levels from resting and dead cell free broth of all 4 strains that ranged between 13.11-23.28 and 11.44 -19.53%, respectively and this report agrees with the findings of this work from *L. mindensis* TMW. Lavanya *et al.*, (2010) stated that the ability of the organism to reduce the cholesterol level was due to assimilation of cholesterol within bacterial cell and increased excretion of bile salts due to de-conjugation by the bile salt hydrolase (Salminen *et al.*, 2002) into amino acid residues and free bile salts. Elevated level of certain blood lipids are a greater risk for cardiovascular disease. A few research reports describe the use of *L. acidophilus* to decrease the serum cholesterol levels in human and animals. The hypocholesteromic potential exhibited by the LAB isolates used in this work is an indication that they will perform well in the area of cholesterol reduction when used as human probiotic.

The two LAB did not exhibit haemolytic activity. This result is in agreement with Kacem and Karam (2006) who had reported that none of the *Lactobacillus plantarum* strains they worked on produced haemolysin

on sheep blood. Naoual *et al.*, (2011) did not observe any strain of lactic acid bacteria that exhibited  $\beta$ -haemolytic activity (clear zones around colonies) when grown in Columbia blood agar. They also found that while most strains were  $\gamma$ -haemolytic (no halo around colonies), only two strains LPAR3 and LPAR12 exhibited  $\alpha$ -haemolytic activity (a green-hued zone around colonies) (Maragkoudakis *et al.*, 2009). The non-haemolytic attributes of the two LAB used in this work suggests that the isolates could be good candidates as human probiotic isolates.

One of the main criteria needed to be fulfilled by a probiotic organism is, it should be non-pathogenic (Dubois *et al.*, 1956; Ljungh and Wadstrom, 2006). Result showed that the LAB isolates will resist broad spectrum antibiotic therapy when they are used as probiotics in the intestine thus ensuring their survival and establishment as part of the microflora so as to elicit their antagonistic effects on the intestinal pathogens. *L. mindensis* TMW was completely resistant to the eight antibiotics tested (Table 4.13). In their work, Lavanya *et al.*, (2011) reported that almost all the strains of lactic acid bacteria they tested were resistant to penicillin and 10% were susceptible to ampicillin, ( $\beta$ -lactam antibiotics). Zhou *et al.*, (2000a) had earlier reported that *Lactobacillus* and *Bifidobacterium* strains were susceptible to  $\beta$ -lactam antibiotics (Penicillin, ampicillin) and this may be due to the difference in sources of isolation.

## CONCLUSION

The study on the in vitro screening for human probiotic potential of the two lactic acid

bacteria: *L. tucseti* CECT 5920 and *L. mindensis* TMW revealed that both isolates had rapid acidification ability as well as production of bacteriocin with varied levels of antimicrobial activities against the two test pathogens: *S. aureus* NCTC 8325 and *E. coli* 0157:H7 used in this work. The LAB grew well over a wide pH range, were not inhibited by pepsin enzyme, assimilated bile salt and cholesterol from growth medium and did not lyse red blood cell. *L. tucseti* CECT 5920 did better than *L. mindensis* TMW, however, both LAB isolates would make good research organisms of study for in vivo studies as human probiotic LAB.

## RECOMMENDATION

The isolation of LAB with probiotic potentials from traditional fermented foods indicates that these foods possess human probiotic values. Hence, their consumption is encouraged beyond the traditional localities

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