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ORIGINAL ARTICLE

Characterization and antioxidant ability of potential probiotic lactic acid bacteria in *ogi* liquor and lemon juice-*ogi* liquor

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Abstract

Purpose *Ogi* is an indigenous edible fermented cereal slurry but the steep liquor is usually wasted or administered as therapeutic to suppress certain illnesses. The combination of lemon juice and *ogi* steep liquor (OSL) is known to possess bioactive metabolites.

Method This study evaluated potential probiotic lactic acid bacteria (LAB) in different OSL (*Zea mays*, *Sorghum bicolor*, and *Pennisetum glaucum* L.) and lemon juice-*ogi* steep liquor (LJOSL) based on low pH, bile and lysozyme tolerances, hydrophobicity and auto-aggregation, antibiotic, cholesterol removal, exopolysaccharide production, β-galactosidase, and antimicrobial and hemolytic activities using standard methods. Presumptive LAB were sequenced and assayed for radical scavenging using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and lipid peroxidation inhibitory (LPI) tests.

Results Presumptive LAB counts were higher in maize OSL (0 h:5.09 log CFU/ml) and combined cereal OSL (24–48 h:7.65 and 7.72 log CFU/ml) but decreased in all steep liquors at 72 h, except in millet OSL (7.72 log CFU/ml). A total of 120 LAB isolates were randomly selected. Based on pH and bile tolerances, 14 isolates were comparable to reference strains. All these isolates demonstrated probiotics properties except for three that did not show γ-hemolysis. Sequenced LAB isolates were identified as *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Pediococcus pentosaceus*, and *Weissella cibara*. DPPH activities of LAB gradually increased during fermentation with the highest activity of DPPH (58.77%) and LPI (57.94%) activity in *L. plantarum*. Strong correlations were found between DPPH and LPI in all the selected isolates.

Conclusion The antioxidant property of probiotic LAB in OSL and LJOSL could contribute to its therapeutic nature.

Keywords Lactic acid bacteria · Probiotics · Lemon juice-*ogi* steep liquor · *ogi* steep liquor · Antioxidant

Introduction

In most of the African countries, traditional fermented cereal foods form an integral part of the human diet because it is readily available and affordable (Obinna-Echem et al. 2014). The fermentation of cereal depends on the biological activities of microorganisms, which convert organic substrates into more desirable substances that impact preferred taste, and enhanced micronutrients. Some fermented beverages like *kunu-zaki*, *burukutu*, and *ogi* are a part of traditional and cultural values of Nigeria (Egwim et al. 2013).

Ogi is made from the fermentation of a variety of cereals but the resulting steep liquor is usually wasted. *Ogi* steep liquor (OSL) has bioactive compounds (Ojokoh 2011; Ajayeoba et al. 2016) which are traditionally believed to change gastrointestinal microbiota and suppress the growth of pathogens (Egwim et al. 2013). Lemon is known to possess medicinal properties. Lemon juice has been used in combination with other antimicrobial products such as honey and OSL for inhibiting microorganisms like *Salmonella*, *Shigella*, *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus* (Dhanavade et al. 2011; Ajayeoba et al. 2016).

The dominant microorganisms reported in OSL are represented by lactic acid bacteria (LAB) such as *Lactobacillus* spp., *Corynebacterium* spp., *Leuconostoc*, *Lactococcus*, *Pediococcus*, and *Weissella* (Ijabadeniyi 2007; Obinna-

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Echem et al. 2014; Okeke et al. 2015). The addition of lemon juice to OSL reduces its pH (Ajayeoba et al. 2016), thus creating an environment for the survival of potential probiotics. Sometimes, OSL and lemon are consumed in the raw form. Although it is believed that OSL can enhance the treatment of certain illnesses, a largely overlooked aspect of its consumption is the probiotic potentials of LAB and antioxidant activities. Therefore, this study intended to characterize the potential probiotic LAB occurring during the fermentation of OSL and lemon juice-*ogi* steep liquor, from a variety of cereal substrates, and determine the antioxidant activity of the isolates.

Materials and methods

Sample collection

White maize (*Zea mays*), white sorghum (*Sorghum bicolor*), and pearl millet (*Pennisetum glaucum* L.) were purchased from Igbona market, Osogbo, Nigeria, while lemon (*Citrus limon*) fruits were procured from Durban, South Africa. The reference strains *Lactobacillus casei* ATCC 393 and *L. plantarum* ATCC 8014, test strain *Escherichia coli* O157:H7, *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 29213, and *Salmonella enterica* ATCC 13076 were obtained from the Food Laboratory, Department of Biotechnology and Food Technology, Durban University of Technology, South Africa. The media, antibiotic discs, and chemicals used for the experiments were products of Oxoid (UK) and Sigma-Aldrich (USA).

Preparation of OSL, LJOSL, and isolation of LAB

OSL and lemon juice-*ogi* steep liquor (LJOSL) were prepared by following the method described by Ajayeoba et al. (2016). *Ogi* samples were prepared in duplicates. A total of 64 liquor samples (white maize steep liquor, white sorghum steep liquor, millet steep liquor, combined cereals liquor, lemon juice-*ogi* steep liquor samples for each liquor) were prepared separately. After preparation, the *ogi* slurry of each processed cereal was allowed to settle for 30 min and 10 ml was taken from each replicate (0 h). About 1 ml of OSL was serially diluted, cultured into 9 ml MRS (de Mann Rogosa Sharpe) broth, and incubated for 24 h at 35 °C. LAB were isolated by spread plating appropriate aliquots on MRS agar and incubating at 35 °C for 48 h. The same procedure was repeated during fermentation of each liquor at 24 h, 48 h, and 72 h respectively. Colonies that had morphologies similar to the LAB were randomly selected from the duplicates for tentative identification. Gram staining, catalase reaction, growth at different temperatures and at different NaCl concentrations, gas production from glucose, hydrolysis of arginine, and carbohydrate fermentation were studied. Potential isolates were sub-cultured and stored at − 80 °C as glycerol stocks for further analysis.

Evaluation of probiotic properties

All of the experiments were carried out in triplicate.

Acid and bile tolerances

The acid tolerance and bile tolerance tests of LAB isolates were carried out as described by Abushelaibi et al. (2017) and Bonatsou et al. (2018), respectively.

Auto-aggregation, hydrophobicity, hemolytic, and antibacterial activity

The LAB isolates were tested for auto-aggregation, cell hydrophobicity, and hemolytic and antibacterial activities according to the modified method described by Angmo et al. (2016). Auto-aggregation percentage was calculated using the formula $(1 - A_t/A_0) \times 100$, where A_t and A_0 represented the absorbance at time t and $t = 0$, respectively. N-hexadecane and xylene were employed in this study to evaluate cell hydrophobicity. Only γ-hemolytic LAB isolates were selected as potential probiotics for further analysis while others were discarded. The supernatants of 18–20-h-old LAB cells were tested against strains such as *B. cereus*, *E. coli*, *S. aureus*, *Salmonella* spp., *Pseudomonas* spp., and *L. monocytogenes*.

Antibiotic susceptibility

Antibiotic resistance of the isolates was performed according to the method of Das et al. (2016). The following antibiotic discs: penicillin G (10 units), gentamicin (30 μg), quinupristin/dalfopristin (15 μg), erythromycin (15 μg), erythromycin (15 μg), streptomycin (10 μg), tetracycline (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), and ampicillin (10 μg) were used and the resulting zone of inhibition (mm) was measured after incubation for 24 h at 37 °C.

Lysozyme tolerance and heat resistance

LAB isolates were tested for lysozyme tolerance by incubation with the enzyme at 30 °C for 3 h and heat resistance at 60 °C for 5 min according to the modified method described by Abushelaibi et al. (2017).

Exopolysaccharide production, β-galactosidase activity, and cholesterol removal

Exopolysaccharide production, β-galactosidase activity, and cholesterol removal of pure LAB isolates were determined in accordance with the method of Abushelaibi et al. (2017).

Identification of the finally selected isolates by *16S rRNA* sequencing

16S rRNA sequences of the selected isolates were amplified by PCR using 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-CGGTTACCTTGTTACGACTT-3′) primers (Altschul et al. 1997). PCR products were sequenced by Inqaba Biotech, South Africa. The resulting sequences were assembled and edited with software BioEdit 7.2.5. Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih.gov/Blast. cgi) was used to compare the consensus sequences with those deposited in the GenBank DNA database. A phylogenetic tree based on *16S rRNA* genes was constructed to determine the closest bacterial species by the neighbor joining method (Saitou and Nei 1987), using MEGA7 (Kumar et al. 2016). Sequence divergence among LAB isolates were quantified using the Maximum Composite Likelihood method (Tamura et al. 2004).

Antioxidant activity of whey from milk fermented with sequenced isolates

Each sequenced isolate was employed to ferment skimmed milk for 24 h at 37 °C. However, the radical scavenging screening activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and plasma lipid peroxidation of whey fractions were carried out only on selected isolates with higher DPPH radical activities (%), according to the modified method of Osuntoki and Korie (2010).

Statistical analysis

Data were statistically analyzed with the SPSS 22.0 version (IBM, New York, USA) and subjected to analysis of variance. Tukey's test was used to separate the differences between the means ($p \leq 0.05$) and Pearson's correlation coefficients were determined to evaluate relationships between radical scavenging activity and lipid peroxidation inhibition.

Results and discussion

Isolation, distribution, and physiological properties of LAB isolates

Although LAB have been reported in OSL (Okeke et al. 2015), the properties and health implications have not been fully reported. Out of 64 samples of fermented OSL and LJOSL, 1388 colonies exhibiting morphological characteristics of LAB were isolated (see supplementary data A1). Generally, presumptive LAB counts (log CFU/ml) progressively increased between 0 and 48 h of fermentation in the steep liquors. In addition, OSL had higher LAB counts than LJOSL. As shown in Table 1, presumptive LAB counts at 0 and 24 h were higher and significantly different ($p \leq 0.05$) in maize and combined cereal steep liquors at 5.09 and 5.02 log CFU/ml and 7.55 and 7.65 log CFU/ml respectively. At 48 h, LAB counts ranged between 7.26 and 7.73 log CFU/ml in millet steep liquor (MSL) and combined cereal steep liquor. At 72 h, LAB counts reduced in all steep liquors, except in MSL (7.72 log CFU/ml). The occurrence of LAB at the onset of fermentation in all OSL has been reported by other authors (Ijabadeniyi 2007; Okeke et al. 2015; Ekwem and Okolo 2017), suggesting that fermentation commenced during steeping cereal, prior to grinding. Furthermore, the slow but gradual progression of LAB population in millet steep liquor and the gradual reduction of LAB in other steep liquors show

Table 1 Distribution of presumptive LAB counts (log CFU/ml) isolated from different *ogi* steep liquor and lemon juice-*ogi* steep liquor

Type of *ogi* steep liquor	LAB counts (log CFU/ml)/fermentation time			
	0 h	24 h	48 h	72 h
White maize steep liquor	5.09 ± 0.07^{c}	7.55 ± 0.03^{c}	7.68 ± 0.01^{b}	7.48 ± 0.03^{b}
White maize steep liquor+ lemon juice	4.00 ± 0.00^{a}	7.27 ± 0.01^{b}	7.41 ± 0.04^{a}	7.30 ± 0.02^{b}
White sorghum steep liquor	4.77 ± 0.10^{b}	7.24 ± 0.05^{b}	7.57 ± 0.05^{b}	7.37 ± 0.04^{b}
White sorghum steep liquor+ lemon juice	4.15 ± 0.21^{a}	6.90 ± 0.08^{a}	7.28 ± 0.06^{a}	6.69 ± 0.30^{a}
Millet steep liquor	4.69 ± 0.12^{b}	7.33 ± 0.04^{b}	7.26 ± 0.17^{a}	7.72 ± 0.03^{c}
Millet steep liquor+ lemon juice	4.59 ± 0.16^{b}	7.49 ± 0.06^{b}	7.52 ± 0.06^{b}	7.20 ± 0.04^{b}
Combined cereal steep liquor	5.02 ± 0.09^{c}	7.65 ± 0.03^{c}	7.73 ± 0.05^{b}	7.45 ± 0.02^{b}
Combined cereal steep liquor+ lemon juice	4.60 ± 0.00^{b}	7.28 ± 0.06^{b}	7.53 ± 0.05^{b}	6.84 ± 0.09^{a}

Values represented as mean ± SD; for each column, different subscripts indicate that the values are significantly different at $p < 0.05$, as measured by Tukey's test

Table 2 Some physiological parameters of LAB from *ogi* liquor and lemon juice-*ogi* steep liquor

Isolate no.	Acid tolerance			Cereal source	Bile tolerance			Auto-aggregation and hydrophobicity			
	pH 7	pH 3	pH 2			0.50%	1%	Auto-aggregation (%)		Hydrophobicity (%)	
	3 h	3 h	3 h		0 h	6 h	6 h	3 h	24 h	xylene	n-hexadecane
TA–001	9.55 ± 0.14^{b}	$8.05 \pm 0.21^{a,b}$	7.60 ± 0.14^{a}	MZL	9.40 ± 0.42^{a}	7.20 ± 0.99^{a}	6.02 ± 0.60^{a}	11.12 ± 0.86^{b}	$43.89 \pm 0.07^{c,d}$	49.80 ± 0.79^{e}	38.48 ± 0.28^{d}
TA–002	10.01 ± 0.28^{a}	8.90 ± 0.42^{a}	7.20 ± 0.14^{a}	MZL	9.90 ± 0.28^{a}	7.10 ± 0.85^{a}	5.85 ± 0.17^{a}	9.08 ± 0.40^{a}	37.57 ± 0.27^{c}	$11.79 \pm 1.68^{a,b}$	5.44 ± 0.31^{b}
TA–023	9.43 ± 0.04^{b}	8.59 ± 0.12^{a}	7.95 ± 0.11^{a}	LJMZL	9.55 ± 0.21^{a}	6.20 ± 0.43^{b}	5.97 ± 0.23^{a}	$18.48 \pm 0.42^{b,c}$	44.21 ± 0.52^{f}	28.50 ± 0.99^{c}	48.99 ± 0.17^{e}
TA–043	9.58 ± 0.04^{b}	$8.46 \pm 0.34^{a,b}$	7.16 ± 0.09^{a}	WSL	9.53 ± 0.11^{a}	6.01 ± 1.18^{b}	$5.78 \pm 0.52^{a,b}$	22.43 ± 0.31^{c}	51.80 ± 0.28^{e}	40.97 ± 0.46^{e}	47.68 ± 0.84^{e}
TA–045	9.53 ± 0.11^{b}	$8.13 \pm 1.10^{a,b}$	7.18 ± 0.08^{a}	WSL	9.58 ± 0.04^{a}	6.89 ± 1.23^{a}	6.84 ± 0.16^{a}	$18.65 \pm 1.03^{b,c}$	40.24 ± 0.31^{d}	$29.97 \pm 0.62^{c,d}$	39.92 ± 0.75^{d}
TA–048	9.93 ± 0.11^{a}	8.60 ± 0.14^{a}	7.09 ± 0.16^{a}	LJWSL	10.00 ± 0.02^{a}	7.23 ± 0.18^{a}	5.93 ± 0.17^{a}	14.87 ± 0.09^{b}	46.45 ± 0.08^{d}	27.15 ± 0.56^{c}	34.44 ± 0.74^{d}
TA–065	$8.93 \pm 1.31^{a,b}$	$7.82 \pm 0.54^{a,b}$	6.92 ± 1.03^{a}	MTL	9.43 ± 0.24^{a}	7.89 ± 0.45^{a}	6.91 ± 1.03^{a}	13.32 ± 0.24^{b}	40.96 ± 0.73^{d}	62.45 ± 0.67^{f}	53.32 ± 1.11^{f}
TA–070	9.92 ± 1.46^{a}	8.73 ± 0.66^{a}	6.53 ± 0.20^{a}	MTL	9.64 ± 0.09^{a}	6.77 ± 1.51^{a}	5.96 ± 0.32^{a}	$15.34 \pm 1.04^{b,c}$	42.92 ± 0.81^{d}	39.31 ± 1.02^{d}	41.41 ± 0.73^{e}
TA–071	9.34 ± 0.94^{b}	7.60 ± 0.85^{b}	6.80 ± 0.13^{a}	MTL	8.59 ± 0.16^{c}	6.07 ± 1.18^{b}	$5.75 \pm 3.28^{a,b}$	12.03 ± 0.29^{b}	46.22 ± 1.02^{d}	$31.40 \pm 1.43^{c,d}$	31.22 ± 0.94^{d}
TA–072	9.12 ± 1.10^{b}	$8.08 \pm 0.18^{a,b}$	6.45 ± 0.21^{b}	MTL	8.49 ± 0.30^{c}	6.74 ± 0.98^{a}	5.81 ± 0.28^{a}	7.19 ± 1.15^{a}	$37.66 \pm 1.36^{c,d}$	28.58 ± 0.53^{c}	40.75 ± 1.59^{e}
TA–075	9.50 ± 0.07^{b}	7.51 ± 2.82^{b}	6.66 ± 0.69^{a}	MTL	9.35 ± 0.14^{b}	6.38 ± 1.34^{b}	6.19 ± 1.47^{a}	$17.35 \pm 0.92^{b,c}$	41.65 ± 0.07^{d}	45.43 ± 0.94^{e}	32.09 ± 0.01^{d}
TA–076	9.19 ± 0.45^{b}	7.13 ± 0.25^{b}	7.05 ± 0.49^{a}	LJMTL	8.32 ± 0.11^{c}	6.72 ± 1.97^{a}	5.91 ± 1.14^{a}	8.20 ± 0.28^{a}	15.00 ± 0.71^{a}	26.54 ± 0.96^{c}	42.61 ± 0.19^{e}
TA–095	9.14 ± 0.01^{b}	8.76 ± 0.82^{a}	7.20 ± 0.63^{a}	CMBL	9.48 ± 0.32^{a}	7.54 ± 0.47^{a}	6.29 ± 0.23^{a}	7.55 ± 0.78^{a}	18.80 ± 0.14^{a}	$30.63 \pm 0.39^{c,d}$	32.33 ± 0.14^{d}
TA–119	9.27 ± 0.03^{b}	$7.96 \pm 0.69^{a,b}$	6.86 ± 1.43^{a}	LJCMBL	9.90 ± 0.92^{a}	6.44 ± 1.49^{b}	5.92 ± 0.14^{a}	$16.89.01 \pm 0.43^{b,c}$	41.34 ± 0.09^{d}	$29.89 \pm 0.65^{c,d}$	58.96 ± 0.60^{f}
L. casei ATCC393	9.10 ± 0.08^{b}	8.64 ± 0.19^{a}	7.27 ± 0.10^{a}		9.24 ± 0.22^{b}	6.89 ± 0.06^{a}	6.21 ± 0.12^{a}	$17.89 \pm 0.04^{b,c}$	46.01 ± 0.12^{d}	$31.89 \pm 0.17^{c,d}$	36.01 ± 0.51^{d}
L. plantarum ATCC 8014	9.26 ± 0.28^{b}	8.63 ± 0.09^{a}	6.96 ± 0.21^{a}		9.47 ± 0.14^{a}	6.76 ± 0.15^{a}	6.29 ± 0.17^{a}	$18.98 \pm 0.35^{b,c}$	52.09 ± 1.02^{e}	47.23 ± 0.63^{e}	50.67 ± 0.17^{e}

Values represented as mean ± SD; for each column, different subscripts indicate that the values are significantly different at $p < 0.05$, as measured by Tukey's test

MZL maize steep liquor, *LJMZL* lemon juice maize steep liquor, *WSL* white sorghum steep liquor, *LJWSL* lemon juice white sorghum steep liquor, *MTL* millet steep liquor, *LJMTL* lemon juice millet steep liquor, *CMBL* combined cereal steep liquor, *LJCMBL* lemon juice combined steep liquor

the competing influence of other microorganisms (Ekwem and Okolo 2017). However, LJOSL displayed a lower number of colonies throughout the fermentation period. This may be due to the natural antimicrobial properties of lemon, which some LAB probably could not withstand (Dhanavade et al. 2011).

One hundred and twenty isolates were randomly selected (see supplementary data A1) to determine physiological properties of LAB. All isolates were found to be Gram-positive and catalase-negative, and they grew at different temperatures (15, 37, 45 °C) and NaCl concentrations. All of the isolates hydrolyzed arginine and showed diversity in their fermenting ability toward different sugars.

Acid and bile tolerance, auto-aggregation, hydrophobicity, and hemolytic activity

The 120 isolates were exposed to simulated in vitro gastric juice and bile. The tolerances of some isolates were significantly reduced while there was little or no impact on the viability of most isolates at pH 3 and 0.5% bile (see supplementary data A2). However, at pH 2 and 1% bile, each of these isolates demonstrated progressive reduction, though 14 LAB isolates did not significantly differ ($p < 0.05$) from *L. casei* ATCC 393 and *L. plantarum* ATCC 8014. The acid and bile profile of 14 LAB is shown in Table 2. For a microorganism to be considered as potential probiotic LAB, it must withstand the hostile conditions of the human gastrointestinal tract and successively colonize it both actively and viably, by demonstrating resistance to acid and bile. The significant decrease in the survival rate at pH 2 and in 1% bile is in accordance with the reports of other authors (Angmo et al. 2016; Abushelaibi et al. 2017). The acid and bile tolerance observed in LAB suggested that the tolerance of LAB to acid or bile was specific for each strain.

Percentages of auto-aggregation and cell surface hydrophobicity suggested that the responses of tested isolates vary. Auto-aggregation of the 14 LAB increased ($p < 0.05$) between 3 and 24 h with values ranging between 7.19–22.43% and 14.34–46.45%, respectively (Table 2). This adhesion characteristic determines its capability to colonize the intestine and prevent pathogen access by specific blockage of cell receptors (Divya et al. 2012). Furthermore, these isolates demonstrated higher hydrophobicity toward n-hexadecane than xylene. For xylene and n-hexadecane, the percentage hydrophobicity ranged between 11.79–62.45% and 5.44–58.96%, respectively. The isolates that exhibited β-hemolysis (TA-002, TA-095) and α-hemolysis (TA-023) were not studied further. The absence of hemolysis indicates that the bacteria are avirulent (Thakkar et al. 2015). The LAB isolates investigated in this study exhibited comparatively higher hydrophobicity and auto-aggregation at varying degrees as compared with previous study (Angmo et al. 2016; Shehata et al. 2016). Dhewa et al. (2010) reported wide variations in cell surface hydrophobicity of LAB isolates, with ranges between 21 and 70%, indicating that higher values could be a sign of adherence to epithelial cells. However, the differences observed in this study may be attributed to the variations in hydrophilic/hydrophobic structures in the bacterial cell wall of LAB isolates (Abushelaibi et al. 2017).

Antimicrobial resistance and antibiotic activity

Table 3 shows the antimicrobial activities and antibiotic resistance of 11 LAB isolates. Antimicrobial activities of LAB

Table 3 Antimicrobial activity against four pathogens and antibiotic-resistant toward nine different antibiotics

Isolates	*S. aureus*	*Salmonella* spp.	*E. coli*	*L. monocytogenes*	P	CN	E	S	C	AMP	VA	CIP	TE	QD
TA–001	aaa	aa	a	aaa	S	S	S	R	S	MS	R	MS	S	S
TA–043	aaa	a	a	aa	S	MS	S	MS	S	MS	R	MS	S	S
TA–045	aa	a	a	aaa	S	S	S	S	S	S	R	S	S	S
TA–048	aa	a	aa	aa	S	S	S	S	S	S	R	S	S	S
TA–065	aaa	a	a	aaa	S	S	S	S	S	MS	R	MS	S	S
TA–070	aaa	aa	aa	aa	S	S	S	MS	S	MS	R	S	S	S
TA–071	aa	a	a	aaa	S	S	S	S	S	S	R	S	S	S
TA–072	aaa	a	a	aa	S	S	S	S	S	S	R	S	S	S
TA–075	aa	a	a	aa	S	S	S	S	S	S	R	S	S	S
TA–076	aaa	a	a	aa	S	S	S	S	S	MS	R	MS	S	S
TA–119	aa	a	a	aa	S	S	S	MS	S	MS	R	MS	S	S
L. plantarum ATCC 8014	aa	a	a	aa	S	S	S	S	S	S	R	S	S	S

a = inhibition zone 0.5–2.0 mm; aa = inhibition zone 2.1–5.0 mm; aaa = inhibition zone > 5.1–10 mm. P = penicillin G (10 units), CN = gentamicin (30 μg), QD = quinupristin/dalfopristin (15 μg), E = erythromycin (15 μg), S = streptomycin (10 μg), TET = tetracycline (30 μg), C = chloramphenicol (30 μg), VAN = vancomycin, CIP = ciprofloxacin (5 μg), and AMP = ampicillin (10 μg)

R resistant, *MS* moderately susceptible, *S* sensitive

Table 4 Heat (60 °C/5 min), lysozyme resistant (log10 CFU/mL), and cholesterol removal (%) of LAB isolates

Isolates	Lysozyme			Heat resistance		Cholesterol removal (%)		
	Control	50 ppm	100 ppm	0 min	5 min	Oxbile	Sodium taurocholate	Cholic acid
TA–001	8.94 ± 0.69^{a}	7.69 ± 0.69^{a}	$5.60 \pm 0.54^{a,b}$	7.27 ± 0.04^{a}	6.98 ± 0.07^{b}	19.76 ± 0.62^{c}	3.76 ± 0.52^{a}	22.37 ± 0.86^{c}
TA–043	8.89 ± 0.79^{a}	7.64 ± 0.79^{a}	5.06 ± 0.22^{b}	7.36 ± 0.05^{a}	7.15 ± 0.04^{a}	32.72 ± 1.41^{d}	5.87 ± 0.20^{b}	33.48 ± 0.14^{d}
TA–045	8.82 ± 0.67^{a}	7.57 ± 0.67^{a}	$5.55 \pm 0.47^{a,b}$	7.36 ± 0.05^{a}	6.49 ± 0.15^{b}	$6.57 \pm 0.99^{a,b}$	1.52 ± 0.16^{a}	18.15 ± 0.18^{b}
TA–048	8.79 ± 0.64^{a}	7.54 ± 0.64^{a}	$5.43 \pm 0.78^{a,b}$	7.38 ± 0.01^{a}	6.31 ± 0.05^{b}	15.69 ± 0.6^{b}	$4.65 \pm 0.38^{a,b}$	22.19 ± 0.04^{c}
TA–065	8.80 ± 0.64^{a}	7.55 ± 0.64^{a}	5.77 ± 0.79^{a}	6.84 ± 0.03^{b}	6.81 ± 0.03^{b}	4.47 ± 0.83^{a}	1.12 ± 0.48^{a}	4.58 ± 0.14^{a}
TA–070	8.77 ± 0.61^{a}	7.52 ± 0.61^{a}	5.88 ± 0.01^{a}	7.32 ± 0.01^{a}	7.29 ± 0.02^{a}	26.67 ± 1.64^{c}	6.45 ± 0.06^{b}	24.61 ± 0.40^{c}
TA–071	9.30 ± 1.35^{a}	8.05 ± 1.35^{a}	$5.21 \pm 1.41^{a,b}$	7.39 ± 0.03^{a}	7.34 ± 0.03^{a}	28.57 ± 0.78^{c}	7.65 ± 0.39^{b}	34.68 ± 0.73^{d}
TA–072	8.45 ± 0.15^{a}	7.20 ± 0.15^{a}	$5.42 \pm 1.12^{a,b}$	7.32 ± 0.02^{a}	7.30 ± 0.03^{a}	$7.67 \pm 0.96^{a,b}$	1.61 ± 0.47^{a}	12.79 ± 0.13^{b}
TA–075	8.90 ± 0.78^{a}	7.65 ± 0.78^{a}	4.70 ± 0.11^{b}	7.35 ± 0.06^{a}	5.15 ± 0.21^{c}	11.06 ± 0.75^{b}	0.35 ± 0.19^{a}	14.71 ± 0.15^{b}
TA–076	8.40 ± 0.08^{a}	7.15 ± 0.08^{a}	6.12 ± 1.16^{a}	7.40 ± 0.01^{a}	7.09 ± 0.01^{a}	4.61 ± 0.78^{a}	1.17 ± 0.15^{a}	8.67 ± 0.19^{a}
TA–119	8.76 ± 0.56^{a}	7.51 ± 0.56^{a}	$5.49 \pm 0.54^{a,b}$	7.36 ± 0.02^{a}	7.24 ± 0.03^{a}	1.93 ± 0.45^{a}	0.44 ± 0.21^{a}	5.83 ± 0.54^{a}
L. plantarum ATCC 8014	8.86 ± 0.70^{a}	7.61 ± 0.70^{a}	6.41 ± 0.75^{a}	7.31 ± 0.02^{a}	7.01 ± 0.01^{a}	21.27 ± 0.14^{c}	5.30 ± 0.08^{b}	$31.82 \pm 0.25^{c,d}$

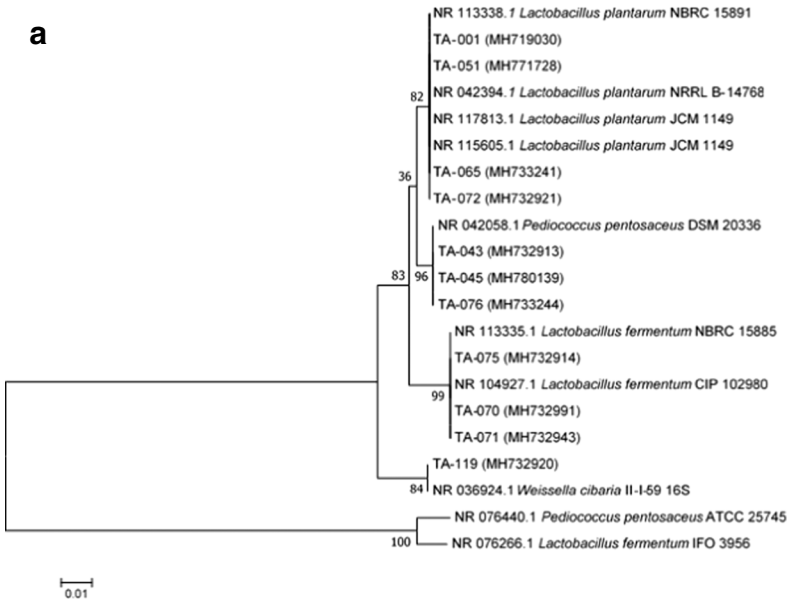
Values are mean ± standard deviation of triplicate. Means in the same column with different lowercase letters differed significantly ($p < 0.05$)

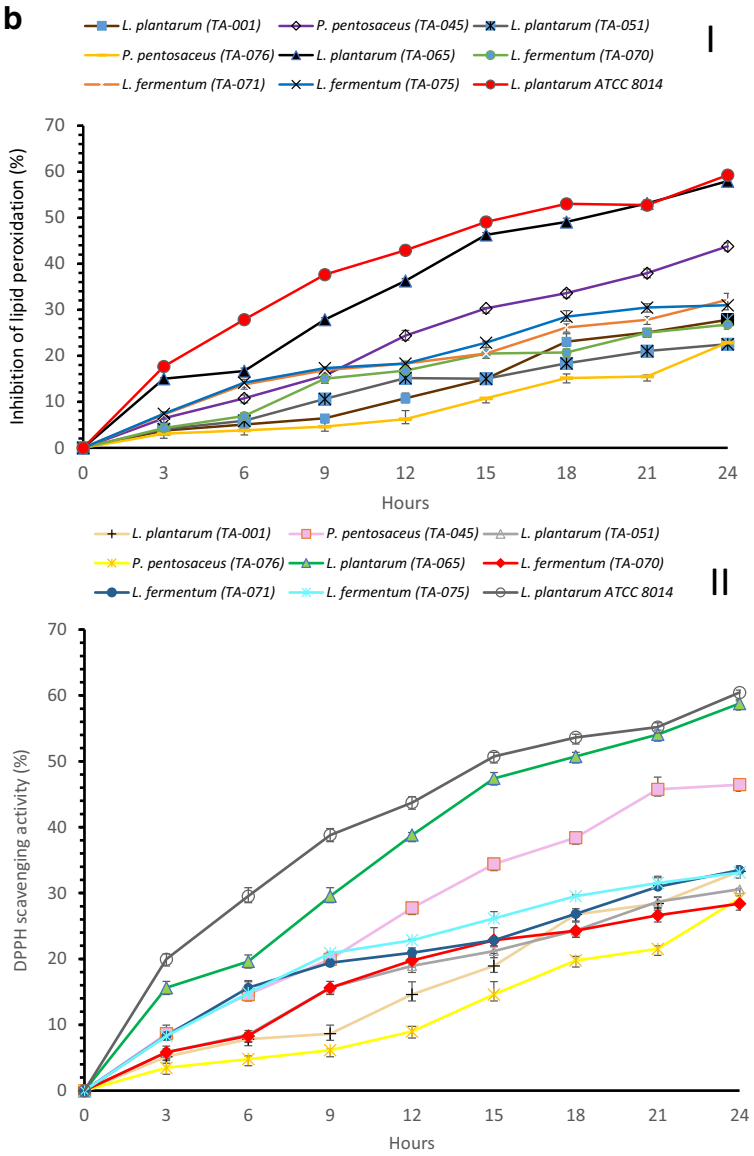
isolates ranged from small zones (0.5 mm) to wider zones (> 10.0 mm). All LAB strains were susceptible to erythromycin, tetracycline, chloramphenicol, and quinupristin/dalfopristin, moderately resistant to other antibiotics but highly resistant to vancomycin. Antimicrobial activity of each isolate varied against the four pathogens with the greatest efficacy against *S. aureus* (Table 3). The examination of other vital features like antimicrobial abilities and absence of antibiotic resistance is also required for the selection of a probiotic isolate, while the antimicrobial potential depicts the inhibitory potentials of harmful or pathogenic intestinal microbes by the production of organic acids from metabolites (Dhewa et al. 2010). The variation observed in the antagonistic activity of LAB against indicator strains has earlier been reported against *E. coli*, *Salmonella*, *Shigella*, *S. aureus*, *E. faecalis*, and *B. cereus* (Dhewa et al. 2010; Das et al. 2016; Shehata et al. 2016). However, LAB resistance to vancomycin has also been observed previously (Gad et al. 2014; Angmo et al. 2016; Abushelaibi et al. 2017). Vancomycin resistance of LAB isolates can be substantiated with the reports supporting the presence of intrinsic resistance mechanism toward this antibiotic family. This intrinsic resistance is usually non-transferable and possesses no risk in LAB (Jose et al. 2015).

Heat resistance, lysozyme tolerance, and cholesterol removal

Resistance to heat and impact of lysozyme on LAB isolates are presented in Table 4. There was a decrease in the growth of all the isolates after heat treatment at 60 °C for 5 min, but the reduction was not significant ($p < 0.05$) in most of the isolates when compared to the positive control. Most of the isolates, except isolate TA-075, exhibited < 65% heat resistance. Conversely, all of the isolates exhibited excellent resistance to 50 μg/mL of lysozyme, while a reduction in the growth of LAB was observed during incubation at a higher concentration of 100 μg/mL. However, no significant difference was observed ($p < 0.05$) between all the isolates (except TA–075) and the positive control. Heat resistance observed in the LAB of this study is a desirable characteristic of any probiotic that is intended for food processing. Similar reports of heat resistant probiotic LAB from cocoa and dairy product have been described (Santos et al. 2016; Abushelaibi et al. 2017). All isolates tested positive for the polysaccharide with characteristic ropy colonies and β-galactosidase activity after incubation at 30 °C for 42 h. The cholesterol removal percentages of the isolates are shown in Table 4. All LAB strains possessed the ability to hydrolyze all three bile salts that were investigated. The uptake of cholesterol by LAB in the medium varied depending on the bile medium. The cholesterol activity was highest in deconjugated bile (cholic acid 4.58–34.68%), a mixture of conjugated (97%) and deconjugated (3%) bile (oxbile 1.93–32.72%) and lowest in conjugated bile (sodium taurocholate 0.35–5.87%). β-Galactosidase activity and the

Fig. 1 **a** Evolutionary relationships of isolated LAB sequences with the existing probiotic *16S rRNA* gene sequences using neighbor joining method. The evolutionary distances were computed using the Maximum Composite Likelihood method as conducted in MEGA7. The DNA sequences retrieved in this work are indicated by their isolate code and accession number. **b** (I) Radical scavenging activity of whey from milk fermented with sequenced *Lactobacillus* spp. (II) Inhibition of lipid peroxidation of whey by *Lactobacillus* spp. Error bar of triplicate determinations


a
NR 113338.1 Lactobacillus plantarum NBRC 15891
TA-001 (MH719030)
TA-051 (MH771728)
NR 042394.1 Lactobacillus plantarum NRRL B-14768
NR 117813.1 Lactobacillus plantarum JCM 1149
NR 115605.1 Lactobacillus plantarum JCM 1149
TA-065 (MH733241)
TA-072 (MH732921)
NR 042058.1 Pediococcus pentosaceus DSM 20336
TA-043 (MH732913)
TA-045 (MH780139)
TA-076 (MH733244)
NR 113335.1 Lactobacillus fermentum NBRC 15885
TA-075 (MH732914)
NR 104927.1 Lactobacillus fermentum CIP 102980
TA-070 (MH732991)
TA-071 (MH732943)
TA-119 (MH732920)
NR 036924.1 Weissella cibaria II-I-59 16S
NR 076440.1 Pediococcus pentosaceus ATCC 25745
NR 076266.1 Lactobacillus fermentum IFO 3956
82
36
83
96
99
84
100
0.01


b
L. plantarum (TA-001)
P. pentosaceus (TA-045)
L. plantarum (TA-051)
P. pentosaceus (TA-076)
L. plantarum (TA-065)
L. fermentum (TA-070)
L. fermentum (TA-071)
L. fermentum (TA-075)
L. plantarum ATCC 8014
I
Inhibition of lipid peroxidation (%)
Hours
II
DPPH scavenging activity (%)
Hours

polysaccharide production observed in these isolates are good properties expected in potential probiotics.

Another important feature of probiotic is its ability to remove cholesterol. LAB hydrolyzes bile salts in human intestine thereby reducing cholesterol absorption inside intestine (Abushelaibi et al. 2017). The decrease in absorption suggests that a significant amount of cholesterol in the medium is precipitated after addition of deconjugated bile acids. Low precipitation of cholesterol was observed in the presence of conjugated bile acids sodium taurocholate. This co-precipitation effect of cholesterol and deconjugated bile acids vis-a-vis reduction in cholesterol level has been reported in some *Lactobacillus* spp. (Angmo et al. 2016). However, minor variation in comparison to our study may be attributed to strain and species differences.

Identification of selected isolates by *16S rRNA* sequencing and screening of LAB isolates for DPPH activity

Eleven potential probiotics identified by *16S rRNA* gene sequence showed PCR amplicons at 1500 bp. The sequence alignment was carried out using BLAST. The isolates basically clustered with specific reference probiotic strains (RPS) as shown in Fig. 1a. TA-001, TA-051, TA-065, and TA-072 had 81% similarity with *L. plantarum* RPS; TA043, TA-045, and TA-076 had 96% similarity with *Pediococcus pentosaceus* DSM 20336; and TA-070, TA-071, and TA-075 had 99% similarity with *L. fermentum* RPS while TA-119 had 84% similarity with *Weissella cibara* II-I-59. The percentage similarity and accession numbers of analyzed isolate are in Table 5.

Skimmed milk was initially fermented for 24 h with 11 sequenced LAB isolates that were obtained from OSL and LJOSL. The whey fractions scavenged between 1.4 and 34.43% DPPH radical (after correction for the activity of unfermented skimmed milk) as shown in Table 5. Eight strains (TA–01, 045, 051, 065, 070, 071, 075, and 076) were selected for further studies based on the results of the first stage of screening for antioxidant activity.

Fermentation of skimmed milk with LAB isolates from non-dairy sources and antioxidant activity of whey have been reported previously (Osuntoki and Korie 2010; Adesulu and Awojobi 2014), which supports the evaluation of this study. Our results show variation in antioxidant activity of each

Table 5 Identity, accession numbers, corresponding radical scavenging activity, and inhibition of lipid peroxidation of whey fractions of selected LAB

Isolate	Identity, accession numbers, and corresponding radical scavenging activity					Radical scavenging activity and inhibition of lipid peroxidation of whey after 24 h		
	Identity	Accession number	Most closely related Genbank sequence	% similarity	Inhibition of DPPH radical (%)	DPPH scavenging activity (%)	Lipid peroxidation inhibition (%)	Pearson's correlation coefficient
TA–001	*Lactobacillus plantarum*	MH719030	*L. plantarum* NBRC 15891	99	32.31 ± 2.01	33.28 ± 1.48	27.82 ± 0.65	0.97
TA–043	*Pediococcus pentosaceus*	MH732913	*P. pentosaceus* DSM 20336	99	9.92 ± 0.75	–	–	–
TA–045	*Pediococcus pentosaceus*	MH780139	*P. pentosaceus* DSM 20336	99	45.10 ± 0.85	46.45 ± 0.13	43.78 ± 0.70	0.92
TA–051	*Lactobacillus plantarum*	MH771728	*L. plantarum* NBRC 15891	99	30.49 ± 1.86	30.57 ± 0.80	22.50 ± 0.68	0.92
TA–076	*Pediococcus pentosaceus*	MH733244	*P. pentosaceus* DSM 20336	99	29.04 ± 0.46	29.20 ± 0.13	22.88 ± 0.78	0.95
TA–065	*Lactobacillus plantarum*	MH733241	*L. plantarum* JCM 1149	99	58.1 ± 1.25	58.77 ± 0.64	57.94 ± 0.39	0.81
TA–070	*Lactobacillus fermentum*	MH732991	*L. fermentum* CIP 102980	99	28.58 ± 0.97	28.39 ± 1.02	26.76 ± 1.21	0.95
TA–071	*Lactobacillus fermentum*	MH732943	*L. fermentum* NBRC 15885	99	33.43 ± 0.83	33.48 ± 0.73	32.15 ± 1.02	0.83
TA–072	*Lactobacillus plantarum*	MH732921	*L. plantarum* NBRC 15891	99	4.45 ± 0.47	–	–	–
TA–075	*Lactobacillus fermentum*	MH732914	*L. fermentum* NBRC 15885	99	32.79 ± 0.98	33.12 ± 0.22	30.97 ± 0.14	0.80
TA–119	*Weissella cibaria*	MH732920	*W. cibaria* II-I-59	99	6.29 ± 0.47	–	–	–
L. plantarum ATCC 8014					60.18 ± 0.56	60.44 ± 0.63	59.27 ± 0.39	0.95

Values are mean ± standard deviation of triplicate. "–" indicates that the test was not carried out on the isolate

isolate, thereby indicating strain-dependent DPPH radical scavenging activity and inhibition of plasma lipid peroxidation. This is in agreement with an earlier report (Osuntoki and Korie 2010). The antioxidant activity was found to be high in a strain of *Pediococcus pentosaceus* (TA-045) and *L. plantarum* (TA-065) while low in another strain of *L. plantarum* (TA-072). LAB cells were found to possess antioxidant activity in vitro. However, using the intact cells as the delivery vehicles in the gastrointestinal tract to release constituents can also be antioxidative (Pieniz et al. 2014).

DPPH radical scavenging activity

The radical scavenging activity of LAB isolates over a 24 h fermentation period was observed to increase from 3 h with the production of different peaks by these isolates (Fig. 1b). The highest DPPH and lipid peroxidation inhibitory (LPI) activity of 58.1 and 57.94%, respectively, was observed in TA−065 (*L. plantarum*—GenBank: MH733241). Additionally, the percentage of lipid peroxidation inhibition progressed similar to the radical scavenging activity, in the whey fraction. A strong correlation was found between the DPPH and plasma lipid peroxidation activity at each interval (see supplementary data A3). The correlation coefficient at the end of 24 h is shown in Table 5.

The human body and food systems produce a wide variety of oxygen-centered free radicals and other reactive oxygen species. LAB possess probiotic characteristics that are continually promoted because of their desired use in the production of functional foods. Radical scavenging activity was an indicator of antioxidant activity since there were strong correlations between the DPPH scavenging activity and the inhibition of plasma lipid peroxidation. As reported in previous studies (Parvez et al. 2006; Wang et al. 2017), the probiotics in OSL and LJOSL with antioxidant potentials may have an influence on the occurrence and duration of some illnesses. They act by decreasing the proportion of unfavorable metabolites and serve as protection for healthy liver function and possibly neutralize the effects of reactive oxygen species.

Conclusion

The increased demand for products having potential health benefits has encouraged the quest to identify microorganisms from relatively unexplored edible food waste, for a potential role in probiotic research. Although a variety of probiotic LAB were identified in this study, their activities were found to be strain-dependent. *Pediococcus pentosaceus* and *L. plantarum* are probiotics identified in this study with high antioxidant potential, which could be used as starter cultures in functional foods. This study indicates that consumption of OSL and LJOSL may render a significant amount of dietary antioxidants. Further studies are needed for exploring the health benefit of these isolates and understanding their behavior under different fermenting conditions.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals N/A. This research did not involve human participants and/or animals.

Informed consent N/A. This research did not involve human participants.

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