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ORIGINAL ARTICLE

Screening and molecular identification of potential probiotic lactic acid bacteria in effluents generated during ogi production

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Abstract

Screening and molecular identification of probiotic lactic acid bacteria (LAB) in effluents generated during the production of ogi, a fermented cereal (maize, millet, and sorghum) were done. LAB were isolated from effluents generated during the first and second fermentation stages in ogi production. Bacterial strains isolated were identified microscopically and phenotypically using standard methods. Probiotic potential properties of the isolated LAB were investigated in terms of their resistance to pH 1.5 and 0.3% bile salt concentration for 4 h. The potential LAB isolates ability to inhibit the growth of pathogenic organisms (*Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhimurium*) was evaluated in vitro. The pH and LAB count in the effluents ranged from 3.31 to 4.49 and 3.67 to 4.72 log cfu/ml, respectively. A total of 88 LAB isolates were obtained from the effluents and only 10 LAB isolates remained viable at pH 1.5 and 0.3% bile salt. The zones of inhibition of the LAB isolates with probiotic potential ranged from 7.00 to 24.70 mm against test organsisms. Probiotic potential LAB isolates were molecularly identified as *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus reuteri*, *Enterococcus faecium*, *Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Enterococcus faecalis*, and *Lactobacillus brevis*. Survival and proliferation of LAB isolates at low pH, 0.3% bile salt condition, and their inhibition against some test pathogens showed that these LAB isolates could be a potential probiotics for research and commercial purposes.

Keywords Cereal · Probiotics · Effluents · Lactic acid bacteria · Fermentation · Antimicrobial

Introduction

Probiotics are live microbial cultures which when consumed by humans can beneficially affect health by improving the original microbiota (Aslam and Qazi 2010; Sathyabama et al. 2014). Probiotics are more preferred when compared to antibiotics in the treatment of infections because prolonged use of antibiotics have resulted in many pathogenic bacteria developing resistance. Probiotic bacteria produce various compounds, such as organic acids (lactic and acetic acids), bacteriocins, and reuterin, which are inhibitory to pathogen's growth. Also, these compounds produced reduce the pH, thereby retarding the growth of pathogens (Tambekar and Bhutada 2010). Microbial isolation and screening from fermented foods with mixed cultures have proven to be a reliable approach in obtaining useful and genetically stable bacterial strains (Adnan and Tan 2006). In many instances, these microbes exhibit stable properties as well as ability to survive under stress conditions due to the complex environment they were isolated from.

Lactic acid bacteria (LAB) are a very important microbial group consisting several probiotic bacteria, among which *Lactobacilli* has been reported to be the most active and non-pathogenic (Salminen and Von Wright 1998). The symbiotic effect of these strains with *Bifidobacteria*, another

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probiotic genus, has also been reported (Kailasapathy and Chin 2000; Saarela et al. 2002). Probiotic bacteria have been widely studied, and this has led to the development of various probiotic foods, such as dairy milk products (Ukeyima et al. 2010) and cereal-based products through the combined use of probiotics, prebiotics, and dietary fibers (Sanni et al. 2013). For probiotic bacteria to survive, grow, and perform their beneficial action efficiently, they have to be able to withstand acidic and bile-containing media, as these are the conditions they would encounter during their passage through the gastrointestinal tract (GIT) (Klaenhammer and Kullen 1999).

Maize (*Zea mays* L.), millet (*Pennisetum typoideum*), and sorghum (*Sorghum bicolor*) are staple foods in many parts of the world including sub-Saharan Africa. In Nigeria and some other West African countries, they were traditionally transformed by submerged fermentation into a fermented porridge called *ogi*. *Ogi* have a smooth texture like a hot blancmange and a sour taste reminiscent of yoghurt. Its color depends on the color of the cereal used: cream or milk white for maize, reddish brown for sorghum, and dirty gray for millet (Onyekwere et al. 1989). *Ogi* is used as a complementary food for weaning infants, convenient food for the sick, convalescent, and elderly, or quick breakfast for low-income earners (Steinkraus 1996). It may also be eaten when made into a very stiff paste called *Eko* (Banigo and Akpapunam 1987). Consumption of this fermented food has many advantages including enhanced nutritional value, digestibility, therapeutic benefits, and safety against pathogens (Oranusi et al. 2003). In some communities in South Western Nigeria, uncooked *ogi* is usually diluted with water and administered to people having diarrhea, so as to reduce the frequency of stooling (Steinkraus 1996; Aderiye and Laleye 2004).

Several authors have reported on ogi production from various varieties of maize (white and yellow), from guinea corn, millet, and sorghum (Odunfa and Adeyele 1985; Teniola and Odunfa 2002; Teniola et al. 2005; Adebayo and Aderiye 2007; Adebayo-tayo and Onilude 2008; Dike and Sanni 2010; Omemu 2011; Banwo et al. 2012; Oyedeji et al. 2013). First and second stages of feremnetation are reported to be the soaking of grains and sedimentation, respectively (Omemu 2011). At large scale or industrial level, ogi is produced by optimizing the processing conditions most especially fermentation time and temperature without compromising product's quality. Hardness of the cereal grains was reduced by soaking (first fermentation), while required tartness or sourness was attained by sedimentation (second fermentation) (Bolaji et al. 2017). Ijabadeniyi (2007) reported molds (*Aspergillus niger*, *Penicillium* sp., *Mucor mucedo*, and *Rhizopus stolonifer*), yeast (*Saccharomyces cerevisiae*), and bacteria (*Corynebacterium* sp., *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Leuconostoc mesenteroides*, *Clostridium bifermentans*, and *Staphylococcus aureus*) as the major fermenting organisms during the first fermentation and only *L. plantarum, L. fermentum*, and *S. cerevisiae* at the second fermentation stage during ogi production. Also, LAB isolates have been previously isolated from effluents of fermented product (Ashe and Paul 2010). However, there are little information on the resistance of isolated LAB strains to acidic pH and bile salt concentration in effluents generated during the production of ogi. This is because most of the effluents obtained after fermentation are usually discarded (Oyewole and Isah 2012). Such information could provide important data to establish the relationship between fermentation and better use of effluents for the isolation of pure bacterial strains. The present study was therefore designed for the screening and molecular identification of potential probiotic LAB in effluents generated during ogi production.

Material and methods

Sources of cereals

Maize, millet, and sorghum used were procured from local markets in *Odeda*, *Ogun* state, Nigeria.

Ogi preparation

Ogi was prepared from maize, millet, and sorghum grains using the wet-milling processing (submerged fermentation) method as described by Omemu (2011) as illustrated in Fig. 1. One hundred grams (100 g) of the sorted maize, millet, and sorghum samples was separately soaked in air-tight container with 200 ml of water for 3 days at room temperature of 28 ± 2 °C. The effluents generated from each steeped grain samples were collected for analyses and tagged as effluents A, B, and C, respectively. The remaining steeped water was discarded by decantation, while steeped grains were wet-milled using a grinder (Kenwood Chef, Japan). The milled slurry was then sieved through a fine mesh sieve to remove

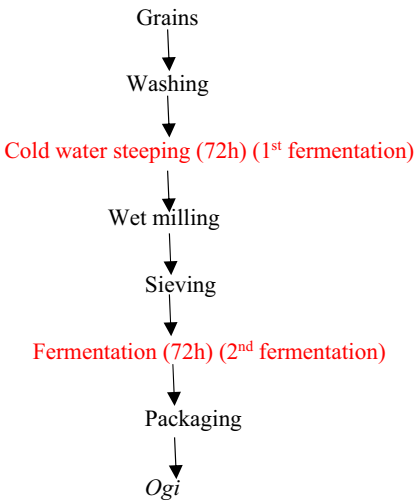

Fig. 1 Flow chart for ogi production

the over tails which were discarded. The troughs were allowed to stand and further fermented for 3 days. The effluents generated from the settling of the milled slurry were also collected for analyses and tagged effluents AA, BB, and CC for maize, sorghum, and millets, respectively.

pH and titratable acidity (TTA) determination

The pH and titratable acidity (TTA) of the effluents generated during the first and second fermentation stages in ogi production were determined using a method described by Omafuvbe et al. (2007).

Isolation of LAB

LAB were isolated from the effluent generated during the first fermentation and settling stage in ogi production from different grains on De Mann Rogosa Sharpe (MRS) agar. One milliliter of each effluent sample was diluted in 9 ml of sterile peptone water to obtain 10^{-1} dilution. The dilution was then made to 10^{-2}, 10^{-3}, until 10^{-5}. One milliliter of 10^{-5} dilutions was inoculated on MRS agar plates. Pour plate method was adopted. The plates were incubated at 37 °C for 48 h under anaerobic condition (using anaerobic jar). Pure cultures of the isolates were obtained by sub-culturing on MRS agar plates. Pure cultures were maintained in MRS agar slants and stored at 4 °C.

Phenotypic identification of the isolates

The isolated LAB were temporarily identified; firstly, preliminary characterization involving Gram staining and microscopic analyses (to determine morphology) of the isolates was done. Then all isolates were tested for the ability to produce catalase and oxidase enzymes. The catalase test was performed by adding a few drops of hydrogen peroxide (3%) to freshly grown bacteria colonies. The formation of gas bubbles indicates a positive result for the test. The oxidase test was done adding 2 to 3 drops of Kovac's oxidase reagent to colonies on plate and observing possible color changes. Appearance of deep purple-blue color indicates positive result.

Test for potential probiotic isolates

Selection of acid and bile salt-tolerant isolates

Acid and bile salt resistance of isolated LAB were assayed using the method of Tambekar and Bhutada (2010) with slight modification. For acid tolerance, strains were grown overnight on MRS broth at 37 °C. One hundred microliters (100 μl) of each overnight cultures was inoculated separately into MRS broth adjusted to pH 1.5 with 5-M HCl and pH 6.0 (which served as control) and incubated anaerobically at 37 °C for 4 h. About 1 ml of each broth cultures was then diluted with sterile peptone water to 10^{-3}, and 1 ml of these dilutions was inoculated on MRS agar plates. Pour plate method was adopted. The plates were then incubated at 37 °C for 48 h under anaerobic conditions. The growth of LAB in the adjusted low pH broth on MRS plates was used to designate isolates as acid tolerant, and the number of colonies counted on the MRS plate was used to determine the survival rate. For bile salt tolerance, the acid-resistant strains were selected and tested for their resistance to bile salt (fresh bile, Himedia, India). The experiment was performed using the same experimental apparatus and protocol described for the acidity resistance test; in this case, bile salt (0.3 *w/v*) was added to the MRS broth.

Test of antimicrobial activity

S. aureus DMST 4745, *Salmonella typhimurium* PSU.SCB.16S.11, and *Escherichia coli* DMST 4212 were obtained from the Food Safety Laboratory, Department of Food Technology, Prince of Songkla University, Hat Yai. Thailand. The antibacterial activity of those LAB isolates tolerant to conditions of high acidity (pH 1.5) and 0.3% bile salt concentration was tested. LAB isolates were grown in MRS broth at 37 °C for 24 h, following which the fully grown cultures were centrifuged (3000 *g*, 4 °C for 45 min, Mini-Centrifuge; LAB kits, China). The supernatant was separated and sterilized by passage through a 0.2-μm membrane filter (Whatman, Sigma-Aldrich, St. Louis, MO). The sterilized supernatant was then tested against 1 g positive and 2 g negative indicator pathogenic microorganisms listed above.

The antibacterial activity of the LAB isolates with potential probiotic properties against these pathogens was tested using the agar well diffusion method, as per the method of Korhonen (2010). Overnight cultures were serially diluted to the final concentration of 10^6 CFU/ml. A 1-ml aliquot of the inoculum was then spread homogeneously on the surface of a Mueller–Hinton agar plate using a sterile swab. A well was made into the plate using a sterile cork bearer (diameter 5 mm), and 30 μl of the LAB with potential probiotic properties supernatant was then placed into each well. The plates were incubated at 37 °C for 24 h. After this time, the diameter of the inhibition halo around the well was measured.

The strains which showed antibacterial activity were then further tested to determine which antimicrobial compound may have caused such activity as described by Ayodeji et al. (2017). The experiments were performed using the same agar well diffusion method, but the LAB supernatant was modified before being tested in three different ways, as follows:

- The pH of each LAB supernatants was adjusted to 6.5 with 1-M NaOH in order to check activity due to the acidity of the supernatant.

- Thirty microliters (30 μl) of catalase (5 mg/ml) was added to each LAB supernatants to determine inhibitory effect due to presence of hydrogen peroxide.
- Thirty microliters (30 μl) of trypsin (9 mg/ml) was added to each LAB supernatant to determine activity due to bacteriocins.

Molecular identification of selected LAB isolates with potential probiotic properties

Genomic DNA extraction

The genomic DNA extraction of the selected LAB with potential probiotic properties was done as described by Ayodeji et al. (2017). The selected LAB isolates were grown overnight in an appropriate liquid media and pelleted by centrifugation at maximum speed for 5 min. The pellets were then washed twice with TE buffer (10-mM Tris-Cl, 1-mM EDTA, pH 8.0). The total genomic DNA of the isolated strains was extracted using the guanidium thiocyanate–*N*-lauroylsarcosine) denaturing method. The quantity and the purity of the total DNA were verified by agarose electrophoresis, and the DNA was stored at − 20 °C until further use.

16s rRNA amplification and sequence data

The method of Ayodeji et al. (2017) was employed for 16s RNA amplification and sequencing of the LAB with potential probiotic properties. The 16s rRNA gene sequencing data for the isolates were obtained for approximately 1500-bp 16S rRNA region extending from nucleotide positions 27 to 1492 (*E. coli* 16S rRNA gene sequence numbering) using the primers 27 F (3′-GAGTTTGATCCTGGCTCAG-5′) and 1492R (3′-TACCTTGTTACGACTT-5′). PCR assays were performed in an automated temperature cycling device (Test Kit, China), using 5 μl of total DNA, 25-μl NzyTaq 2× Green Master Mix (Genaxxon Bioscience, Germany) and 2 μl of each primer in a total volume of 50 μl. The amplification cycling program consisted of a 5-min initial denaturation at 94 °C, followed by 35 cycles of a 2-min denaturation at 94 °C, a 1-min annealing at 51 °C, and a 2-min extension at 72 °C, with a final extension at 72 °C for 5 min. After the amplified fragments were verified by electrophoresis. The amplicons were purified and sequenced by Magrogen (Korea). Sequences were manually proofread, and nBLAST searches were performed using the GenBank Internet server (http://www.ncbi.nlm.nih.gov), for comparison with other strains deposited in the public databases, to identify the species taxon of each isolate. Sequences that showed more than 98% similarity were considered as belonging to the same taxonomy unit. The sequences obtained were deposited to the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) to allow public access.

Results and discussion

Isolation of LAB strains

The pH, TTA, and LAB counts of the effluent samples generated during the fermentation stages in ogi production are presented in Table 1. The pH ranged from 3.31 to 5.10, which was highest in effluent generated during the second fermentation of ogi in millets and lowest in effluent generated during first fermentation of sorghum ($p < 0.05$). The TTA of the effluents ranged from 0.21 to 0.24%. The pH and TTA of the effluents show clearly that they were acidic. Reports abound as to the fact that ogi from maize, millet, and sorghum were lactic acid fermented foods with the production of lactic acid produced. Omemu (2011) reported that LAB and yeast were the major fermenting organisms during the production of ogi. The resulting lactic acid produced by the fermentation of sugar present in cereals, the raw materials of ogi, must have occasioned the low pH in the fermented food product. By implication, certain changes occur in the food due to low pH, such as reduction in microbial community, as those which cannot tolerate the raised acid level will not be able to survive or multiply. The LAB counts ranged from 3.67 to 4.72 log cfu/ml ($p < 0.05$). High counts of LAB in ogi give more evidence that LAB are among the major fermenting organisms in the microbial community of the effluents. It also shows that the low pH did not affect the proliferation of LAB, demonstrating LAB ability to adapt to the substrate environment and utilization of the available organic compound for growth (Ayodeji et al. 2017). The LAB isolates characterized by cultural and morphological characteristics appeared small, creamy, and whitish. All of the isolates are Gram positive, catalase negative, and oxidase negative. A

Table 1 pH, titratable acidity (TTA), and LAB count of effluents generated during the fermentation stages in ogi production from maize, millets, and sorghum

Samples	Initial pH	TTA (%)	LAB count (log cfu/ml)
A	4.11^{d}	0.23^{a}	4.17 ± 0.33^{ab}
B	5.10^{f}	0.21^{a}	3.67 ± 0.28^{a}
C	3.63^{b}	0.23^{a}	4.31 ± 0.21^{ab}
AA	3.98^{c}	0.24^{a}	4.32 ± 0.33^{ab}
BB	4.40^{e}	0.22^{a}	4.12 ± 0.37^{bc}
CC	3.31^{a}	0.24^{a}	4.72 ± 0.40^{c}

Means values of replicate values, data with the same superscripts with the column are not significantly difference ($p > 0.05$) A: effluents of the first fermentation in ogi production from maize grains, B: effluents of the first fermentation in ogi production from sorghum grains, C: effluents of the first fermentation in ogi production from millet grains, AA: effluents of the second fermentation in ogi production from maize grains, BB: effluents of the second fermentation in ogi production from sorghum grains, CC: effluents of the second fermentation in ogi production from millet grains

total of 88 LAB isolates were obtained in effluents generated from the production of ogi from maize millets and sorghum grains.

Tolerance against low pH

The result of this present study showed that only 10 LAB isolates could survive the pH 1.5 for 4 h. Figure 2a–b presented the survival rate counts of LAB isolates from effluents generated during the fermentation stages of maize, millet, and sorghum under acidic condition compared to the control. It was observed that the survival rate for the LAB isolates with potential probiotic properties in pH 1.5 was lowered than that of the control (pH 6) ($p < 0.05$). LAB over the years have been known to have ability to survive and grow in acidic medium. Particularly in lactic acid fermentation food products, such as ogi, fufu, nunu, and some other rapidly African fermented foods, they ferment sugar with the production of gas (Omemu 2011; Oyedeji et al. 2013). These characteristics allow the organisms to be established in the intestinal tract as well as enable them to survive, grow, and perform their actions in the GIT of the hosts. Before reaching the intestinal tract, probiotic bacteria must first survive transit through the stomach where the pH can be as low as 1.5 to 2.0. The ability of the selected LAB to grow at pH 1.5 suggests that these LAB could be a potential probiotic, although the survival rate of selected LAB at pH 1.5 is lower than the control. The resistance to an acidic environment shown by the selected LAB strains is in agreement with data published in the literature. Haller et al. (2001), Argyri et al. (2013), and Kuda et al. (2014) reported the resistant of LABs isolated from different sources to acidic media.

Tolerance against bile salts

The result of the tolerance of the LAB isolates with potential probiotic properties from effluents generated during fermentation stages in ogi production showed that all the isolates that survived pH 1.5 were still viable at 0.3% bile salt concentration. Figure 3a–b represents the survival rate count of LAB

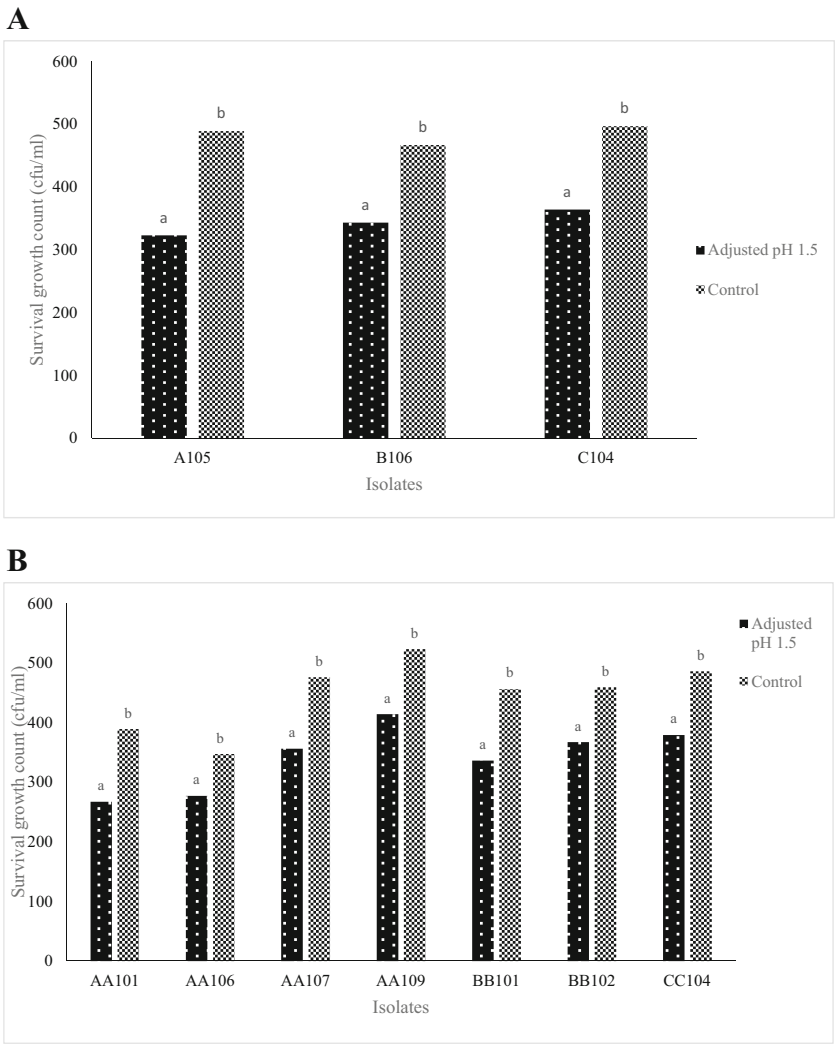

Fig. 2 Survival rate count of LAB isolates obtained from effluents generated during first fermentation (**a**) and second fermentation (**b**) in ogi production that survived pH 1.5 compared with the control. Data represent mean of replicate ($n = 3$). Different lower cases on the bar within the same microorganism indicate significant difference ($p < 0.05$). A: effluents of the first fermentation in ogi production from maize grains, B: effluents of the first fermentation in ogi production from sorghum grains, C: effluents of the first fermentation in ogi production from millet grains, AA: effluents of the second fermentation in ogi production from maize grains, BB: effluents of the second fermentation in ogi production from sorghum grains, CC: effluents of the second fermentation in ogi production from millet grains

isolates with potential probiotic properties at bile salt condition (0.3%) compared to the control (no bile salt). The survival rate of LAB isolates was a little lower than that of the control ($p < 0.05$). Tolerance to bile acids was considered to be a prerequisite for colonization and metabolic activity of bacteria in the small intestine of the host. Therefore, when evaluating the potential of using LAB as effective probiotics, it is generally considered necessary to evaluate their ability to resist the effects of bile acids. The concentration of the bile salts used in this study represents the extreme concentration obtained in human intestine during the first hour of digestion (Gotcheva et al. 2002). LAB have been reported to have complete resistance bile salt (Haller et al. 2001) and in some cases, non-complete resistance (Solieri et al. 2014). Selected LAB isolates demonstrated complete resistance against 0.3% bile salt, which suggested that these LAB isolates could be able to colonize the GIT when applied as probiotics.

Antimicrobial activity of isolates

Figure 4a–b shows the antimicrobial spectrum of the LAB isolates with potential probiotic properties on different indicator pathogenic bacteria. The tests were applied two times and the averages of zones of inhibition were given. The inhibitory test showed that all the selected strains of LAB significantly inhibited the growth of *S. typhimurium*, *E. coli*, and *S. aureus* used for this study. Zones of inhibition ranged from 10 to 24.7 mm for *E. coli*, 10.17 to 17.89 mm for *S. typhimurium* and 7.10 to 14.16 mm for *S. aureus.* Antibacterial activity is an important and desirable property for probiotic microorganisms,

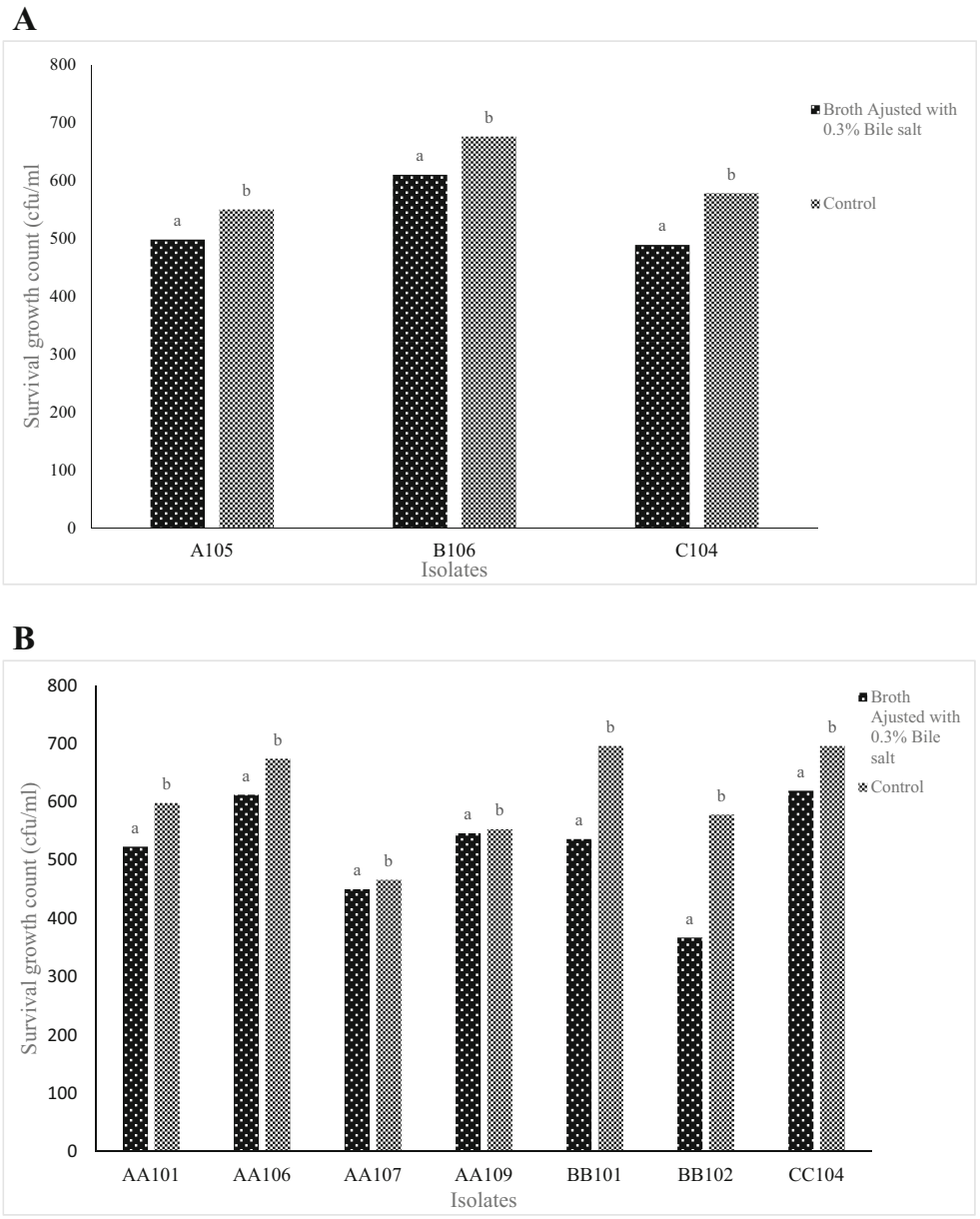

Fig. 3 Survival rate count of LAB isolates obtained from effluents generated during first fermentation (**a**) and second fermentation (**b**) in ogi production to bile salt 0.30% compared with control. Caption: see Fig. 2

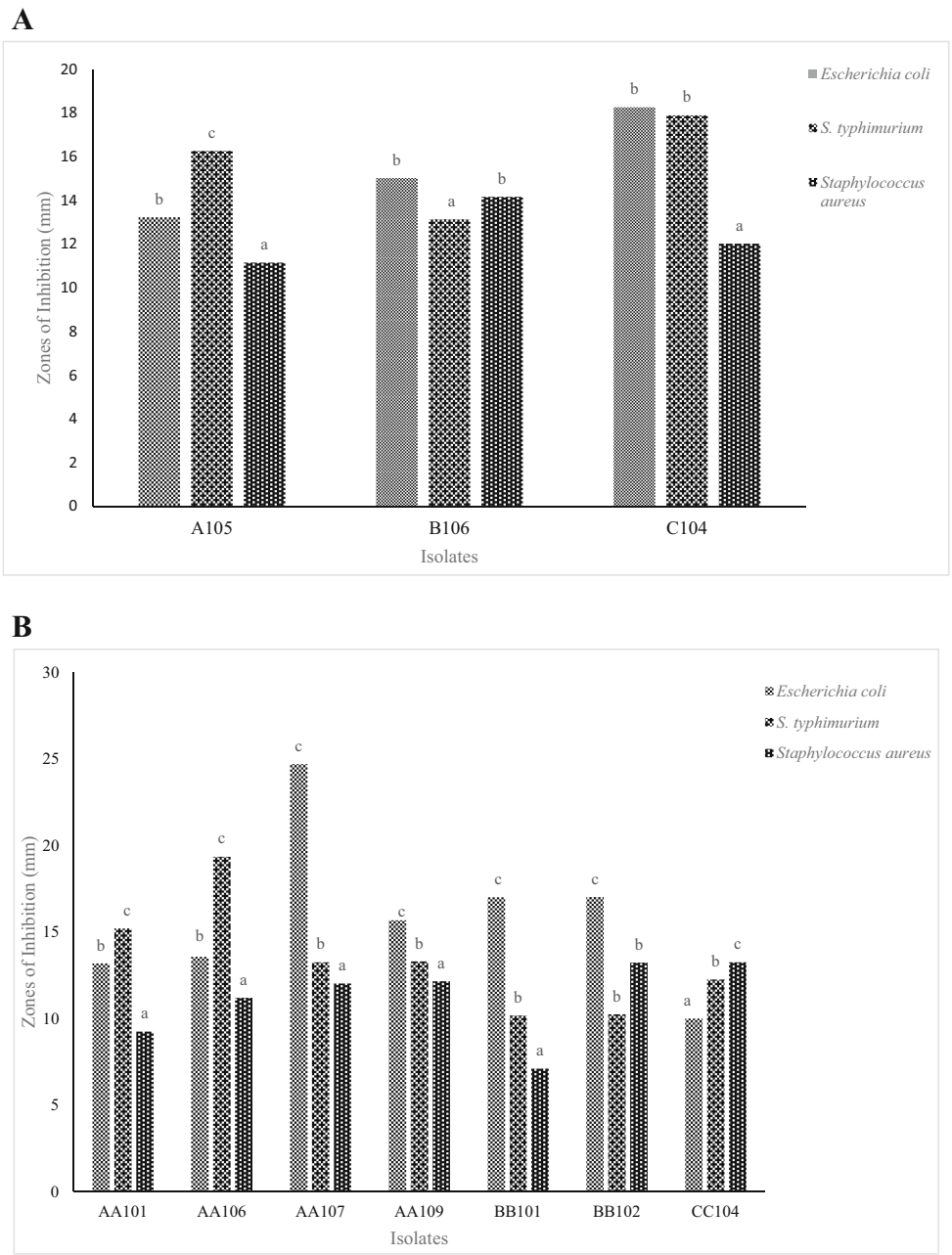

Fig. 4 Zones of inhibition demonstrated by LAB isolates from effluents during first fermentation (**a**) and second fermentation (**b**) in ogi production against test organisms. Caption: see Fig. 2

as a reduced growth of pathogens in the large intestine can lead to a decrease in gastroenteritis and food poisoning (Chapman et al. 2011). Previous studies reported that LAB isolated from fermented food showed antibacterial properties towards Gram-positive and Gram-negative strains (Grosu-Tudor et al. 2014; Iranmanesh et al. 2014). The ability of active compounds in penetrating bacteria cell was a major factor in determining antimicrobial properties (Corona and Martinez 2013). However, bacteria can resist the penetration of tested compounds by either modifying the lipopolysaccharide on their cell membrane or increasing the production of membrane vesicles on membrane surface (Fernández and Hancock 2012). The inhibitory test showed that all the selected LAB isolates significantly inhibited the growth of *S. typhimurium*, *E. coli*, and *S. aureus* used for this study. The high zones of inhibition observed for Gram-negative bacteria are attributed to the ability of the LAB supernatant to penetrate the cells more rapidly than the Gram-positive counterpart because of their thin peptidoglycan layers of the former (Abdollahzadeh et al. 2014). Lawalata et al. (2011) investigated the inhibitory activity of some LAB on some Gram-positive and Gram-negative pathogenic bacteria such as *E. coli*, *S. aureus*, and *Pseudomonas fluorescens* and reported the zones of inhibition in the range of 3.00 to 15.00 mm. These LAB are known to produce antimicrobial substances that are active against pathogenic bacteria (both Gram positive and Gram negative). These antimicrobial

Table 2 Effect of trypsin, catalase, and pH 6.5 on the antagonistic property of cell-free supernatant isolates obtained from first fermentation on indicator organisms

Indicator organism	Isolates	Untreated supernatant	Trypsin treated	Catalase treated	pH treated
Escherichia coli DMST 4212	A105	+++	−	+	+
	B106	+++	−	++	+
	C104	+++	−	++	+
Staphylococcus aureus DMST 4745	A105	++	−	+	+
	B106	++	−	+	+
	C104	+++	−	++	+
Salmonella typhimurium PSU.SCB.16S.11	A105	+++	−	+	+
	B106	+++	−	++	+
	C104	+++	−	+	+

(−) no zone of inhibition; (+) zones of inhibition < 7 mm in diameter; (++) zones of inhibition between 7 and 10 mm; (+++) zones of inhibition > 10 mm

A: effluents of the first fermentation in ogi production from maize grains, B: effluents of the first fermentation in ogi production from sorghum grains, C: effluents of the first fermentation in ogi production from millet grains

substances include organic acids (lactic acid, acetic acid, propionic acid, and butyric acid), hydrogen peroxide, and bacteriocins (Lonkar et al. 2005). These compounds not only may reduce the number of viable pathogenic bacteria but also

Table 3 Effect of trypsin, catalase, and pH 6.5 on the antagonistic property of cell-free supernatant isolates obtained from second fermentation on indicator organisms

Indicator organisms	Isolates	Untreated supernatant	Trypsin treated	Catalase treated	pH treated
Escherichia coli DMST 4212	AA101	+++	−	+	+
	AA106	++	−	+	+
	AA107	+++	−	+	+
	BB101	+++	−	+	++
	BB102	+++	−	+	++
	BB104	++	−	+	+
	BB101	++	−	+	+
	CC102	+++	−	+	++
Staphylococcus aureus DMST 4745	AA101	++	−	+	+
	AA106	++	−	++	++
	AA107	+++	−	+	++
	BB101	++	−	+	++
	BB102	++	−	+	+
	BB104	++	−	+	+
	BB101	++	−	+	++
	CC102	+++	−	+	+
Salmonella typhimurium PSU.SCB.16S.11	AA101	++	−	+	+
	AA106	++	−	+	+
	AA107	+++	−	+	++
	BB101	++	−	+	++
	BB102	++	−	+	+
	BB104	+++	−	+	+
	BB101	++	−	+	++
	CC102	++	−	+	+

(−) no zone of inhibition; (+) zones of inhibition < 7 mm in diameter; (++) zones of inhibition between 7 to 10 mm, (+++) zones of inhibition of > 10 mm.

AA: effluents of the second fermentation in ogi production from maize grains, BB: effluents of the second fermentation in ogi production from sorghum grains, CC: effluents of the second fermentation in ogi production from millet grains

Table 4 Molecular identification of potential probiotic LAB strains

Isolates/codes	Identification	Accession number
A105	*Pediococcus pentosaceus*	KY861324
AA101	*Lactobacillus fermentum*	KY940560
AA106	*Pediococcus acidilactici*	KY940561
AA107	*Lactobacillus reuteri*	KY940562
CC102	*Enterococcus faecium*	KY940563
B106	*Lactobacillus fermentum*	KY940564
BB101	*Lactobacillus fermentum*	KY940565
BB102	*Lactobacillus fermentum*	KY940566
BB104	*Enterococcus faecalis*	KY940567
C104	*Enterococcus faecalis*	KY940568

A: effluents of the first fermentation in ogi production from maize grains, B: effluents of the first fermentation in ogi production from sorghum grains, C: effluents of the first fermentation in ogi production from millet grains, AA: effluents of the second fermentation in ogi production from maize grains, BB: effluents of the second fermentation in ogi production from sorghum grains, CC: effluents of the second fermentation in ogi production from millet grains

may affect the bacterial metabolism and toxin production (Rolfe 2000). The inhibition of pathogens by LAB could also be due to low pH of the medium as a result of organic acid production (Draksler et al. 2004)

Tables 2 and 3 depicted the antagonistic property of the LAB strains with potential probiotic properties. After neutralizing the possible effects of organic acidity on the pathogens, most of them were still sensitive to strains with inhibition zones less than what was observed before neutralization. It shows that organic acid is partly responsible for the inhibition. The antimicrobial potential of isolates was not eliminated after reacting with catalase. It could be deduced that the antimicrobial properties of these strains were not caused by hydrogen peroxide alone. From the results, inhibitive abilities of LAB isolates were eliminated by trypsin. This suggest that the inhibitory properties of LAB isolate were mostly due to the inhibitory substance (protein) produced (Vandenberg 1993). The inhibitory properties of the LAB isolates treated with trypsin were lost mostly because of the proteolytic properties of the enzyme. Millette et al. (2004) reported that LAB produce nisin that inhibited bacterial growth in semi-synthetic media. Both Gram-positive and Gram-negative bacteria were inhibited by nisin and plantaricin 35d produced from *L. plantarum* (Messi et al. 2001). However, it could be postulated that antagonistic properties of the selected LAB with potential probiotic properties can be due to pH, bacteriocin, and hydrogen peroxide production. This result agrees with the conclusion of Cabo et al. (2002) that the inhibitory effect of bacteriocins is enhanced by other components of probiotics, such as hydrogen peroxide and organic acid produced as secondary metabolites.

Molecular identification of probiotic potential LAB isolates

Amplified selected LAB DNA showed distinct single DNA bands with molecular weight ranging from 1200 to 1500 bps. The 16S rDNA gene sequencing identified all 10 isolates to be *L. plantarum*, *L. fermentum*, *Lactobacillus reuteri*, *Enterococcus faecium*, *Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Enterococcus faecalis*, and *Lactobacillus brevis* (confidence degree, $E = 0.0$, homology of between 99 and 100% for all). The 16S rDNA sequences obtained were deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank) under accession numbers as reported in Table 4. Identification of probiotic potential LAB by 16S rRNA has been referred to as a very reliable method by several authors, among them are Kostinek et al. (2005) and Oguntoyinbo and Narbad (2012). Molecular techniques, especially polymerase chain reaction (PCR)-based methods, are important for the specific characterization and detection of LAB strains (Mohania et al. 2008; Adiguzel and Atasever 2009; Lawalata et al. 2011). The 16S rRNA gene amplification revealed that the isolates were all bacteria and the lengths of amplification varied from 1500 to 1700 bp. These sizes were almost similar to the sizes of 1500 to 2000 bp previously obtained by Bulut (2003). Many strains of these LAB have been reported by many authors as probiotic bacteria and to a certain extent are used in the production of probiotic preparations for animal and human health benefits (Bhattacharyya 2009; Hoque et al. 2010 and Sarkono et al. 2010).

Conclusion

The survival and proliferation of LAB strains isolated in this study under very low pH and bile salt concentration show that these strains could be able to withstand the conditions of the GIT and exert their beneficial effects on human bowel microbiota. Also, the metabolite produced by the selected LAB strains has been shown to be effective against both Gram-positive and Gram-negative pathogens tested in this study, although the results are preliminary but very promising when used in food to prevent infections or as potential probiotics.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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