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Probiotic potential and safety analysis of lactic acid bacteria isolated from Ethiopian traditional fermented foods and beverages

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Abstract

Background Probiotics are live microorganisms that effectively combat foodborne pathogens, promoting intestinal health when consumed in sufficient amounts. This study evaluated the probiotic potential and safety of lactic acid bacteria isolated from selected Ethiopian traditional fermented foods and beverages (Kotcho, Bulla, Ergo, Cabbage-Shamita, Borde, and Bukuri). To assess the isolates' probiotic activity, tolerance, and survival rate under various stressful conditions, including low pH, intestinal inhibitory substances, salt concentration, bile salt, and simulated gastric/intestinal juice. The isolates were also tested for antagonistic activities against common bacterial and fungal pathogens (*Staphylococcus aureus*, *Salmonella* Typhimurium, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Candida albicans*) and safety (auto-aggregation, co-aggregation, cell source hydrophobicity, hemolytic activity, DNase, and antibiotic susceptibility). The best probiotic lactic acid bacteria (LAB) were characterized to species level following standard MALDI TOF/mass spectrometry analysis.

Results A total of 125 potentially probiotic LAB were isolated of which 17 (13.60%) isolates survived low pH (2, 2.5, and 3), bile salt (0.3%), intestinal inhibitory chemicals (phenol, bile, low acidity, pepsin, and pancreas), and simulated gastro-intestinal settings with near 60–94% survival rate. In addition, 11 best LAB isolates were further screened based on additional screening including their antimicrobial efficacy, preservative efficiency, bacteriocin production besides resistance to low acid and bile salts, and survival potential under simulated gastrointestinal conditions. All 11 LAB isolates were resistant to ampicillin, vancomycin, gentamicin, kanamycin, clindamycin, and chloramphenicol, while they were susceptible to streptomycin and tetracycline. The MALDI TOF mass spectrometry analysis result of efficient probiotic LAB grouped them under the genus *Pediococcus*, *Enterococcus*, and *Lactococcus* including *Pediococcus pentosaceus*, *Enterococcus faecium*, *Lactococcus lactis*, and *Pediococcus acidilactici*.

Conclusion Ethiopian traditional fermented foods and beverages are good sources of promising probiotic lactic acid bacteria. These isolates could serve as potential starter cultures and bio-preservative for the enhancement of the shelf life of foods. This study established the groundwork for the selection of excellent probiotics for the development and application of LAB for antibacterial action, starter culture production, and preservation activities.

Keywords Antimicrobial activity, Ethiopian, Lactic acid bacteria, Probiotic potential, Traditional fermented products

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Introduction

Probiotics are living microorganisms that have beneficial aspects for human health if administered in adequate amounts (Mulaw et al. 2020). *Lactic acid bacteria* (LAB) are a group of bacteria that are generally recognized as safe (GRAS) and are widely used in fermented foods. They produce antimicrobial substances such as acids, hydrogen peroxide, and bacteriocins and have great potential as food biopreservatives (Mokoena et al. 2016). In addition to their preservative properties, LAB have also been shown to have immunomodulatory effects and may improve gut health. Studies have suggested that consuming probiotics containing LAB can alleviate symptoms of gastrointestinal disorders such as irritable bowel syndrome and inflammatory bowel disease (M. Mokoena et al. 2021; Mulaw et al. 2019).

The growing number of food-borne illness outbreaks caused by various pathogens is a significant concern for food safety and regulatory agencies (Akbar et al. 2019). Foodborne diseases are the most serious and expensive issues in the food industry, and their prevention and control mechanisms require a multidisciplinary approach (Hoelzer et al. 2018). Probiotic LAB, such as *Lactobacillus, Bifidiobacteria*, *Lactococcus*, and *Leuconostoc* have been reported to have probiotic potential (Birri et al. 2010). In addition, *Pediococcus* species are also known to display promising probiotic properties. Among *Pediococcus* species, *Pediococcus pentosaceus* and *Pediococcus acidilactici* are associated with promising potential probiotic activities (Altermann et al. 2005; Federici et al. 2014; Haghshenas et al. 2017).

The possible mechanisms by which probiotic LAB protect enteric pathogens are related with the production of antimicrobial substances, competition for limited resources, and anti-adhesive effects (Oelschlaeger 2010). In addition, probiotic LAB play an important role in traditional fermented foods and beverages by producing excellent flavor, aroma, and texture of fermented foods and beverages (O'Bryan et al. 2015; Ricci et al. 2019). Probiotic LAB play an important role in traditional fermented foods and beverages by producing excellent flavor, aroma, and texture. In addition to their culinary benefits, probiotic LAB have also been shown to have potential health benefits such as improving digestion and boosting the immune system. These microorganisms can be found in a variety of fermented foods and beverages. Ethiopian traditional fermented foods and beverages can harbor native and beneficial probiotic LAB, as most of these products are consumed without further heat processing. Ethiopian fermented foods and drinks with the potential to deliver probiotic LAB include Ergo and Kotcho (Mulaw et al. 2020), Borde (Negasi et al. 2017), Bukuri (Chali and Bacha 2014), and injera (Tilahun et al. 2018). These products not only provide unique flavors but also offer potential health benefits to those who consume them regularly.

However, there is still a lack of genetic diversity in traditional fermented foods and beverages, which presents opportunities to study food microbes and understand how their potential functions can be changed or modulated. Furthermore, this research can lead to the development of new fermentation techniques that can enhance the nutritional value and safety of these products. Additionally, it can also provide insights into how microbial communities interact with each other and with their environment, which has implications for both food science and ecology. The findings could have applications as starter cultures for large-scale production of traditional products and have desirable functional properties for industrial applications. By understanding the metabolic processes involved in fermentation, scientists can identify ways to optimize production and reduce environmental impact. This has important implications for the future of food security and sustainability. This study aimed to evaluate the probiotic potential and safety analysis of LAB isolated from Ethiopian traditional fermented foods and beverages to determine their potential inhibiting activity against foodborne pathogens like *Staphylococcus aureus*, *Salmonella* Typhimurium, *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Candida albicans.*

Materials and methods

Isolation and Characterization of lactic acid bacteria

About 500 mL or 500 mg of each food sample was separately collected using sterile polyethylene bags and homogenized in 225 mL of sterile buffered peptone water. The diluted samples were aseptically transferred to MRS agar and incubated for 48 h at 37 °C under an anaerobic jar (Model No. HV-2-AJ, New Delhi, India). To ensure the purity of the isolates, snow white colonies' characteristics of typical LAB were further sub-cultured in MRS broth, incubated overnight, and then finally transferred onto MRS agar to pick distinct colonies from well-grown colonies for further characterization (Lobo et al. 2010).

Twenty-four-hour-old cultures of LAB were gram-stained and observed under an oil immersion objective to characterize the presumptive isolates of LAB for their cell shape and cell configurations (Thairu et al. 2014). Biochemical tests including the oxidase test, the catalase test, and the test for spores were performed as confirmation tests. Finally, the presumptive LAB isolates were further identified at species level using MALDI TOF (Zybio, EXS300, and China).

Bacterial strains

Lactic acid bacteria used in this study were isolated from Ethiopian traditional fermented foods and beverages. The standard strains, *Escherichia coli* ATCC25922, *Staphylococcus aureus* ATCC25923, *Listeria monocytogens* ATCC7644, *Pseudomonas aeruginosa* ATCC27853, *Salmonella* Typhimurium ATCC13311, and *Candida albicans* ATCC 14053 were kindly obtained from Ethiopian Public Health Institute (EPHI).

Characterization of probiotic lactic acid bacteria

Resistant to low pH

LAB isolates were grown in MRS broth at different pH values (2, 2.5, and 3) and their optical density was determined using UV-spectrophotometry after 0 h, 3 h, and 6 h after incubation at 37 °C under anaerobic conditions. The survival rate was calculated as the percentage LAB survival rate as follows: $SR(\%) = \frac{OD03h/6h}{\text{OD600nm}} * 100$

where OD600nm = the bacterial cell density at 0 h, OD3h/6 h = bacterial turbidity or cell density at 3 h or 6 h (Grosu-Tudor & Zamfir 2012). The bacterial density (Log 10^{6-8} CFU/ml).

Tolerance to bile salts

The isolates were cultured overnight in MRS broth at 37 °C to determine bile (0.3% (w/v; Oxgall, USA) tolerance in probiotic LAB. After adjusting cell density, they were inoculated in sterile MRS broth supplemented with 0.3% bile salt. Samples were collected after 24 h to measure the survival rate of the isolates (Grosu-Tudor & Zamfir 2012). The survival rate was calculated as follows: $SR(\%) = \frac{OD024h}{\text{OD600nm}} \times 100$, where OD600nm = the bacterial cell density at 0 h, O24h = bacterial turbidity or cell density after 24-h incubation, with the bacterial density (Log 10^{6-8} CFU/ml).

Growth at different temperatures

About 50µL of selected LAB cultures was placed into separate tubes containing 5 ml of MRS broth with 0.12 g/l bromocresol purple indicator. They were cultured for 7 days after inoculation at 25, 37, and 45 °C. Growth at any temperature was seen throughout this incubation period by the transformation of the cultures from purple to yellow (Yavuzdurmaz 2007).

Growth at different NaCl concentrations

The LAB tolerance to salt was evaluated by adding 4% and 6.5% NaCl to MRS broth. Test tubes were filled with 1% overnight culture and incubated at + 37 °C for 2–3 days. The color change from purple to yellow indicated cell development (Yavuzdurmaz 2007).

Gas production from glucose

The LAB homo-fermentative and hetero-fermentative characteristics by determining CO_2 production from glucose in modified MRS broth. Gas production was observed in Durham tubes after 5 days of incubation at + 37 °C recorded as a positive result (Bulut 2003).

Antimicrobial activity of lactic acid bacteria

The study examined the antibacterial activity of selected probiotic LAB strains against foodborne pathogens using the Agar well diffusion method. The selected LAB isolates were incubated overnight in a fresh MRS broth. The cell suspension (Log 107 CFU/ml) of pathogenic bacteria was used to assess the antibacterial activity of LAB isolates. Cell-free supernatant was obtained as a crude extract after centrifuged at 10, 000 rpm for 10 min at 4 °C (Fontana et al. 2015). Antimicrobial activity was detected after incubation at 37 °C, with a zone of inhibition around the wells showing antimicrobial activity.

Production of bacteriocins

The bacteriocin producing potential of isolates was evaluated using the agar-well diffusion assay against *Staphylococcus aureus* ATCC@25923, *Listeria monocytogenes* ATCC@7644, *Salmonella* Typhimurium ATCC@13311, and *Escherichia coli* ATCC@25922 (Yang et al., 2014). The inhibition due to acids and hydrogen peroxides was ruled out by adjusting the CFS PH to 7.00 using 1 NaOH and adding 5 g/ml of catalase, respectively. To confirm the formation of bacteriocin, CFS was treated with proteinase K (Sigma, St. Louis, USA) before evaluating the bacteriocin bioassay. As a result, 3 µl of proteinase K were added to each test tube containing 5 ml of CFS. The test tubes with and without enzymes (control) were incubated for 2 h at 37 °C (Saad et al. 2015a, b).

Tolerance to intestinal inhibitory substances

The study assessed the probiotic qualities of LAB isolates by examining their tolerance and survival ability to intestinal inhibitor chemicals. The isolates were cultured individually in MRS broth overnight at + 37 °C under anaerobic conditions. The survival potential was tested under adverse growth conditions, including phenol (0.3, 0.4, and 0.5 v/v %), bile salt (0.3, 0.5, and 0.8 w/v %), low pH (2 and 2.5), and various enzymes (pancreatic and pepsin). The cells were cultured for 24 h at 37 °C in static conditions, and their growth was observed. The survival rate of the isolates was calculated as follows: % tolerance(survival)rate $= \frac{\text{OD0h} - \text{OD24h}}{\text{OD0hr}} \times 100$ where OD_0 and OD_{24} refer to bacterial density at 0 h and

bacterial density after 24-h incubation period, respectively (Yasmin et al. 2020).

Survival of LAB in simulated gastrointestinal environment

Simulated stomach and intestinal fluids were made by combining 13.3 mg/L pepsin with 0.5% (w/v) sterile saline and changing the pH to 2.5, while simulated small intestinal juice was made by combining 250 mg/L pancreatic with 0.5% (w/v) sterile saline and adjusting the pH to 7.5. In 5 mL of freshly produced test solution, 106 CFU/mL of LAB cells were injected. For 3 h, the cultures were incubated under anaerobic conditions at 37 °C. After being exposed to gastric and intestinal juices, 1% v/v of each test sample was transferred to sterile MRS broth and incubated for 24 h under anaerobic conditions at 37 °C. In all cases, growth was measured at 600 nm at 0 h, 3 h, and 24 h, and the percentage was calculated. $SR(\%) = \frac{(OD0h-24h)}{OD0h} \times 100$ where OD 0 h and OD 3 h/24 h refer to bacterial optical density at 0 h and at 3 or 24 h, respectively (Kondrashina et al. 2023).

Survival of LAB to simulated stomach-duodenum passage (SSDP)

Simulated stomach-duodenum passage represents a complete environment for the survival of LAB in the stomach and duodenum of human (Mathara et al. 2008). To evaluate the survival potential of LAB to SSDP, MRS broth (pH 3.0), synthetic duodenum juice (Na_2HCO_3 (6.4 g), KCl (0.239 g), NaCl (1.28 g), distilled water (1000 ml), pH-7.5), and bile salt solution (10 g/100 ml) were prepared. MRS broth was inoculated with a 16–18-h-grown culture of LAB isolates. After 0, 3, and 24 h, samples were collected, and the optical density of the isolates was measured using a spectrophotometer. After 1 h of incubation, 4 ml of bile salt and 17 ml of duodenal juice were added. Samples were incubated at 37 °C for 2–3 h before being extracted for optical density assessment and percent survival was estimated after 3 h. Finally, the percent survival rate was calculated using the following formula:

$$SR(\%) = \frac{(OD0h - OD24h)}{OD0h} \times 100$$

where OD 0 h and OD 3 h/24 h refer to a bacterial optical density at 0 h and at 3 or 24 h, respectively (Yadav et al. 2016).

Safety evaluation of LAB isolates

Antibiotic susceptibility

The antibiotic susceptibility of LAB was assessed using an antibiotic disc diffusion method on MRS agar plates. Broth cultures of LAB were prepared using MRS and adjusted to 0.5 McFarland standards. 100 µl suspensions of freshly grown LAB cultures were spread on MRS agar plates. The antibiotic susceptibility pattern of the isolates was assessed using ampicillin (10 µg/disc), vancomycin (30 µg/disc), gentamicin (10 µg/disc), kanamycin (30 µg/disc), streptomycin (10 µg/disc), chloramphenicol (30 µg/disc), erythromycin (15 µg/disc), clindamycin (2 µg/disc), and tetracycline (30 µg/disc) (Zhang et al. 2016). The diameter of the zone of inhibition was measured using the antibiotic zone scale (CLSI scale). The results obtained are presented in terms of susceptibility, moderate susceptibility, or resistance. These results were compared with the interpretative zone diameters as described in Performance Standards for Antimicrobial Disc Susceptibility Tests (Callan and Westblade 2020).

Hemolytic activity

To determine the hemolytic activity of the LAB, the isolates were streaked onto blood agar plates containing 5% (w/v) sheep blood and incubated at 37 °C for 48 h. After incubation, the plates were examined for β-hemolysis, α-hemolysis, and non-hemolytic activities (Yadav et al. 2016).

DNase activity

The LAB isolates were streaked onto a deoxyribonuclease (DNase) agar medium to test for the production of the DNase enzyme. The plates were then incubated at 37 °C for 48 h and observed for the zone of DNase activity. A clear pinkish zone around the colonies was considered as positive DNase activity (Shuhadha et al. 2017).

Auto-aggregation

To evaluate the auto-aggregation potential of LAB isolates, an overnight culture was harvested by centrifugation (at 8000 rpm, 4 °C for 10 min), washed with PBS twice, and re-suspended in PBS buffer. The sample was allowed to stand for a while and incubated under anaerobic condition (BBL, anaerobic system) at 37 °C. Then, the supernatant was checked for absorbance at 600 nm at intervals of 0, 1, 2, 3, 4, and 5 h. The % auto-aggregation was measured using the formula: auto aggregation $(\%) = [1 - (A \text{ time}/A0) \times 100]$, where A time represents the absorbance at a particular time and $A0$ represents the absorbance at time 0 h (Zommiti et al. 2017).

Co-aggregation

For evaluation of co-aggregation of LAB, the selected isolates were grown in 10 mL of MRS broth while selected indicator bacteria pathogens (in this case, *S. aureus* ATCC@25,923) were grown in brain heart infusion at 37 °C. Co-aggregation was calculated using the following equation:

$$Co-aggregation(\%)=\frac{\frac{AODx+AODy}{2}-A\left(ODx+ODy\right)}{AODx+\frac{AODy}{2}}\times 100$$

where OD = optical density, OD0 = initial density at 0 h (the initial OD measurement taken immediately after the relevant strains were paired), ODt = optical density at a defined time (OD of the supernatant at time 0, 1, 5, and 24 h) (Zommiti et al. 2017), while x and y represent each of the two strains in the control tubes and in the $(x+y)$ mixture.

Cell surface hydrophobicity

In-vitro cell surface hydrophobicity OF LAB isolates was evaluated by measuring the microbial cell adhesion to hydrocarbons according to the method described by Rokana and colleagues (Rokana et al. 2018). Briefly, overnight cultures of LAB isolates grown in MRS broth were separately harvested by centrifugation (8000 rpm, 4 °C for 10 min) and washed twice with PBS before its re-suspension in PBS buffer, Finally, absorbance (A0) was measured at 600 nm. About 3 ml of the cell suspension was blended with 1 ml of hydrocarbon (xylene) and incubated at 37 °C without shaking for 1 h for separation of the aqueous and organic phases. The aqueous phase (1 ml) was removed carefully and the absorbance (A1) was measured at 600 nm. The percent hydrophobicity of the isolate was determined by the decrease in level of absorbance and calculated using the following formula: percentage cell surface hydrophobicity = $(1-A1/A0)\times 100$).

Data analysis

The data were analyzed statistically using the SPSS Statistics software package (version 23.0, IBM Corp., Armonk, NY, USA). The statistical significances between isolates with respect to their activities towards the evaluated probiotic criteria or parameters were analyzed using analysis of variance (ANOVA) post hoc, multiple variation comparison. In all cases, statistical significance was determined at $p<0.05$.

Results

Characterization of probiotic lactic acid bacteria (LAB)

Resistance to low pH

A total of 125 LAB isolates that tolerated and survived under various acidic conditions (pH 2, 2.5, and 3) and 0.3% bile salt concentrations were identified as candidate probiotics in this study (data not shown). For screening of better probiotics, isolates with the highest inhibition zone (>15 mm), acid and bile tolerance, and potential survival rate (>65%) were considered besides their antimicrobial efficiency, bacteriocin production, resistance to low acid and bile salts, and survival potential under simulated gastrointestinal conditions. As a result, only 54 LAB were identified as more promising probiotics out of the original 125 candidate probiotic isolates. After further extension of the incubation periods for 3 and 6 h, only eleven LAB isolates were found more capable of surviving pH values of 2, 2.5, and 3 with survival rates ranging between 34.92 and 98.82%. The survival rate of the isolates showed significant variation at $P=0.05$. This variation in survival rate suggests that pH levels have a significant impact on the viability of the isolates. The best probiotic isolates were finally subjected to MALDI TOF/mass spectrometry analysis for species identification.

Moreover, the study found that all isolates survived under specific acidic conditions, but their survival rates were higher under higher pH values and low time exposure. Accordingly, JULABB16 was the most tolerant isolate with 75.35% survival rate, followed by JULAB01 (74.23%) and JULABE31 (42.92%). Most isolates survived at pH 2 for 3 h and 6 h, similar to the 11 isolates with the highest tolerance to pH 2.5 and 3. The survival rates of the isolates ranged from 34.92 to 98.82% when exposed for 6 h under pH 2.5. However, the isolates had survival rates ranging from 61.92 to 98.82% for 6 h under pH 3. JULABB16 was the most tolerant isolate at pH 2 and 3 for 6 h, with a survival rate of 75.00 and 98.82%, respectively (Table 1).

Tolerance to bile salts

All 11 LAB isolates survived 0.3% bile salt concentration and were chosen as good probiotic candidates, with survival rates ranging from 57.56 to 99.20%. JULABB16 was the most tolerant with 99.20% survival potential, while JULABK37 had the least. These isolates had high resistance to low pH and recorded low death rates, making them promising candidates for further probiotic characterization (Table 1).

Antimicrobial activities of Lactic Acid Bacteria (LAB)

Acid-bile tolerant probiotic LAB demonstrated significant antimicrobial activity against foodborne pathogens, displaying significantly different diameters of zone of inhibitions ($P<0.05$). The average zone of inhibition ranged from 13.55 to 35.50 mm, with six of the LAB isolates showing the highest inhibition against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* Typhimurium, *Pseudomonas aeruginosa*, and *Candida albicans.* Accordingly, isolates, JULABB01, JULABE05, JULABE35, JULABK37, and JULABB16 showed the most effective antagonistic activity against all food spoilage and fungal pathogens (Table 2).

Bacteriocin production potential of LAB isolates

In this study, 11 LAB isolates were selected as potential bacteriocin producers. All eleven potential probiotic LAB

Table 1 Acid and bile tolerance of LAB isolated from Ethiopian traditional fermented foods and beverages

LAB isolates	pH tolerance rate (percentage of survival rate)						Bile salt
	3 h			6 h			
	pH2	pH2.5	pH3	pH2	pH2.5	pH3	
JULABB01	74.23 ± 0.69^{bc}	89.80 ± 0.69^{a}	95.42 ± 0.72^{a}	63.26 ± 0.65^{efg}	85.33 ± 0.58^{b}	97.67 ± 0.58^{ab}	98.00 ± 1.00^{a}
JULABB16	75.35 ± 0.56^{c}	80.33 ± 0.58^{bcd}	84.67 ± 0.58^{c}	73.00 ± 2.65^{c}	83.00 ± 6.24^{b}	98.82 ± 1.00^{a}	99.20 ± 0.72^{a}
JULABBk33	50.80 ± 0.28^{gh}	78.91 ± 0.13^{cde}	78.80 ± 0.28^{d}	52.80 ± 0.28^{ghi}	58.80 ± 0.28gh	79.80 ± 1.13^{e}	88.93 ± 0.11^{e}
JULABBk39	46.12 ± 1.02^{i}	69.00 ± 1.00^{f}	84.67 ± 0.58^{c}	65.29 ± 0.61^{e}	78.33 ± 0.58^{cd}	85.67 ± 0.58^{d}	81.33 ± 1.53^{fgh}
JULABBk40	47.97 ± 0.04^{hij}	74.62 ± 0.53^{ef}	75.97 ± 0.04^{d}	49.97 ± 0.04^{h}	55.97 ± 0.04^{h}	76.97 ± 1.37^{e}	71.77 ± 0.33^{g}
JULABE05	71.20 ± 0.72^{cde}	74.87 ± 0.81^{def}	89.08 ± 0.14^{bcde}	49.32 ± 0.59^{h}	78.33 ± 0.58^{cd}	88.00 ± 1.00^{cde}	84.21 ± 0.71^{fg}
JULABE31	42.92 ± 0.12^{j}	68.84 ± 0.22^{f}	60.92 ± 0.12^{fgh}	34.92 ± 0.12^{k}	40.92 ± 0.12^{kl}	61.92 ± 1.29^{h}	70.79 ± 0.30^{gh}
JULABE33	46.70 ± 0.42^{i}	69.64 ± 0.51^{f}	67.20 ± 0.29^{f}	41.70 ± 0.42^{jkl}	47.70 ± 0.42^{j}	68.70 ± 1.00^{fg}	73.63 ± 0.53^{g}
JULABE35	43.88 ± 0.17^{j}	69.60 ± 0.57^{f}	61.88 ± 0.17^{fgh}	35.88 ± 0.17^{k}	41.88 ± 0.17^{k}	62.88 ± 1.25^{h}	74.71 ± 0.41^{g}
JULABK37	59.17 ± 1.17^{defgh}	65.53 ± 0.66^{h}	86.17 ± 0.25^{cde}	60.67 ± 0.46^{f}	66.67 ± 0.46^{efg}	87.67 ± 0.95^{cde}	57.56 ± 0.62^{kl}
JULABK50	63.25 ± 1.09^{defgh}	75.00 ± 1.00^{d}	84.83 ± 1.04^{c}	44.40 ± 0.53^{ijk}	68.27 ± 0.64^{efg}	87.86 ± 0.24^{cde}	82.12 ± 0.82^{fgh}

[a-l] Values with different letters within a column are significantly different with regards to tolerance to a particular pH at $p < 0.05$. All values are mean or average ($n = 3$) ± SD (standard deviation)

Table 2 Antimicrobial activities of probiotic LAB against selected foodborne pathogens

LAB isolates	*E. coli* ATCC®25922	*S. aureus* ATCC®25923	*L. monocytogenes* ATCC®7644	*P. aeruginosa* ATCC®27853	*S.* Typhimurium ATCC®13311	*C. albicans* ATCC®14053
JULABB01	30.13 ± 0.18^{ab}	28.30 ± 0.40^{b}	25.40 ± 0.53^{c}	29.67 ± 0.33^{a}	20.50 ± 0.71^{abc}	29.67 ± 0.33^{a}
JULABB16	26.00 ± 0.57^{b}	32.50 ± 0.71^{a}	35.50 ± 0.71^{a}	30.50 ± 0.71^{a}	20.50 ± 0.71^{abc}	30.50 ± 0.71^{a}
JULABBk33	16.50 ± 2.12^{cdef}	18.50 ± 0.71^{cde}	23.00 ± 1.40^{c}	20.50 ± 0.71^{cde}	20.45 ± 0.63^{abc}	20.50 ± 0.71^{c}
JULABBk39	22.83 ± 0.58^{c}	12.17 ± 0.74^{fg}	20.50 ± 0.66^{d}	21.00 ± 0.5^{c}	19.17 ± 0.81^{b}	21.17 ± 0.50^{cde}
JULABBk40	14.58 ± 0.9^{f}	19.74 ± 0.65^{ij}	16.66 ± 0.18^{ef}	16.16 ± 0.2^{fg}	16.81 ± 0.63^{c}	16.50 ± 0.5^{d}
JULABE05	20.28 ± 0.40^{c}	20.30 ± 0.37^{cd}	29.33 ± 0.66^{bc}	17.50 ± 0.71^{e}	19.25 ± 0.47^{b}	15.75 ± 1.43^{d}
JULABE31	16.00 ± 0.63^{ef}	10.33 ± 0.54^{gh}	16.67 ± 0.63^{ef}	18.67 ± 0.06^{cdef}	20.00 ± 0.40^{b}	24.33 ± 0.43^{bcd}
JULABE33	16.67 ± 0.38^{ef}	14.00 ± 0.42^{f}	18.67 ± 0.28^{de}	20.67 ± 0.32^{cde}	19.33 ± 0.63^{b}	18.33 ± 0.76^{d}
JULABE35	21.28 ± 1.02^{c}	20.30 ± 0.40^{cd}	19.33 ± 0.66^{d}	17.00 ± 0.00^{e}	24.00 ± 1.41^{a}	20.50 ± 0.71^{c}
JULABK37	21.68 ± 0.45^{c}	17.80 ± 0.30^{d}	21.40 ± 0.53^{cde}	20.33 ± 0.33^{cde}	26.83 ± 0.71^{a}	18.50 ± 0.71^{c}
JULABK50	$16.62 \pm .04^{ef}$	15.62 ± 0.02^{ef}	23.28 ± 0.78^{cd}	25.28 ± 0.47^{b}	21.28 ± 0.28^{ab}	18.28 ± 0.71^{c}
Gentamicin (30mg/ml)	22.75 ± 0.35^{c}	20.30 ± 0.71^{cd}	18.96 ± 0.53^{cde}	21.25 ± 0.35^{cde}	22.67 ± 0.94^{ab}	19.00 ± 0.00^{c}

Mean values denoted by the same letter within a column are not significantly different in their antimicrobial activities against selected foodborne pathogens at $p < 0.05$,using gentamicin (30 mg/ml) as standard

isolates showed promising inhibition against *E. coli*, *S. aureus*, *Salmonella* Typhimurium, *P. aeruginosa*, and *C. albicans*. The inhibition zone diameters of crude extracts of the isolates ranged from 14.50 to 35.50 mm, while isolates JULABE05 and JULABBk40 exhibited the highest and the lowest antagonistic activities, respectively, against all foodborne pathogens evaluated in the study. The observed antimicrobial activities, potentially associated with bacteriocins produced by the isolates were inactivated after treatment of the extracts with proteinase K, confirming the proteinaceous nature of the substance responsible for the observed antimicrobial activities, hence bacteriocins (Table 3).

Antibiotic susceptibility test

Probiotic lactic acid bacteria were tested for their antibiotic susceptibility and resistance profiles. Accordingly, the isolates revealed varying degrees of susceptibility to five of the tested antibiotics including tetracycline, ampicillin, chloramphenicol, and erythromycin but all are resistant to kanamycin (Table 4).

Survival potential of probiotic LAB under stress conditions

Survival potential of LAB under simulated stomach and duodenum passage (SSDP) All LAB isolates showed potential survival rates ranging from 46.15 to 90.24%

Table 3 Antimicrobial activities of putative bacteriocin-producing probiotic LAB against selected foodborne pathogens

LAB isolates	*E. coli ATCC®25922*	*S. aureus ATCC®25923*	*L. monocytogenes ATCC®7644*	*P. aeruginosa ATCC®27853*	*S.* Typhimurium *ATCC®13311*	*C. albicans ATCC®14053*
JULABB01	17.75 ± 0.35^{c}	30.29 ± 0.41^{a}	35.94 ± 1.32^{a}	34.13 ± 0.18^{a}	33.49 ± 0.69^{a}	18.50 ± 0.71^{de}
JULABB16	18.25 ± 0.35^{c}	30.00 ± 0.00^{a}	30.13 ± 0.18^{b}	13.25 ± 0.35^{e}	24.82 ± 0.69^{b}	14.50 ± 0.71^{e}
JULABBK40	19.75 ± 0.35^{abc}	20.75 ± 1.06^{c}	20.29 ± 0.41^{de}	17.13 ± 0.18^{d}	19.49 ± 0.69^{bc}	25.38 ± 0.53^{cd}
JULABBK33	15.67 ± 0.66^{cde}	13.00 ± 0.00^{e}	14.67 ± 0.34^{e}	19.33 ± 0.34^{de}	16.67 ± 0.34^{c}	19.67 ± 0.34^{de}
JULABBK39	19.67 ± 0.82^{abc}	17.00 ± 0.00^{def}	25.33 ± 0.33^{c}	13.33 ± 0.33^{fg}	17.34 ± 0.07^{bcd}	23.33 ± 0.33^{c}
JULABE31	17.75 ± 0.35^{c}	20.50 ± 0.71^{c}	23.25 ± 0.35^{cd}	17.13 ± 0.18^{d}	20.13 ± 0.18^{bc}	14.26 ± 0.37^{e}
JULABE05	17.75 ± 0.35^{c}	17.50 ± 0.71^{def}	20.26 ± 0.37^{de}	19.13 ± 0.18^{de}	20.13 ± 0.18^{bc}	20.38 ± 0.53^{c}
JULABE33	17.25 ± 0.35^{c}	20.50 ± 0.71^{c}	18.43 ± 0.60^{d}	19.13 ± 0.18^{de}	18.13 ± 0.18^{bcd}	16.26 ± 0.37^{d}
JULABE35	20.00 ± 0.00^{abc}	13.00 ± 0.0^{e}	12.00 ± 0.00^{f}	17.00 ± 0.00^{cd}	13.00 ± 0.00^{d}	23.00 ± 0.00^{c}
JULABK37	20.87 ± 0.18^{abc}	14.75 ± 0.35^{d}	14.13 ± 0.18^{e}	18.50 ± 0.71^{d}	19.88 ± 0.18^{bc}	18.13 ± 0.18^{de}
JULABK50	16.00 ± 0.57^{cd}	15.00 ± 0.00^{d}	17.33 ± 0.33^{d}	17.33 ± 0.33^{d}	16.67 ± 0.34^{c}	15.33 ± 0.34^{ef}
Gentamicin (30mg/ml)	19.75 ± 1.06^{c}	17.50 ± 0.71^{def}	17.43 ± 0.53^{d}	15.13 ± 0.18^{e}	18.13 ± 0.18^{bc}	20.26 ± 0.37^{cd}

Mean values denoted by the same letter within a column are not significantly different in terms of the antimicrobial activities (inhibition) of putative bacteriocins produced by the isolates against the test pathogens at $p < 0.05$, gentamicin (30 mg/ml) as standard

Table 4 Antibiotic susceptibility profile of potential probiotic lab isolated from Ethiopian traditional fermented foods and beverages

Isolates	Antimicrobial susceptibility profile					
	TET	STR	KAN	AMP	ERY	CHL
JULABB01	S	S	R	S	S	S
JULABB16	S	S	R	S	R	S
JULABBk33	S	S	R	R	R	S
JULABBk39	S	R	R	S	R	S
JULABBk40	S	R	R	S	S	S
JULABE05	S	S	R	R	S	S
JULABE31	S	S	R	S	S	S
JULABE33	S	S	R	S	R	S
JULABE35	S	S	R	S	R	S
JULABK37	S	R	R	S	S	S
JULABK50	S	R	R	S	S	S

The zone of inhibition (diameter in mm) for each antibiotic measured and expressed as susceptible, S (≥ 21 mm); intermediate, I (16–20 mm) and resistance R (≤ 15 mm)

TET tetracycline, *STR* streptomycin, *KAN* kanamycin, *AMP* ampicillin, *ERY* erythromycin, *CHL* chloramphenicol

under simulated stomach duodenum passage. Isolates JULABB01 and JULAB16 had the highest survival rates, with significant variation at $p < 0.05$ (Table 5). These findings suggest that these LAB isolates have the ability to survive and potentially colonize the human gastrointestinal tract.

Tolerance to simulated gastro-intestinal juice

The study tested the survival potential of LAB isolates in vitro under simulated gastro-intestinal juice. Isolates JULABB01 and JULAB16 showed the highest rates, with 85.45% and 83.06%, respectively. JULABK37 had the lowest survival rate (43.42%), with significant variation at $p < 0.05$ (Table 5).

Simulated intestinal juice tolerance

Out of 125 potential LAB evaluated for their survival under simulated intestinal juice, 11 showed the highest survival rate, with isolates JULABB01 and JULABB16 having the highest survival rates, accounting for 85.18 and 78.35% of the total screened probiotics, respectively. The survival rates showed significant variation at $P < 0.05$ (Table 5).

Intestinal inhibitor substances (phenol, pancreatin, and pepsin)

The eleven isolates were screened for their ability to tolerate 0.3% phenol concentration and showed survival

Table 5 Percentage survival rates of probiotic LAB under conditions of simulated gastro-intestinal environment

LAB isolates	Survival rate (percentage survival)					
	SGIJ	SIJ	SSDP	Intestinal inhibitor substance		
				Phenol	Pepsin	Pancreatin
JULABK37	59.00 ± 0.00^{e}	46.05 ± 0.06^{hij}	51.34 ± 0.48^{gh}	48.93 ± 0.10^{k}	56.37 ± 0.52^{fg}	56.14 ± 0.20^{hi}
JULABE31	61.21 ± 0.29^{def}	48.00 ± 0.00^{hi}	54.45 ± 0.63^{g}	50.95 ± 0.07^{k}	58.01 ± 0.01^{e}	58.15 ± 0.22^{h}
JULABE35	61.28 ± 0.39^{def}	48.06 ± 0.08^{h}	56.34 ± 0.49^{fgh}	50.45 ± 0.63^{k}	58.02 ± 0.03^{e}	58.37 ± 0.52^{h}
JULABE33	61.49 ± 0.70^{def}	48.15 ± 0.22^{h}	56.38 ± 0.54^{fgh}	51.02 ± 0.03^{k}	58.02 ± 0.03^{e}	59.76 ± 0.34^{h}
JULABBK39	67.40 ± 0.57^{cd}	57.30 ± 0.43^{fg}	61.37 ± 0.52^{efg}	54.44 ± 0.62^{j}	69.04 ± 0.05^{de}	66.09 ± 0.13^{gh}
JULABE05	43.42 ± 0.71^{ij}	49.39 ± 0.31^{h}	59.54 ± 0.29^{f}	98.67 ± 0.33^{a}	69.03 ± 0.55^{de}	70.03 ± 0.55^{f}
JULABBK40	68.01 ± 0.02^{cd}	57.36 ± 0.51^{fg}	62.23 ± 0.32^{efg}	55.02 ± 0.03^{j}	69.29 ± 0.41^{de}	66.48 ± 0.68^{gh}
JULABK50	58.07 ± 0.07^{ef}	70.19 ± 0.19^{c}	46.15 ± 0.15^{i}	89.35 ± 0.33^{c}	69.78 ± 0.22^{de}	70.44 ± 0.29^{f}
JULABBK33	68.45 ± 0.64^{cd}	59.23 ± 0.33^{f}	63.26 ± 0.36^{efg}	56.19 ± 0.26^{ij}	70.02 ± 0.03^{cde}	71.10 ± 0.14^{f}
JULABB16	83.06 ± 0.08^{ab}	78.35 ± 0.50^{b}	86.44 ± 0.63^{abc}	77.16 ± 0.22^{e}	88.09 ± 0.13^{a}	98.06 ± 0.09^{b}
JULABB01	85.45 ± 0.64^{a}	85.18 ± 0.26^{a}	90.24 ± 0.34^{a}	79.82 ± 0.25^{e}	88.21 ± 0.30^{a}	106.50 ± 0.71^{a}

SGIJ simulated gastro-intestinal juice, *SIJ* simulated intestinal juice, *SSDP* simulated stomach duodenum passage. Mean values denoted by the same letterwith similar superscripts within a column are not significantly different at $p < 0.05$ with regards to the isolates' tolerance to different inhibitory substances

rates ranging from 48.93 to 98.67%. JULAB01 had the highest survival rate at 98.67%, while JULABK37 had the lowest at 48.93%. These high resistance rates make them promising probiotic candidates for potential applications (Table 5).

Pancreatin was used to assess probiotic LAB survival rates in the simulated gastrointestinal tract. JULABB16 and JULABB01 showed the highest survival rates, with JULABK37 having the least. JULABB01 exhibited the highest pancreatin tolerance (Table 5).

Screening probiotic lactic acid bacteria for potential applications requires tolerance to conditions in the gastro-intestinal environment. Pepsin resistance was examined and it revealed that all the tested isolates showed survival rate ranging from 56.37 to 88.21%. Isolates JULABB01 and JULABB16 had the highest survival rate at 88.21% and 88.09%, respectively, with statistically significant differences ($P < 0.05$) in their survival rates (Table 5).

Safety evaluation of LAB isolates

Auto-aggregation and co-aggregation

All isolates showed potential auto-aggregation ability and a survival rate that ranged from 45.19 to 120.39%. Isolates JULABB01 and JULABB16 had the most auto-aggregation potential, while JULABBr40 had the lowest auto-aggregation potential with about 45.35% auto-aggregation potential. The mean auto-aggregation value of the isolates showed significant variation at $P < 0.05$ (Table 6). LAB isolates were evaluated by testing their co-aggregation with selected pathogens (*S. aureus* ATCC[@]25,923), ranging from 53.37 to 116.20%.

All isolates exhibited potential auto-aggregation abilities, with survival rates ranging from 45.19 to 120.39%. JULABB01 and JULABB16 had the highest auto-aggregation potential, 120.39 and 102.34%, while JULABBr40 had the lowest with 45.35% of auto-aggregation potential. Significant variation was observed in mean value auto-aggregation. Co-aggregation of selected pathogens (*S. aureus* ATCC@25,923) was evaluated, ranging from 53.37 to 116.20%. Co-aggregation is crucial for LAB's adhesion to oral, gastrointestinal, and urogenital spaces, preventing colonization by pathogens. JULABB01 and JULABB16 exhibit the highest potential, while JULABBk40 has the lowest rate at 53.37% (Table 6).

Cell surface hydrophobicity

The cell surface's hydrophobicity was demonstrated through high adherence to xylene, with isolates showing a percentage of hydrophobicity greater than 65.26%. Isolates JULABB01 and JULABB16 showed the most adherence capacity, with 106 and 97% adherence rates, respectively. JULABBE35 had the lowest cell-surface hydrophobicity rate and the mean value of hydrophobicity showed significant variation at $P < 0.05$(Table 6).

Discussion

The study identified 125 probiotic LAB, but only eleven were screened as the most promising candidates based on their acid-bile tolerance, antimicrobial activity, bacteriocin production capacity, and survival rate under simulated gastro-intestinal conditions. All eleven isolates showed a promising tolerance rate and were able to grow at 4 and 6.5% salt concentrations, and different temperature ranges (15 °C and 37 °C). However, only a few were

Table 6 Aggregation, hydrophobic, safety analysis, and co-aggregation potential of probiotic lactic acid bacteria isolated from Ethiopian traditional fermented foods and beverages

Isolates	Hemolytic activity	DNase activity	Auto-aggregation	*Co-aggregation	Hydrophobicity
JULABE35	γ-hemolytic	-	55.25 ± 0.36^{k}	65.26 ± 0.37^{i}	65.26 ± 0.71^{ghi}
JULABE33	γ-hemolytic	-	63.74 ± 0.36^{j}	76.43 ± 0.61^{gh}	67.43 ± 0.12^{f}
JULABBk33	γ-hemolytic	-	64.55 ± 0.63^{j}	77.39 ± 0.55^{g}	67.39 ± 0.22^{f}
JULABBk40	γ-hemolytic	-	45.35 ± 0.50^{m}	55.37 ± 0.52^{j}	68.37 ± 0.28^{h}
JULABBk39	γ-hemolytic	-	73.16 ± 0.22^{hij}	83.43 ± 0.61^{fg}	73.43 ± 0.81^{e}
JULABK37	γ-hemolytic	-	79.38 ± 0.54^{h}	87.36 ± 0.52^{e}	87.36 ± 0.21^{e}
JULABK50	γ-hemolytic	-	91.07 ± 0.10^{def}	97.14 ± 0.19^{cde}	89.14 ± 0.12^{cde}
JULABE31	γ-hemolytic	-	67.92 ± 0.12^{ij}	80.31 ± 0.44^{fgh}	90.31 ± 0.04^{ef}
JULABE05	γ-hemolytic	-	93.47 ± 0.66^{de}	99.29 ± 0.40^{c}	95.29 ± 0.20^{c}
JULABB01	γ-hemolytic	-	120.39 ± 0.55^{a}	116.20 ± 0.29^{a}	106.50 ± 0.14^{a}
JULABB16	γ-hemolytic	-	102.34 ± 0.48^{bc}	106.19 ± 0.26^{bc}	97.19 ± 0.80^{b}

Mean values with similar superscript within a column are not significantly different at $p < 0.05$ in terms of the activities displayed.

* The co-aggregation potential of LAB isolates was evaluated against selected food-borne pathogen (*S. aureus* ATCC@25923)

capable of growing at 45 °C. This finding is similar to previous research on LAB isolated from Ethiopian traditional fermented products like *Ergo*, *Shamita*, *Borde*, and fermented teff (Azadnia & Khan Nazer 2009; Negasi et al. 2017). Recently, Mulaw et al. (2019) reported that all 34 probiotic LAB isolated from *kotcho*, *ergo*, and fermented teff showed remarkable growth at 4 and 6.5% salt concentrations, as well as at 10 and 15 °C temperatures, which is more similar to findings of this study, This shows that Ethiopian traditional fermented foods and beverages are good sources of probiotics.

The survival rate of probiotic LAB to pH 2, 2.5, and 3 was found to be the best for all 11 isolates. However, as the pH decreased to 2, the survival rate decreased. The survival rate ranged from 34.92 to 98.82%, with significant variations in their ability to survive under acidic conditions ($P < 0.05$). Abushelaibi et al. (2017), Ribeiro et al. (2014), and Song et al. (2021) found that some *Pediococcus* species have a 70% survival rate at pH 2 for 3 h. However, Akbar et al. (2019), Pan and Zhang (2008), and Zielińska et al. (2015) demonstrated that most *Lactococcus lactis* have a survival rate from 65 to 70% after exposure to pH 2 for 3 h, which is relatively lower than the present study conducted at Jimma University, Ethiopia. It is important to note that survival rates can vary depending on the specific strain of bacteria and the conditions of the experiment, highlighting the need for further research in this area (Anker-Ladefoged et al. 2021).

The human gastrointestinal tract's bile concentration is 0.3% w/v, with a staying time of 3–4 h. All eleven LAB isolates were highly resistant to bile salt, indicating their survival in the small intestine. Their survival rate ranged from 82.30 to 99.20%, consistent with Mulaw et al. (2019) in which 34 LAB isolates showed the survival rate ranging from 82.58 to 99.44% survival rate. Similarly, Dutra Rosolen et al. (2021), Harnentis et al. (2020), Jatmiko et al. (2017), and Ribeiro et al. (2014) found that most *Pediococcus* and *Lactococcus* species exhibit bile tolerance, with survival rates ranging from 42.50 to 85.30%. LAB's resistance to 0.3% bile salt is due to its ability to produce bile salt hydrolase, which de-conjugates bile acid, making it less soluble and reabsorbed less efficiently. This leads to a reduction in serum cholesterol (de Melo Pereira et al. 2018).

The study found that eleven LABs demonstrated the most significant antagonistic effect, with a zone of inhibition ranging from 18 to 29.96 mm. In line with this, Mulaw et al. (2019) found 34 probiotic LABs had effective antagonistic activity, with inhibition zones ranging from 12 to 22 mm. Similarly, Tadesse et al. (2005) demonstrated that LAB isolated from *borde* and *shamita* inhibited *Staphylococcus aureus*, *Salmonella Typhimurium*, and *Escherichia coli* growth with inhibition zones ranging from 15 to 17 mm in diameter. On the other hand, Negasi et al. (2017) discovered that LAB from Ethiopian fermented dairy product, *Ergo*, had potential antibacterial action against *Salmonella Typhimurium*, with inhibitory zones ranging from 10 to 14.5 mm in diameter. Probiotic isolates exhibit variation in antagonistic activity, indicating they are pathogen-specific and require bioactive compounds like organic acids, hydrogen peroxides, and bacteriocins. These compounds enable probiotic LAB to survive and tolerate adverse conditions, inhibiting the growth and proliferation of food-borne pathogenic bacteria (Mulaw et al. 2019).

This study found only 11 isolates showed qualitative evidence for bacteriocin production and antimicrobial activity. This is similar to previous research by Zhang

et al. (2013), which found that out of 300 LAB isolates screened for bacteriocin production, only six probiotic strains produced bacteriocins and displayed antagonistic activity against foodborne pathogens like *Listeria monocytogenes*. Mulaw et al. (2019) reported that out of 34 potential probiotic LAB isolates, only nine were capable of producing bacteriocins and showed antagonistic activity against *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella* Typhimurium. Furthermore, Musikasang et al. (2012) and Saad et al. (2015a, b) also reported that probiotic LAB isolated from various sources were capable of producing bacteriocins and showing effective antagonistic activity against selected foodborne pathogens. This highlights the potential of probiotic LAB as a natural alternative to chemical preservatives in food products. However, further research is needed to determine the safety and efficacy of these probiotic lactic acid bacteria.

Antibiotic resistance is a significant concern in the food industry due to foodborne diseases and the spread of multidrug-resistant pathogens. Intrinsic antibiotic resistance in bacteria poses minimal risk for horizontal spread, while acquired resistance is high. But most probiotic LAB are considered safe due to their acquired resistance genes, making them a major concern in the food industry (Kim & Ahn 2022), because they are not the problem of food-borne pathogens and thus why, they are generally regarded as safe(GRAS).

The study found that eleven probiotic lactic acid bacteria (LAB) were more resistant to kanamycin and more sensitive to tetracycline and chloramphenicol. However, all LAB were resistant and susceptible to ampicillin and streptomycin. This finding is consistent with previous studies in Ethiopia (Mulaw et al. 2019; Negasi et al. 2017) where LAB isolated from traditional fermented foods and beverages were more resistant to kanamycin and susceptible to antibiotics like tetracycline, ampicillin, and erythromycin. Similarly, LAB isolated from traditional fermented milk in Spain (Niazi Amraii et al. 2014 and Rajoka et al. 2019) were more susceptible to chloramphenicol, erythromycin, tetracycline, and ampicillin. These antimicrobial susceptibility patterns align with previous literature, making LAB a potential candidate for the food industry and human health.

In this study, we evaluated the probiotic LAB potential for survival and tolerance in the gastrointestinal tract and pancreatic juice stimulation. All 11 isolates showed survival rates ranging from 32 to 100%. A good probiotic LAB should have to survive and tolerate barriers like low pH, gastric juice, bile salts, and pancreatin (Pérez Montoro et al. 2018; Sanhueza et al. 2015) to be selected as a good probiotic candidate. Studies have shown that *Pediococcus acidilactici* isolates can survive in gastric juice at pH 3, with a survival rate of 68%. Therefore, in line with this result, Doğan and Ay (2021) and Sarkar et al. (2020) reported that *Pediococcus pentosaceus* and *Pediococcus acidilactici* survived in acidic gastric juice with a pH of 2–3, making them potential probiotic LAB strains. *Lactoccoccus lactis* from various food items showed potential survival rates in simulated gastric juice at pH 3(Dobson et al. 2011; Naissinger da Silva et al. 2021). Furthermore, studies have shown that certain strains of *Pediococcus* and *Lactococcus* can also survive in gastric juice at low pH levels, indicating their potential as probiotics for gut health (Ouwehand et al., 2002) and treatment of bacterial vaginosis(Wang et al., 2019). However, it is important to note that survival in gastric juice alone does not guarantee probiotic efficacy and further research is needed to evaluate the health benefits of these strains.

The secretion of gastric juice with a pH of 2.0 is not favorable for most pathogenic bacteria, leading to the death of pathogens when ingested into the gastrointestinal tract (GIT) (Vizoso Pinto et al. 2006). In this study, all eleven LAB isolates were able to survive in simulated gastric juice with a survival rate ranging from 43.42 to 85.45%. This is similar to previous research by Blajman et al. (2015), García-Hernández et al. (2016), and Musikasang et al. (2012), which reported survival rates ranging from 20 to 80%. LABs should survive gastrointestinal conditions and reach the small intestine alive, where they colonize and provide health benefits to the host and gastric and intestinal juice resistance is a crucial factor in selecting potential probiotics (Larsen et al. 2018). In this study, all LAB isolates had the potential to survive and tolerate stimulation of the gastro-intestinal tract and intestinal juice, making them potential candidates for starter cultures and natural food preservatives.

Lactic acid bacteria from Ethiopian fermented foods were tested for survival under simulated stomach duodenum passage. Most LAB isolates showed a survival rate greater than 45%, indicating their ability to tolerate stomach conditions and be potent probiotics. In the duodenum, the physiological concentration of human bile is around 0.3%, and therefore, this concentration is usually selected as an essential criterion for the assessment and selection of probiotic strains (Shehata et al. 2016). FAO guidelines recommend using microbial strains as probiotics with hemolytic activity as a safety criterion. All LAB isolates were chosen for evaluation due to their safety as probiotics (FAO 2016). The study found that none of the selected probiotics showed α- or β-hemolytic activity when grown in Columbia blood agar. All strains were γ-hemolytic, consistent with previous observations (Argyri et al. 2013; G Pavli et al. 2016; Maragkoudakis

et al. 2006; Oyewole et al. 2018; Pisano et al. 2014). This suggests that most LAB isolates are non-hemolytic, making them safe for use as good probiotic candidates.

Auto- and co-aggregation are crucial characteristics for identifying potential probiotic LAB, as they influence bacterium adhesion to the intestinal mucosa and formation of biofilms on surfaces (Krausova et al., 2019). According to the study, the auto-aggregation potential of LAB varied significantly at $P < 0.05$, ranging from 45.19 to 120.39%. The highest auto-aggregation was recorded by *Pediococcus pentosaceus* JULABB01, followed by *Pediococcus pentosaceus* JULABB16 at 120.39% and 10234%, respectively. The least auto-aggregation was observed in *Lactococcus lactis* JULABB (45.19%). Mathara et al. (2008), Nallala & Jeevaratnam (2015), and Taheri et al. (2009) found that certain LAB species have auto-aggregation potential exceeding 40% adhesion capacity, similar to the study's findings. Auto-aggregation is crucial for biofilm formation, facilitating probiotics' adhesion and colonization of host intestinal cells.

Co-aggregation prevents enteric pathogen adhesion to intestinal cells. Auto-aggregation and surface hydrophobicity promote cell adhesion, binding probiotics to the intestinal lining, acting as a barrier against pathogen colonization. This binding is essential for pathogen omission and immunomodulation (Nallala & Jeevaratnam 2015). Auto-aggregation ability and surface hydrophobicity are related to cell adhesion, which promotes the binding of probiotics to the intestinal lining. As a result of this binding, it acts as a barrier and pathogens are unable to colonize. This adhesion of probiotics to the intestinal cell is essential for pathogen omission and immunomodulation (Russell et al. 2011).

The study assessed LAB isolates' cell surface hydrophobicity, revealing significant variation between 69.08 and 116.20%, indicating their ability to adhere to the intestinal epithelium. Similar to this study, Iñiguez-Palomares et al. (2008) found that probiotic LAB isolates have 40% cell surface hydrophobicity against xylene. In pigs and chickens, LAB species have the highest potential adhesion capacity to duodenal epithelium cells.

This study examined the cell surface hydrophobicity of six LAB isolates to determine their capacity to adhere to the intestinal epithelium. The cell surface hydrophobicity was ranged from 69.08 to 116.20%, with significance variation at $P < 0.05$. Similar to this study, Iñiguez-Palomares et al. (2008) report that large numbers of probiotic LAB showed the cell surface hydrophobicity greater than 40%against xylene(Iñiguez-Palomares et al. 2008). In addition, according to Gabriel, LAB species showed the most potential adhesion capacity to duodenal epithelium cells of pig and chicken, respectively (Marks et al. 2022). The study on cell surface hydrophobicity is very important, because the experiments help in studying the colonization and adhesion of probiotic bacteria to epithelial cells in the gastrointestinal tract, which lead to the prevention of colonization by pathogens through their interaction (Abushelaibi et al. 2017).

Conclusion

In this study, eleven LAB isolates, belonging to seven *Pediococcus pentosaceus*, two *Pediococcus acidilactici*, and two *Lactococcus lactis*, *were* identified from four Ethiopian traditional fermented foods and beverages (*Bulla*, *Kotcho*, *Ergo*, and Bukuri). These selected isolates were found to have the most promising probiotic candidate, which can be applied as a novel and natural food preservative in food industry to improve food shelf life and for starter culture development. However, further research activities are needed to evaluate their probiotic and potential activities under in vivo conditions and whole genomic sequencing to determine and analyze their detailed antimicrobial resistance genes.

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Authors' contributions
DA and KB developed the research concept and solicited the necessary research inputs. DA designed and conducted the experiments, analyzed the data, and wrote the draft manuscript. KB supervised the experiment and reviewed the draft manuscript. All authors read, reviewed, and approved the manuscript.

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Declarations

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Competing interests
The authors declare that they have no competing interests.

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