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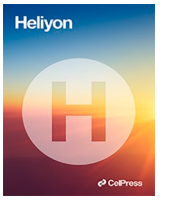

Characterization of lactic acid bacteria isolated from street pickles of Dhaka, Bangladesh

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ABSTRACT

Traditionally fermented pickles are a popular street food in Bangladesh famous for their unique flavors and health benefits. Pickles are often prepared by fermentation using lactic acid bacteria (LAB) that can act as probiotics. The study was aimed to isolate and characterize lactic acid bacteria from pickle samples collected from streets of Dhaka city, as well as assess the microbial quality of pickles for food safety.

A total of 30 pickle samples of different kinds were collected from streets of Dhaka city. Isolation and identification were conducted using conventional cultural and biochemical tests, followed by molecular confirmation of identity. Antibiotic susceptibility of isolates was investigated against 7 antibiotics of different groups. Antimicrobial activity of LAB isolates was analyzed by well-diffusion assay and phenotypic enterocin activity assay. Physiological characterizations of LAB were performed to determine their tolerance to temperature, salt, pH, bile, carbohydrate fermentation pattern, proteolytic activity and biofilm formation.

Fifty isolates were obtained from pickle samples, of which 18% was identified as LAB, including *Enterococcus faecalis* (6) and *Enterococcus faecium* (3). The rest included *S. aureus* (18), *E. coli* (11), *Klebsiella* spp. (5), *Salmonella* (3), *Shigella* (3) and *Pseudomonas aeruginosa* (1). Antibiotic resistance pattern revealed higher occurrence of resistance against azithromycin among the non-LAB isolates, but none of the LAB isolates were found to resist any of the antibiotics used. Antimicrobial activity of LAB isolates was not observed against the foodborne isolates. All LAB isolates fermented a wide range of carbohydrates and showed adequate tolerance to salt, pH, temperature and bile. Out of 9 isolates, 5 displayed proteolytic activity, and 6 were found as strong biofilm producer.

These results suggest that although the LAB isolates from street pickles collected from Dhaka does not have antimicrobial activities, they still have potential to be used as probiotics. It also shows high occurrence of antibiotic resistant foodborne pathogens in pickles, indicating that consumption of such street food can be serious health hazard.

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1. Introduction

In recent times, probiotics have become one of the most sought-after factors to enhance the quality of food consumed. Based on the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [1]. Several species of the genera *Lactobacillus* and *Bifidobacterium*, as well as some *Bacillus, Streptococcus, Pediococcus*, and *Enterococcus* species, are widely considered as probiotic bacteria [2–4]. They come with a number of advantages including resistance to gastric acidity, bile acid resistance, adherence to mucus and/or human epithelial cells and cell lines, ability to reduce pathogen adhesion to surfaces, as well as antimicrobial activity against potentially pathogenic bacteria [5,6]. Although the health benefits of probiotics are still being discovered, they have been a part of human diet for a long time. In the past, it was thought that the Gastrointestinal tract of humans and animals were the main source of probiotic strains since such strains of host origin would be more suited to colonize those tracts [7,8].

Dairy and fermented food products are the main source of probiotics, but since dairy products might be inconsumable to some due to lactose intolerance and high cholesterol levels [9], non-dairy fermented products like pickles have drawn greater attention. Fermented food products can serve legitimate and diverse source of probiotic bacterium [10], but the restricted availability of probiotic strains with various functional characteristics for the industrial production of innovative probiotic foods presents certain difficulties [11,12]. Fermented fruits and vegetables are often studied as a source of probiotic strains [2,3,13], but they are still in little supply when compared to dairy products. In this situation, pickles, a conventional fermented fruit or vegetable product, may be a potential source of probiotic bacteria.

Pickles are traditional appetizers in South Asian countries like Bangladesh, where it is also sold as "Aachar", a popular street food. Pickling is one of the oldest ways to preserve fruits through fermentation [14]. Unlike Western pickling that involves brining with spices [15], Aachar is prepared with a wide range of components including salt, sugar, vinegar, jaggery, spices like chili, turmeric, cumin, fenugreek, and oil [2]. The high salt and sugar content of pickles aids to prolong the shelf life of fruits and vegetables for food preservation and has been a part of Indian, Chinese and Egyptian cuisine since ancient time [16]. It may contain a diverse population of lactic acid bacteria (LAB) that improve digestion of vegetables and grains, provide vitamins, minerals and carbohydrates as well as produce a unique flavor profile by supplying components like bacteriocins and exopolysaccharides [17–19]. Considered as Generally Recognized as Safe (GRAS) additive, LAB may also prevent infectious and spoiling microbes in food [10,20]. Significant LAB responsible for traditional pickle fermentation includes *Lactobacillus plantarum*, *L. brevis, Leuconostoc mesenteroides, Pediococcus cerevisiae, Pediococcus pentosaceus,* and *Enterococcus faecalis* [21,22]. Several studies have reported LAB being used to manufacture different traditionally fermented foods like boza and cider fermentation using *Limosilactobacillus fermentum* and *Leuconostoc mesenteroids* in Egypt, sourdough fermentation using *Lactobacillus sanfranciscensis* in Italy and cheese fermentation using *Lactococcus lactis* in Egypt [57–59]. LAB isolated from traditionally fermented African foods like fufu and garri has been seen to have therapeutic effect against SARS-CoV-2 and other viral infections [60].

Since pickles are often consumed as street food sold by vendors, it can also serve as a source of food-borne pathogens like *E. coli, Salmonella* spp., *Staphylococcus aureus* and *Klebsiella* spp [23]. Food-borne diseases caused by these potentially pathogenic bacteria may cause diarrheal diseases, and an estimated 1.7 billion cases of child deaths worldwide annually are attributed to consumption of contaminated food and water [24]. Pickles can serve as a reservoir for such bacteria if production, processing and serving process is somehow contaminated. Open displays, contamination from insects, dust and hands of vendor, unhygienic conditions and lack of access to basic sanitary facilities may lead to cross-contamination and cause food-borne diseases including diarrhea, gastroenteritis and Typhoid fever [25,26]. Thus, monitoring of microbial quality of pickles is essential to ensure the presence of beneficial bacteria like LAB and absence of potentially pathogenic bacteria.

Information on the microbial quality of traditional pickles or Aachar sold on streets of Bangladesh is low. Keeping this in view, the goals of the present study was to isolate, identify and characterize the bacterial isolates from pickles samples collected from the streets of Dhaka. Additional physiological characterization of potential LAB isolates was conducted in order to learn more about the role of LAB as a potential probiotic option, and antimicrobial resistance & biofilm formation of potentially pathogenic isolates were also screened to characterize them.

2. Methodology

2.1. Sample collection

In this study, a total of 30 different locally produced pickled fruit samples including pickled jujube *(Ziziphus jujube)* (8), tamarind *(Tamarindus indica)* (6), mixed fruit (tamarind, olive and jujube) (5), olive *(Olea europaea)* (2), elephant apple *(Dillenia indica)* (2), green mango *(Mangifera indica)* (2), plantain *(Musa acuminata)* (2), garlic *(Allium sativum)* (2) and hog plum *(Spondias dulcis)* (1) were collected from mobile street vendors selling pickles at different locations across Dhaka North and Dhaka South over a period of 3 months. Pickles were produced using fruits grown locally and conventional pickling ingredients. Samples were aseptically collected and put into sterile bags for immediate transportation to laboratory for bacterial isolation while maintaining temperature and other physicochemical conditions [23].

2.2. Sample processing and pre-enrichment

To process the sample, 10 g of pickle sample was added to 90 ml of sterile 0.9% normal saline, followed by homogenization and

Table 1
Primers used for molecular identification in this study.

Primer name	Purpose	Primer Sequence	Amplicon size (bp)	Annealing T (°C)	References
*invA*139 (F) *invA* 141 (R)	*Salmonella* spp.Invasion Antigen	GTG AAA TTA TCG CCA CGT TCG GGC AA TCA TCG CAC CGT CAA AGG AAC C	284	63	[28]
uidA (F) *uidA* (R)	*E.coli*-specific β-Glucuronidase enzyme	TAT GGA ATT TCG CCG ATT TT TGT TTG CCT CCC TGC TGC GG	166	55.2	[29]
fd1 (F) rp2 (R)	16S rDNA Amplification	AGA GTT TGA TCC TGG CTC AG ACG GCT ACC TTG TTA CGA CTT	1500	49	[30]

serial dilution up to 10^{-6} decimals. Diluted samples were spread over Plate Count Agar (Oxoid, UK) to determine total viable bacterial count. Pre-enrichment was conducted to detect specific food-borne pathogens including *E. coli, Salmonella* spp. and *S. aureus* by inoculating 25 g of each sample in 225 ml of buffered peptone water (Oxoid, UK), homogenization and incubation at 37 °C for 18–24 h. Following incubation, 1 ml of pre-enrichment culture was inoculated into 9 ml organism-specific medium, including Henja Tetrathionate Broth (HiMedia Laboratories, India) for *Salmonella* spp. and lactose broth (Oxoid, UK) for *E. coli,* and incubated at 37 °C for 18–24 h.

2.3. Presumptive identification

For presumptive identification, a series of cultural and biochemical tests were performed. Cultural characterizations included MacConkey Agar for selective isolation of Gram-negatives, Mannitol Salt Agar (MSA) for selective isolation of Gram-positives, Eosine-Methylene-Blue Agar (EMB) for selective isolation of *E. coli, Salmonella-Shigella* Agar for selective isolation of *Salmonella* and *Shigella,* and Bile-Esculin Agar for selective isolation of *Enterococcus* (all from Oxoid, UK). Presumptive identification by biochemical tests was conducted by tests including Gram staining, IMViC test (Indole- Methyl Red- Voges Proskauer- Citrate utilization), Oxidase test, Catalase test and Carbohydrate utilization profiling (Reagents were from HiMedia, India). All isolates were identified presumptively up to the Genus level according to Bergey's Manual of Determinative Bacteriology [27].

2.4. Molecular identification

Presumptively identified *E. coli* and *Salmonella* spp. isolates were identified on the basis of PCR amplification of species-specific genes *uidA* and *invA,* respectively. Rest of the isolates was subjected to 16S rDNA amplification using a universal primer. Ten isolates from different biochemical profiles were selected for this experiment. DNA was extracted using commercially available DNA Extraction Kit by Favorgen, Taiwan. PCR was conducted using PCR Ready-Mix, followed by PCR product purification and sequencing. Sequences were blasted using an online database to confirm the identity of the bacterial isolates [56]. Sequences obtained were aligned using ClustalW alignment and phylogenetic tree based on sequence homology was constructed by Neighbor Joining method using MEGA 6.06 software. Primers used in this study are described in Table 1.

2.5. Antibiotic susceptibility testing

Bacterial susceptibility test to antibiotics was performed by disc-diffusion method [31] followed by determination of MIC by agar dilution method and interpreted according to CLSI standards and guidelines [32]. Each non-LAB isolate was checked for resistance against seven commonly used antibiotics including Cephalosporin (Ceftriaxone; CRO), Penicillin + β-lactamase inhibitor (Amoxicillin + Clavulanic Acid; ACL), Carbapenem (Imipenem; IMP), Fluoroquinolone (Ciprofloxacin; CIP), Aminoglycoside (Gentamicin, CN); Tetracycline (Tetracycline, TET); and Macrolide (Azithromycin, AZM). As control for the antibiogram, *E. coli* ATCC 25922 was used.

2.6. Characterization of LAB

2.6.1. Antimicrobial activity of LAB

For preparation of culture extract, LAB isolates were incubated in MRS broth for 24 h at 37 °C anaerobically. Following incubation, cell free supernatants were obtained by centrifugation at 2800*g* for 15 min, followed by filter sterilization using 0.22 μm pore-size filter. Supernatant fluid was preserved at −20 °C. To determine the antimicrobial activity of the culture extract, well diffusion method was employed [33]. For this experiment, *E. coli* ATCC 25922 and representative foodborne bacteria obtained from the samples collected in this study (*E. coli, S. aureus, Klebsiella* spp., *P. aeruginosa* and *Salmonella*) were used as indicator strains, against which antimicrobial activity of LAB was tested. Overnight test culture (0.1 ml) was inoculated in 20 ml molten appropriate soft agar and poured into a sterile Petri dish. After hardening, wells with 1 cm in diameter were cut into the agar and 100 μL of the prepared LAB culture extract were placed into each well. Following incubation at 37 °C overnight, inhibition zones were observed and measured.

2.6.2. Screening for enterocin activity

To determine the phenotypic bacteriocin activity of LAB against foodborne bacterial isolates, the enterococcal strains were streaked on BHI agar plates (Oxoid, UK), and incubated at 37 °C for 24 h. The following day, the plates were inverted and 1 ml of chloroform was

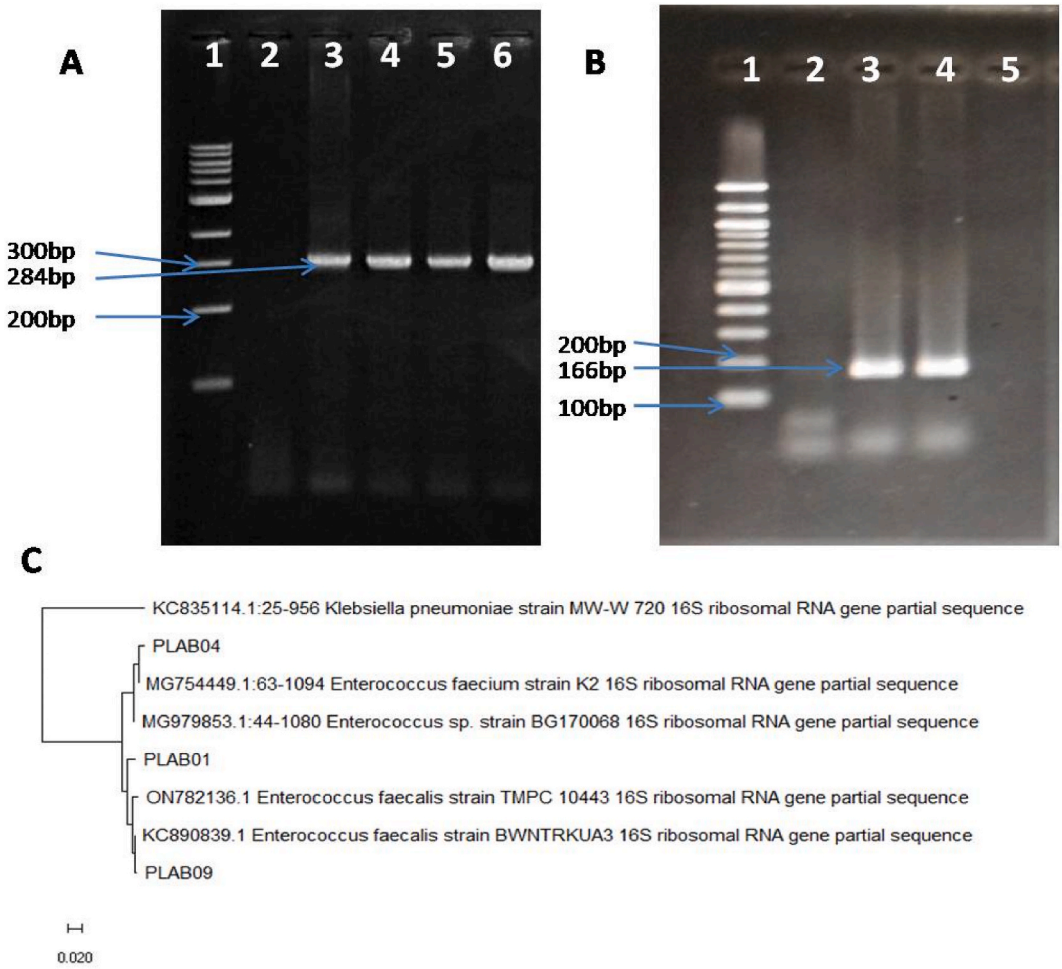

Fig. 1. Molecular identification of bacterial isolates collected from pickle samples; Polymerase chain reaction (PCR) was employed to amplify the genes *invA* and *uidA* for identification of *Salmonella* and *E. coli,* respectively. PCR products underwent electrophoresis through 1% agarose gel, stained with ethidium bromide, and visualized under UV light. The 100 bp DNA molecular marker (Promega Corporation, WI) were run alongside to estimate the size of the amplified PCR products. A) PCR amplification of *Salmonella* invasion antigen-encoding gene *invA* (284 bp)*;* Lane 1: 100 bp DNA Marker, Lane 2: Negative control, Lane 3: Positive control, Lane 4–6: Amplified PCR products. B) PCR amplification of *E. coli*-specific β-Glucuronidase enzyme-encoding gene *uidA* (166 bp)*;* Lane 1: 100 bp DNA Marker, Lane 2: Negative control, Lane 3: Positive control, Lane 4: Amplified PCR product. (Original gel photos are available in Supplementary Materials) C) Phylogenetic tree showing the relative position of representative LAB isolates (PLAB01, PLAB04, PLAB09) with reference sequence as inferred by neighbor joining method with 16S rRNA amplified sequences. *Klebsiella pneumoniae* is used as an outgroup. A 0.02 scale length is applied to indicate evolutionary distance between isolates.

added to the plate covers. After 20 min at room temperature, the plates were opened to evaporate any residual chloroform. Using pour plate method, 10^8 cells/ml of indicator organism (*E. coli* ATCC 25922, representative *E. coli, S. aureus, Klebsiella* spp. *P. aeruginosa* and *Salmonella* obtained from pickle samples) were inoculated into BHI agar and poured over prepared enterococcal plates to form an overlay. After incubation at 37 °C for 24 h, the plates were observed. Enterococcal isolates were considered positive id zone of inhibition were visible around the colonies, and measured in millimeters [34].

2.6.3. *Effect of temperature on LAB*

The effect of different temperature on lactic acid bacterial growth was assessed by inoculating 10 ml MRS broth with freshly grown LAB cultures, followed by incubation in anaerobic jar at temperature 15 °C, 30 °C, 45 °C and 60 °C for 72 h. After results were recorded, positive growth in the plate was observed after 72 h [35].

2.6.4. *Salt and pH tolerance of LAB*

The level of salt tolerance of LAB was determined by inoculating the isolates on MRS agar containing 1.5%, 3.5%, 5.5%, 7.5%, 9.5%, and 10.5% NaCl (w/v) for 3 days. For determining pH tolerance, modified MRS agar medium with pH adjusted from pH 1 to pH 7 using 1 M HCl and 1 M NaOH was used for lactic acid bacterial isolates. After inoculation, the growth rate was checked after 6, 12, 18,

24 h respectively [36].

2.6.5. Bile tolerance test

MRS broth supplemented with two different concentrations (0.15 and 0.3% (w/v)) ox gall (Oxoid, UK) and adjusted for four different pH (1, 3, 5, 7) was used to test the bile tolerance of the lactic acid bacterial isolates. The isolates were prepared for this experiment by overnight incubation in MRS broth at 37 °C, followed by adjusting the final bacterial concentration to 0.05 McFarland. From the standardized culture, 200 μL were inoculated into each of the two concentrations of the bile solutions with four different pH adjusted. The total presenting count was determined by serial dilution and spreading on MRS agar plates in triplicates [37].

2.6.6. Carbohydrate fermentation

The carbohydrate fermentation experiments were conducted by Jayne–William's method [38]. Total 15 different carbohydrates were used to determine the fermentation pattern of the bacterial isolates. MRS Broth and Nutrient Broth supplemented with selected carbohydrates (0.5% W/V) and chlorophenol red as the test indicator was used for LAB and other isolates, respectively. The prepared medium was inoculated with bacterial suspension at a defined density of about 50 μl in a microplate. Finally, to create anaerobic conditions for LAB, the micro-plate was sealed with sterile paraffin. Inoculated microplate was incubated at 35 °C for around 72 h. Positive carbohydrate fermentation was indicated by changing of color from red to yellow. The results of carbohydrate fermentation were checked according to the information of Bergey's manual systematic Bacteriology [27,39].

2.6.7. Proteolytic activity of LAB

Qualitative screening of LAB isolates for their proteolytic activity was conducted on skim milk agar plate containing 0.1 g/ml skim milk powder as a source of casein. LAB isolates were inoculated on prepared skim milk agar and incubated at room temperature for 3 days. Degradation of casein was indicated by the appearance of clear zone on agar [40].

2.6.8. Biofilm production

The qualitative detection of biofilm production was conducted by using Congo Red agar. In this experiment, 37 g/L Brain Heart Infusion Agar was supplemented with 0.8 g Congo Red and 36 g of sucrose to prepare Congo Red agar. Following overnight incubation, colony morphology was observed to determine biofilm producer isolates. Black colonies with crystalline consistency were considered as strong biofilm producers, whereas isolates with red or pink colonies were considered to be non-biofilm producers. Isolates displaying darkening of media, but red or pink colonies were considered as moderate biofilm producers [41].

2.7. Statistical analysis

Collected data were verified and entered into IBM SPSS Statistics Data Editor (Version 21) and STATA 15 for subsequent analysis.

3. Results and discussion

3.1. Isolation and identification of bacterial isolates

A total of 50 non-duplicate bacterial isolates were isolated from pickled fruit samples collected from street vendors across Dhaka city. Among the 50 isolates, *Staphylococcus aureus* was predominant (36%; 18 of 50), followed by *E. coli* (22%; 11 of 50), *Klebsiella* spp. (10%; 5 of 50), *Salmonella* (6%; 3 of 50), *Shigella* (6%; 3 of 50) and *Pseudomonas aeruginosa* (2%; 1 of 50). Presence of coliform in food served to children is a major cause of gastroenteritis and diarrheal disease, which contributes to high level of mortality in the developing countries like Bangladesh [42]. Similar studies done on street food collected from the periphery of Dhaka city revealed an alarming number of coliforms and food-borne pathogens, which indicates the contamination of food to be a persistent issue [2]. Alongside about 18% of isolates (9 of 50) were identified as lactic acid bacteria (LAB), among which *Enterococcus faecalis* was predominant (6 of 9). The rest were identified as *E. faecium* (3 of 9) (Fig. 1). These isolates are categorized as lactic acid bacteria or LAB, and are responsible for fermentation in pickle preparation. Although they can have beneficial effects as a probiotic and can have antimicrobial effects, *E. faecalis* and *E. faecium* can also become pathogenic and show antibiotic resistance, which can be detrimental to health upon consumption [44]. Pickles are traditionally fermented food available all over the country with varieties in raw ingredients and flavor. Compared to other traditional sources probiotic like yogurt and fermented buttermilk, pickles are cheaper and more readily available also it is traditionally consumed as an appetizer as well as a snack in southeast Asian countries like Bangladesh [61]. Moreover, they can be consumed by people with lactose intolerance who can't have dairy-based probiotics [62]. Besides its properties to improve digestive health, pickles can be an excellent source of probiotics like *Lactobacillus, Lactococcus and Enterococcus* as observed in this study. This makes pickles an excellent alternative to dairy-based probiotics [2].

3.2. Antibiotic resistance in isolates

Antibiotic susceptibility pattern among the 41 non-LAB and 9 LAB isolates investigated in this study revealed that majority of the isolates showed minimal antibiotic resistance. Highest occurrence of resistance to Azithromycin was observed in *Klebsiella* spp. isolates (100%), followed by *E. coli* (84%), *Salmonella* (66%), *Shigella* (66%) and *Staphylococcus aureus* (25%). Third generation cephalosporin Ceftriaxone showed better efficiency, as none of the *Klebsiella* spp., *Salmonella, Shigella* and *E. coli* were resistant to the antibiotic, but

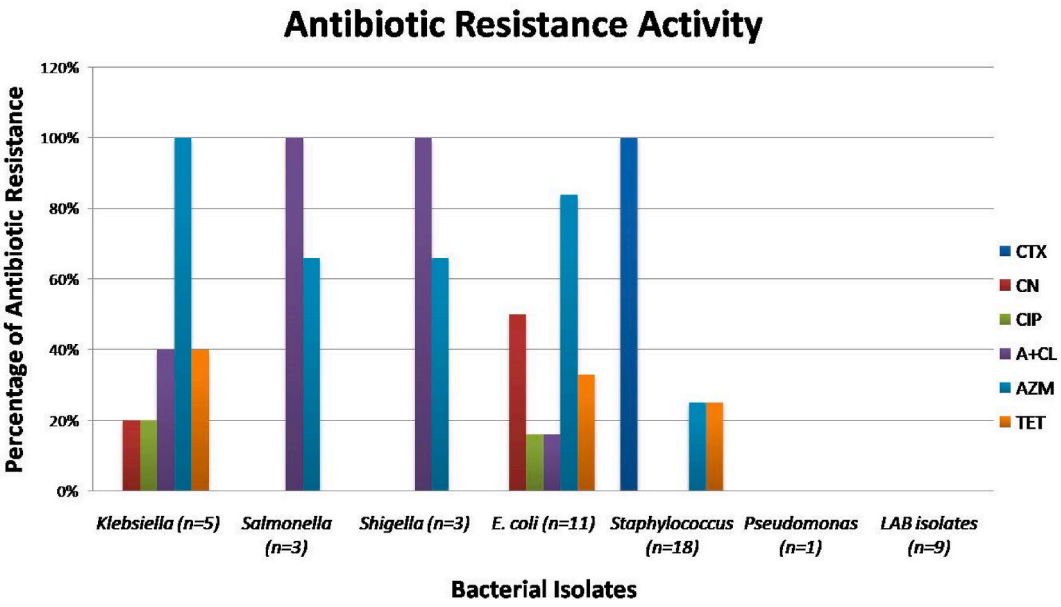

Fig. 2. Graphical representation of resistance percentages of bacterial isolates from pickle samples against different antibiotics. Here, X-axis represents bacterial isolates against different antibiotics, and Y-axis represents the percentage of resistant isolates. CTX= Cefotaxime; CN= Gentamycin; CIP= Ciprofloxacin; A + CL= Amoxicillin + Clavulanic acid; AZI = Azithromycin; TET = Tetracycline.

all of the *S. aureus* isolates showed phenotypic resistance to Ceftriaxone. The combination of Amoxicillin and Clavulanic acid was most effective against *Staphylococcus aureus,* but was resisted by *Salmonella* (100%), *Shigella* (100%), *Klebsiella* (40%) and *E. coli* (16%). Most of the isolates were susceptible to Gentamycin and Ciprofloxacin, and Tetracycline resistance was observed in 40% *Klebsiella,* 33% *E. coli* and 25% *Staphylococcus* isolates. No resistance was observed in LAB and *Pseudomonas aeruginosa* isolates. Susceptibility to antibiotics is one of the important criteria for lactic acid bacteria as probiotics. All the LAB isolates were found to be susceptible to all antibiotics tested; however, antimicrobial agents like vancomycin and teicoplanin are not investigated in this study. Lactic acid bacteria tend to resist these antibiotics more frequently as seen in previous studies [45,46], and these antibiotics are to be tested yet. *Klebsiella* isolates were found to show comparatively higher resistance against all antibiotics tested. Widespread antimicrobial resistance in *E. coli, S. aureus* and resistance to inhibitor like clavulanic acid in *Salmonella* and *Shigella* poses as a potential threat (Fig. 2). Similar results were observed in previous studies in Bangladesh [2,23,43], indicating consuming street foods like pickles may not be safe and is a potential health risk for consumers [47].

3.3. Antimicrobial activity and enterocin production by LAB

Not much of antimicrobial activity of LAB isolates against representative isolates was observed in the agar well diffusion assay method. None of the indicator organism exhibit inhibition of growth when exposed to culture extracts of LAB isolates. Similar results were observed in Bacteriocin production assay, where no apparent zone of inhibition was observed, indicating absence of bacteriocin activity. The antimicrobial activity of LAB depends on a number of factors, including changes in pH, production of substrates like enterocins, diacetyl, organic acids and competitive growth environment [33,48,49]. Absence of antimicrobial activity in LAB isolated in this study may indicate the lack of antimicrobial substrate production ability as evident in bacteriocin production assay. Environmental growth parameters may also affect the antimicrobial activity of the isolates, like pH, salinity and temperature [2].

3.4. Physiological properties of LAB isolates

Identified LAB isolates were subjected to a series of tests to analyze their physiological properties including temperature sensitivity, pH and salt tolerance, bile tolerance, and carbohydrate fermentation. Results of these tests are presented in Table 2. All the isolates were found to be Gram positive, catalase negative cocci with ability to ferment a wide range of carbohydrates, including myo-inositol and complex starch. This facilitates their ability to produce significant flavor and acidity during fermenting fruits to produce pickles [50].

3.5. Proteolytic activity of LAB isolates

Among 9 LAB isolates, 5 displayed positive casein degradation ability in skim milk agar (PLAB01, PLAB02, PLAB05, PLAB06 and PLAB07), with PLAB01 and PLAB05 showing better proteolytic activity (Table 2). Proteolytic activity of LAB depends on the species

Table 2
Physiological properties of LAB isolated from pickle samples.

Isolates	Source	Cell shape	Catalase test	Carbohydrate fermentation															Proteolytic activity
				Xylose	Lactose	Dextrose	Sucrose	Galactose	Raffinose	Rhamnose	Sorbitol	Mannitol	Mannose	Salicin	Inulin	Myo inositol	Ribose	Starch	
PLAB01	Jujube	Cocci	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PLAB02	Elephant Apple	Cocci	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PLAB03	Hog plum	Cocci	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
PLAB04	Hog plum	Cocci	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
PLAB05	Mixed fruit pickle	Cocci	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PLAB06	Mixed fruit pickle	Cocci	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PLAB07	Mixed fruit pickle	Cocci	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PLAB08	Pineapple	Cocci	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
PLAB09	Garlic	Cocci	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−

Here, '+' indicates positive result; '−' indicates negative result.

Table 3
Phenotypic characteristics of LAB isolated from pickle samples.

Isolates	Temperature Tolerance				NaCl Tolerance					Bile Tolerance						pH Tolerance			
	15 °C	30 °C	45 °C	60 °C	1.5%	3.5%	5.5%	7.5%	9.5%	0.15%			0.30%			pH 1	pH 3	pH 5	pH 7
										pH 3	pH 5	pH 7	pH 3	pH 5	pH 7				
PLAB01	+	+	−	−	+++	+++	++	+	−	−	−	+	−	−	+	−	−	++	+++
PLAB02	+	+	−	−	+++	++	++	+	−	−	−	+	−	−	+	−	−	+	++
PLAB03	+	+	−	−	+++	+++	+++	++	−	−	+	+	−	+	+	−	++	++	+++
PLAB04	+	+	−	−	++	++	+	+	−	−	+	+	−	+	+	−	++	++	+++
PLAB05	+	+	−	−	+++	+++	++	+	−	−	+	+	−	+	+	−	+	+	+++
PLAB06	+	+	+	−	++	+	+	+	−	−	+	+	−	+	+	−	+	+	++
PLAB07	+	+	−	−	+++	++	+	+	−	−	+	+	−	+	+	−	+	+	++
PLAB08	+	+	−	−	+++	++	++	+	−	−	+	+	−	+	+	−	−	+	+++
PLAB09	+	+	−	−	+++	+++	++	+	−	−	−	+	−	−	+	−	−	+	++

Here, '+' indicates growth; '++' indicates moderate growth; '+++' indicates excellent growth; '−' indicates no growth.

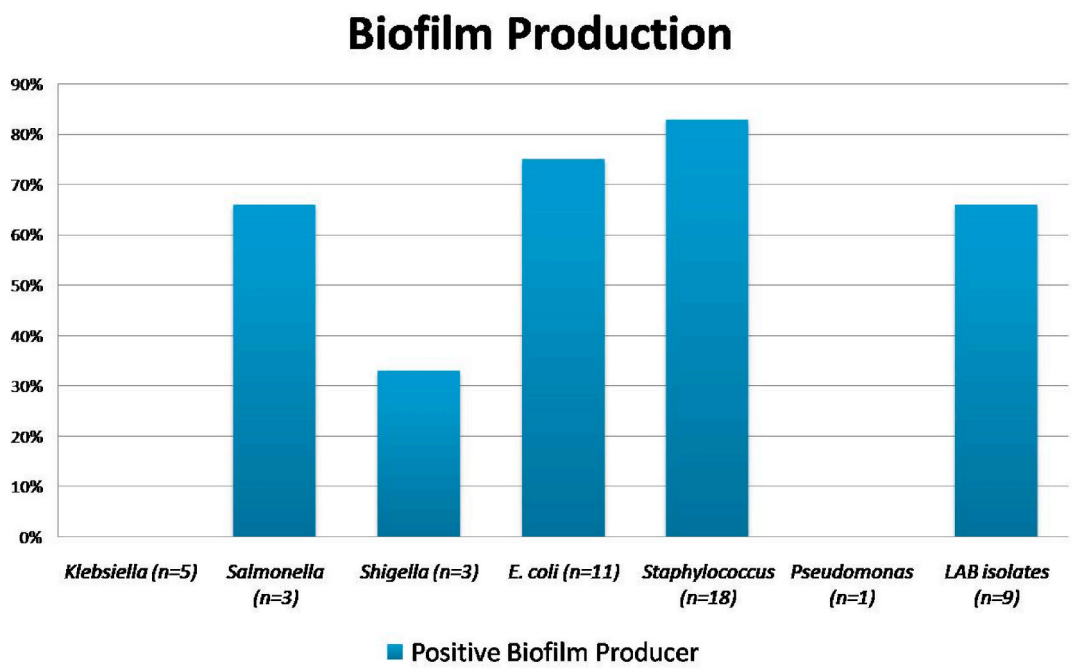

Fig. 3. Biofilm production in bacterial isolates collected from pickle samples. Here, X-axis represents different types of bacteria isolated in this study; Y-axis represents the percentage of isolates positive for biofilm production. Isolates with black, crystalline colonies on Congo Red Agar were considered as positive biofilm producer. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

and strain of the isolate, and further quantitative assays are to be done to assess proteolytic enzyme activity in each strain. Proteolysis by LAB mainly targets proteins like casein, α-lactalbumin and β-lactoglobulin, and can be used to reduce protein antigenicity [2,53].

3.6. Phenotypic characteristics of LAB isolates

Analysis of phenotypic characteristics revealed majority of the isolates to be able to grow from 15 °C to 30 °C, 7.5% NaCl, pH 7 and can tolerate 0.3% bile in pH 7 (Table 3). LAB require expressing high tolerance to acid and bile to be able to colonize the gastrointestinal tract and act as probiotic bacteria [51]. Isolates obtained from the study displays satisfying phenotypic characteristics with tolerance level adequate to act as probiotic [51,52].

3.7. Biofilm production

Analysis of biofilm production on Congo Red agar revealed that 66% of LAB isolates (6 of 9) were positive for biofilm production (Fig. 3), among which 4 showed stronger biofilm activity. Other bacterial isolates obtained from pickle samples showed that majority of *Staphyloccus aureus* were positive for biofilm production (78%; 14 of 18), while 75% of *E. coli* were biofilm producer. None of the *Klebsiella pneumoniae* isolates showed biofilm positive results. Formation of biofilm in lactic acid bacteria is often associated with food spoilage, formation of dental plaques, to reduce environmental stress and as a virulence factor for pathogenicity [54,55].

4. Conclusion

Pickles can be a better alternative to commercial probiotics, as it can serve as an inexpensive and easily available source of probiotics. Pickles are produced from locally available fruits and enjoyed as a common street snack. In contrast, commercially available probiotics are both expensive and not available everywhere in the country. Compared to other fermented sources of probiotics like yogurt, pickles are more popular and consumed widely. The study reports isolation, identification and characterization of lactic acid bacteria from traditionally fermented pickled fruits sold by street vendors in Dhaka, Bangladesh. The isolates have been characterized for their potential as probiotics, which revealed them to be able to tolerate a wide range of growth parameters, but they did not show antimicrobial activity against any foodborne isolates. The study also found the high level of microbial contamination and antibiotic resistance in foodborne isolates, indicating the consumption of such pickles to be a potential health hazard. However, this study has limitations for its geographic values, as only samples from Dhaka city were collected. A more elaborate study across the country in required to analyze the effect of LAB present in street pickles.

Added in word

Avijit Banik, Hasnain Anjum: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Humayra Habib: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Maruf abony: Analyzed and interpreted the data; Wrote the paper.

Anowara Begum, Zakaria Ahmed: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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