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Evaluation of the Probiotic Potential of *Lactobacillus delbrueckii* ssp. *indicus* WDS-7 Isolated from Chinese Traditional Fermented Buffalo Milk *In Vitro*

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Abstract

The present study aimed to evaluate the probiotic potential of lactic acid bacteria (LAB) isolated from Chinese traditional fermented buffalo milk. Out of 22 isolates, 11 were putatively identified as LAB preliminarily. A total of six LAB strains displayed strong adhesion to HT-29 cells and all these strains showed preferable tolerance to artificially simulated gastrointestinal juices. WDS-4, WDS-7, and WDS-18 exhibited excellent antioxidant capacities, including DPPH radical, $ABTS^+$ radical, and superoxide anion scavenging activities. Compared with the other two LAB strains, WDS-7 had a stronger inhibition effect on four pathogens. Based on the 16S rRNA gene sequencing and phylogenetic analysis, WDS-7 was identified as *Lactobacillus delbrueckii* ssp. *indicus* and selected to assess the potential and safety of probiotics further. The results revealed that WDS-7 strain had a strong capacity for acid production and good thermal stability. WDS-7 strain also possessed bile salt hydrolase (BSH) activity. Compared to LGG, WDS-7 was a greater biofilm producer on the plastic surface and exhibited a better EPS production ability (1.94 mg/ml as a glucose equivalent). WDS-7 was proved to be sensitive in the majority of tested antibiotics and absence of hemolytic activity. Moreover, no production of biogenic amines and β-glucuronidase was observed in WDS-7. The findings of this work indicated that *L. delbrueckii* ssp. *indicus* WDS-7 fulfilled the probiotic criteria *in vitro* and could be exploited for further evaluation *in vivo*.

K e y w o r d s: fermented buffalo milk, lactic acid bacteria, probiotic potential, *Lactobacillus delbrueckii* ssp. *indicus*, food application

Introduction

In recent years, the consumers' interest in health-promoting foods containing probiotics has risen due to probiotics' capability to promote human health (Nair and Dubhashi 2016). Probiotics are defined as living microorganisms that, when given in sufficient number, confer a health benefit to the host (Hill et al. 2014). Probiotics are generally applied in the fermentation of foods as starter cultures and are considered safe with application in the food industry. The most widely used probiotic in the food industry is lactic acid bacteria (LAB). LAB is a heterogeneous group composed of Gram-positive, non-spore-forming bacteria, including *Lactococcus* spp., *Streptococcus* spp., *Pediococcus* spp., *Enterococcus* spp., *Oenococcus* spp., and *Lactobacillus* spp., which have been traditionally used in the fermented food industry, due to their capacity to transform sugars into lactic acid (Chapot-Chartier 2014; Chapot-Chartier and Kulakauskas 2014; Mahony and van Sinderen 2014). *Lactobacillus* spp., as the LAB with probiotic potential, has received considerable attention over the past few years. In addition, *Lactobacillus* spp. is a member of the healthy microbiota of the gastrointestinal tract. Therefore, the selected strains can be used as probiotics (Ren et al. 2014). Probiotic *Lactobacillus* spp. are generally considered safe by the US Food and Drug Administration (FDA) and qualified as presumed safe by the European Food Safety Agency (EFSA). Probiotic *Lactobacillus* spp. is widely used in the food industry because of its unique characteristics and lactic acid fermentation capacity of different foods, such as vegetables, dairy products, and meat products (Pringsulaka et al. 2015; Motahari et al. 2017).

A worldwide spectrum of *Lactobacillus* spp. has been originated from different fermented foods. Based on their potential biological activities, *Lactobacillus* spp. has been applied in the food and pharmaceutical

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industry. Common probiotic carriers are fermented milk products at present due to the favorable conditions provided by the acid environments for the survival of probiotics. Shakibaie et al. (2017) isolated a LAB strain belonging to the species *Lactobacillus brevis* from an Iranian traditional dairy product named spar. Bao et al. (2012) found that *Lactobacillus helveticus* and *Lactobacillus casei* screened from traditional yak milk products of Gansu Province in China were considered the predominant populations in the yak milk products and may be a valuable source for further starter selection. *Lactobacillus fermentum* SJRP30 and *L. casei* SJRP145 and SJRP146 isolated from water buffalo mozzarella cheese were revealed to be safe and to possess similar or superior probiotic characteristics (Casarotti et al. 2017). Traditional fermented dairy products are rich in wild LAB as they are generally fermented with local microbiota from raw milk in the process of natural fermentation without participation with industrial starters. This microbial community plays an important part in their specific characteristics and indigenous flavor and texture (Chapot-Chartier and Kulakauskas 2014; Zuo et al. 2014). Traditional fermented milk products provide potential affordable functional products with probiotic properties. Traditional fermented dairy products, mainly manufactured in rural areas, offer potential functional products with probiotic characteristics.

Furthermore, the efficacy of probiotics has been proved to be species- or even strain-dependent, as different LAB strains can affect hosts in different ways (Cani and Van Hul 2015). Isolation and identification of such wild LAB from traditional dairy foods can be a good opportunity to develop new starter cultures and new probiotics (Bao et al. 2012). Consequently, exploration for new probiotics, especially from the relatively undeveloped rural areas, has become a hot research topic (Bajaj et al. 2014; Gupta and Bajaj 2016).

Chinese traditional buffalo milk is a kind of fermented yogurt with a unique flavor, which is made from raw milk of buffalo raised in the Changjiang river basin and fermented in clay pots by natural LAB. It is different from the yogurt products in Northwest China made from the raw milk of yak, cow, and sheep as the primary raw material. Rare reports have been available on the usage of Chinese traditional fermented buffalo milk as a potential probiotic carrier so far. A detailed study on the probiotic potential of Chinese traditional fermented buffalo milk can provide valuable information and clarify its potential use in a broader range.

Although LAB is generally considered safe to consume in the food industry, a series of *in vitro* tests have been carried out and applied to identify microorganisms with the probiotic-rich potential to establish criteria for probiotic screening (Leahy et al. 2005). Consequently, several criteria have been employed to estimate probiotic properties of a newly isolated strain, including antibacterial properties, sensitivity to antibiotics, ability to adhere to epithelial cells, and absence of hemolytic activity when used in food fermentation (Verdenelli et al. 2009; Diosma et al. 2014; Khan 2014). In addition, potential probiotics should also exhibit some specific probiotic properties, such as biofilm formation and exopolysaccharide (EPS) production. It is well known that LAB forms biofilm under specific environmental conditions, which is advantageous for intestinal colonization and probiotic potential of LAB (Elhadidy and Zahran 2014; Johansson and Rasmussen 2013; Popović et al. 2018). What is more, some studies have found that biofilms formed by LAB exhibit the ability to influence the survival and the multiplication of pathogens. In addition, the biofilms formed by LAB exhibit the ability to repress the survival and proliferation of pathogens (Guerrieri et al. 2009). EPS plays a vital role in its rheological and physicochemical properties during fermentation (Widyastuti and Febrisiantosa 2014). EPS has also been investigated as an antiviral drug (Katsuraya et al. 1995) and antitumor agent (Sun et al. 2018).

This study's objecives were to provide a more comprehensive investigation of the probiotic properties of LAB strains obtained from Chinese traditional fermented buffalo milk through various *in vitro* tests to be used in the food industry.

Experimental

Materials and Methods

Enrichment, isolation and screening of LAB. Five home-made traditional fermented buffalo milk was collected from the households of Digang Town, Fanchang County (E 118°20″, N 31°08″), Wuhu City, Anhui Province, China. The samples did not deteriorate, showed porcelain white, had milk fragrance, and no peculiar smell. To enrich LAB, the samples were added at 2% volume into 50 ml de Man, Rogosa, and Sharpe (MRS, Qingdao Hope Bio-Technology Co., Ltd.). The sample suspensions of an appropriate dilution were inoculated onto MRS plates (containing 1% $CaCO_3$), and placed in an anaerobic environment for culture at 37°C for 48 h. The separate white colonies, which showed calcium-dissolving circle by streaking on the MRS plate were selected and purified. Biochemical features were used for the identification of isolated LAB. All the experiments in this study have been carried out in triplicates. *Lactobacillus rhamnosus* GG ATCC 53103 (LGG) provided by the Institute of Microbiology, Anhui Academy of Medical Sciences, China, was used as the reference strain.

Adhesion to HT-29 cell. Adhesion to HT-29 cell was performed following the method described previously (Lee et al. 2015) with minor modifications. Human colon cancer cells HT-29 (Hunan Fenghui Biotechnology Co., Ltd.) were maintained in Dulbecco's Modified Eagle Medium F-12 (DMEM/F12, Gibco, USA), supplemented with 10% (v/v) fetal bovine serum (Zhengjiang Tianhang Biotechnology Co., Ltd.) in a carbon dioxide incubator of 5% CO_2 at 37°C for 48 h. The HT-29 cells were harvested and added into the 24-well plate (2×10^6 cells/well), and grown for 48 h. The medium was refreshed daily. A volume of 0.5 ml LAB (1×10^8 CFU/ml) was added into the wells, and the suspension was incubated at 37°C for 2 h. The wells were washed with PBS three times, then the cells were treated with Triton X-100 (BioFRoxx, Germany), and the bacteria were inoculated on the MRS agar plate. The number of the adherent bacterial cells was counted on the plates and the adhesion rate was calculated.

Tolerance to artificial simulated gastrointestinal conditions. The determination of resistance to simulated gastrointestinal juices was carried out according to the method described previously with minor modifications (Cao et al. 2018; Iraporda et al. 2019). The cultures of LAB strains incubated in MRS broth, supplemented with 0.1% (w/v) ascorbic acid at 37°C for 48 h on anaerobic condition were centrifuged at 10,000 × *g* for 10 min at 4°C, and the pellets were washed twice with PBS buffer (pH 7.4). The artificial simulated gastric juice at a volume of 4 ml (125 mM NaCl, 7 mM KCl, 45 mM $NaHCO_3$ and 3 g/l pepsin (Shanghai Lanji Technology Development Co., Ltd.), adjusted to pH 2.0 with HCl, filtrated by 0.22 μm filter membrane) was used to suspend the cell pellets. The bacterial concentration was adjusted to 10^8 CFU/ml, incubated at 37°C in a water bath for 3 h (a period, which simulated the gastric transit time for humans). After tenfold serial dilutions, the suspension of appropriate concentration was coated on MRS plates, and the number of viable bacteria was determined by colony counting. After treatment with artificial simulated gastric juice for 3 h, the LAB strains were obtained by centrifugation at 10,000 × *g* for 10 min, and the pellets were washed twice with PBS buffer. Four milliliters of artificial simulated intestinal juice (22 mM NaCl, 3.2 mM KCl, 7.6 mM $NaHCO_3$, 1.0 g/l pancreatin (Shanghai Lanji Technology Development Co., Ltd.) and 0.3 g/l bile oxgall (Beijing Solarbio Science and Technology Co., Ltd.), pH adjusted to 8.0 with NaOH, filtrated by 0.22 μm filter membrane) were used to suspend the cell pellets, incubated at 37°C in a water bath for 5 h (a period that simulated the intestinal transit time for humans). After tenfold serial dilution, the suspension of appropriate concentration was coated on the MRS plates; the number of viable bacteria was determined by colony counting. The survival rate of the LAB strains was calculated as follows:

$$Survival\ rate\ (\%) = \frac{N_1}{N_0} \times 100$$

where N_0 is the initial inoculated viable cell numbers, and N_1 is the cell numbers after treatment with artificial simulated gastric juice (3 h) or artificial simulated intestinal juice (5 h).

Antioxidant capacity. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was assessed according to the methodology used (Yu et al. 2018) with slight modifications. 0.2 ml of LAB culture (1×10^9 CFU/ml) was mixed with 1 ml DPPH solution in methanol (100 μM). After being placed against exposure to light at 37°C for 20 minutes, the mixture was centrifugated at 8,000 × *g* for 5 minutes. Finally, the absorbance value at 517 nm was measured. 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) cation radical scavenging activity was investigated according to the methodology used (Cao et al. 2018) with simple modifications. Seven mM of $ABTS^+$ solution was prepared with 2.45 mM potassium persulfate solution and incubated in the dark at room temperature for 12 h as the $ABTS^+$ working solution. 0.1 ml of LAB culture (1×10^9 CFU/ml) was mixed with 1 ml of $ABTS^+$ working solution. After being placed against exposure to light at 37°C for 20 minutes, the mixture was centrifuged at 8,000 × *g* for 5 minutes. Finally, the absorbance value at 734 nm was measured. Superoxide anion scavenging activity was ascertained according to the previous methodology (Tang et al. 2017) with slight modifications. 0.5 ml of LAB culture (1×10^9 CFU/ml) was mixed with 1.5 ml Tris-HCl (pH = 8), and placed in water bath at 25°C for 20 min, 0.2 ml pyrogallol solution in deionized water (25 mM) was added into the suspension. A volume of 0.25 ml hydrochloric acid was used to terminate the reaction after 5 min. The mixture was centrifuged at 8,000 × *g* for 5 min. Finally, the absorbance value at 325 nm was measured. In the above three tests, PBS solution was set as blank control, and scavenging activity was calculated as the following formula:

$$Scavenging\ rate\ (\%) = (1 - \frac{A_{sample}}{A_{sample}}) \times 100$$

where A_{sample} represent the OD_{517}, OD_{734} and OD_{325} values of sample, respectively. $A_{control}$ represent the OD_{517}, OD_{734} and OD_{325} values of control, respectively.

Antibacterial activity. Antibacterial activities of LAB strains were investigated by a disc diffusion method (Piyadeatsoontorn et al. 2019) with some modifications. The pathogens were used as indicator strains in this study, including *Escherichia coli* ATCC8099,

Staphylococcus aureus ATCC6538, *Salmonella enterica* ATCC9120, and *Shigella sonnei* BNCC192105, respectively. One hundred microliters of fresh indicator bacterial culture (10^7 CFU/ml) were inoculated on the LB plate; the sterilized disc was placed on the plate after the surface of plate was left to dry. A volume of 20 µl cell-free supernatant (CFS) was carefully added onto the disc, and the plates were kept in the incubator at 37°C for 24 h, subsequently. The antibacterial activity of LAB was assessed by measuring the diameter of the inhibition circle with a vernier caliper.

16s rRNA gene sequencing for molecular identification. The LAB strain isolated was subjected to the 16S rRNA gene identification at the species level. The genomic DNA was extracted according to the manufacturer's instructions of the bacterial DNA extraction kit purchased from TIANGEN Biotech Co., Ltd., and the 16S rRNA gene was amplified with universal primers 27F (5'-AGAGTTGATCCTGGCTCAG-3') and 1492R (5'-GGTATCCTTGTTACTACTT-3') by using gradient PCR instrument (ABI, USA) (Piyadeatsoontorn et al. 2019). A total volume of 50 µl consisted of 1.0 µl template DNA, 1.0 µl Taq DNA polymer, 5.0 µl 10 × PCR buffer, 1.0 µl 10 mM dNTP, 1.5 µl 10 µM upstream and downstream primers, respectively, 39.0 µl ddH_2O. The thermal cycling parameters were as follows: initial denaturation at 95°C for 300 s, 35 denaturation cycles at 95°C for 30 s, annealing at 58°C for 30 s, elongation at 72°C for 90 s, and final elongation at 72°C for 420 s. Three microliters of PCR amplicons were visualized at 100 V for 1 h by using 1% agarose for electrophoresis. The PCR product was sent to Shanghai Personal Gene Technology Co., Ltd. for sequencing. The 16S rRNA gene sequences were compared and matched using BLAST with the available sequences in NCBI GenBank. The homology of the target gene sequence was analyzed, and the phylogenetic tree was constructed by MEGA 7.0 with bootstrap values based on 1,000 replications. The sequence identified was uploaded to the NCBI Gene Bank database.

Scanning electron microscope (SEM) observations. Observations on the morphology of isolated LAB strains exhibiting probiotic potential by SEM were conducted as described previously (Prasanna and Charalampopolous 2018). The LAB strains were placed in the incubator at 37°C for 18 h. The cultures were centrifuged at 8,000 × *g* for 10 min; the cell pellets were harvested and fixed with PBS buffer (0.1 M, pH = 7.2) containing 2.5% (w/v) glutaraldehyde at 4°C overnight. Afterward, the samples were washed twice with PBS and then dehydrated with a graded ethanol series (30%, 50%, 70%, 80%, 90%, 100%) at 4°C for 20 min, respectively. The samples were transferred into anhydrous acetone and dried in a critical point dryer (Emitech-K850, UK). The dried bacterial powders were smeared evenly using a sterile cotton swab onto the stage with electric conductive adhesive and sputter-coated with gold with a sputter-coater (Hitachi E-1010, Japan). Eventually, the samples were observed using a scanning electron microscope (Hitachi S-4800, Japan) under standard operating conditions at an accelerating voltage of 0.5–30 kV.

Determination of LAB strain's growth curve and acid production curve. The growth curve and acid production curve were drawn based on the method described (Xia et al. 2019) with modifications. The fresh culture of isolated LAB strain was subcultured twice for 24 h and added into the conical flask (250 ml) containing 150 ml MRS broth at 2% inoculation amount for static culture in an anaerobic incubator (Shanghai Longyue Biotechnology Co., Ltd.). Two milliliters of culture were taken out through the rubber gloves fixed and sealed on the operating hole left on the anaerobic incubator quickly and carefully every 2 h until 24 h, and try not to shake the conical flask to keep the static condition. The absorbance value at 600 nm and pH value were measured. The growth curve and acid production curve were drawn according to the absorbance value at 600 nm, and pH value, respectively, determined at different culture times.

Heat resistance test. According to Bacon et al. (2003), the heat resistance test was performed with modifications. The strain was cultured in MRS broth until the absorbance value at 600 nm reached 1.5 at 37°C anaerobically. A total of 1 ml culture was inoculated into 4 ml PBS solution and treated in the water bath at 40, 50, 60, 70, or 80°C for 3 minutes, respectively. As soon as the heating was over, the solution was immediately cooled on ice and diluted to 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}, subsequently. The sample suspensions of appropriate dilution were plated onto MRS and placed in the incubator at 37°C for 18 h anaerobically.

Bile salt hydrolase (BSH) activity. BSH activity was performed as described by Shehata et al. (2016). The LAB strain was incubated in the incubator at 37°C for 18 h, and the culture was coated on the MRS plate with 0.5% (w/v) sodium taurodeoxycholic acid (TDCA) or MRS plate without TDCA, followed by incubation at 37°C for 72 h anaerobically. The appearance of precipitation indicated the BSH activity of the strain.

Biofilm formation assay. Biofilm formation on the glass and plastic surfaces was detected according to Gheziel et al. (2019). After LAB incubation the biofilm rings were collected, washed with distilled water, and stained with crystal violet (0.5%, v/v). Followed by dissolving the biofilms with acetic acid (30%, v/v), the absorbance value at 590 nm was measured. LAB was defined as strongly biofilm formation ($OD_{590} > 0.2$), weakly biofilm formation ($0.1 < OD_{590} < 0.2$), or no biofilm formation ($OD_{590} < 0.1$).

Exopolysaccharide (EPS) production capability. According to Adesulu-Dahunsi et al. (2018), the EPS production capability was detected. The strain was cultured in an MRS broth medium with 2% sucrose (w/v) at 37°C for 18 h. After the culture was centrifugated at 8,000 × *g* for 15 min, the supernatant was retained and ethanol was added into the supernatant. After an ice bath for 4 for 24 h and centrifugation at 12,000 × *g* for 15 min, the precipitate was dissolved in distilled water. The sugar equivalent of EPS (calculated as glucose) was determined.

Hemolysis test. The hemolysis test was assessed according to Menezes et al. (2020). The appearance of the hydrolytic circle (β-hemolysis) was regarded as a positive result. The appearance of the green circle (α-hemolysis) or no hydrolytic circle appearing (γ-hemolysis) was regarded as non-hemolysis.

Antibiotic susceptibility assay. Antibiotic susceptibility was assessed as Maldonado et al. (2012) with minor modifications. Under aseptic condition, 100 µl of LAB culture (1×10^7 CFU/ml) was coated on MRS plate. After 5 min, the discs containing antibiotics were placed on the plate, and the media were incubated at 37°C for 24 h. Types and dosages of antibiotics were as follows: metronidazole 5 µg, chloramphenicol 30 µg, streptomycin 10 µg, kanamycin 30 µg, ampicillin 10 µg, gentamicin 10 µg, tetracycline 30 µg, erythromycin 15 µg, rifampicin 5 µg, ciprofloxacin 5 µg, doxycycline 30 µg, vancomycin 30 µg. The diameter of the inhibition zone was measured with a vernier caliper, and the results were declared in accordance with the microbiological breakpoints for antimicrobials issued (CLSI 2012).

Biogenic amines production. Biogenic amines production was investigated following Bover-Cid and Holzapfel (1999). Pyridoxal-5-phosphate (0.005%) was added to the medium as a cofactor for decarboxylation reaction, and the pH was adjusted to 5.3. The LAB strain was streaked on MRS plate supplemented with amino acids (lysine, histidine, arginine, tyrosine, and ornithine) (Beijing Solarbio Science & Technology Co., Ltd.) at 0.5% final concentration. Bromocresol purple was used as a color indicator, and the plate was placed in the incubator at 37°C for 72 h, subsequently. The positive result was confirmed by changing the indicator from yellow to purple.

Enzyme production. Enzyme production was determined by using an API-ZYM kit (Biomerieux, France). After incubation of the strain, the culture was centrifuged at 4°C at 12,000 × *g* for 10 min, and the precipitate was resuspended with sterile saline to a concentration of 10^5 CFU/ml, and added to each cupule. The cupules were placed in the incubator at 37°C for 5 h. One drop of enzyme A and enzyme B reagents was added continuously to each cupule. According to the manufacturer's instruction, the enzyme production was declared as 0 to 5.

Statistical analysis. Experiments were conducted in triplicate in our research, and all data were expressed as means and standard deviation. SPSS version 23.0 was employed for data analysis. One-way ANOVA estimated the difference with Duncan's multiple range tests, and statistical significance was set at $p < 0.05$.

Results

Adhesion to HT-29 cell. Through preliminary screening of LAB, out of a total of 22 isolates, 11 isolated strains were putatively identified as LAB (Table I), and their adhesion to HT-29 cells was evaluated. The results

Table I
Physiological and biochemical features of 11 isolated strains.

Characteristic	WDS	WDS-7
Glucose gas production	–	–
Gelatin	3	–
Nitrate reduction	–	–
Catalase	–	–
Arginine hydrolysis	–	–
Motile	–	–
H_2S production	–	–
Indole	–	–
15°C growth test	–	–
45°C growth test	+	+
Arabinose	3	–
Cellobiose	7	–
Esculin	5	–
Fructose	+	+
Gluconate	4	–
Lactose	9	+
Mannose	6	+
Mannitol	1	–
Sorbitol	–	–
Melezitose	–	–
Melibiose	5	–
Raffinose	3	–
Rhamnose	–	–
Salicin	6	–
Glucose	+	+
Sucrose	8	+
Trehalose	6	–
Xylose	–	–
Ribose	–	–
Maltose	8	–

WDS – WDS-2, WDS-3, WDS-4, WDS-8, WDS-9, WDS-10, WDS-11, WDS-15, WDS-18, WDS-20; 10 isolates
+ – positive or weakly positive reaction
– – negative reaction
number – the number of positive reactions

are shown in Table II. The adhesion rate to HT-29 cell ranged from 2.47 ± 0.46% to 11.50 ± 1.22%. Among the 11 LAB strains, six strains displayed strong adhesion capacity to HT-29 cells, including WDS-3, WDS-4, WDS-7, WDS-9, WDS-10, and WDS-18. WDS-4 had the strongest adhesion capacity to HT-29 cells with an adhesion rate of 11.50 ± 1.22%.

Tolerance to artificial simulated gastrointestinal conditions. Tolerance to artificial simulated gastrointestinal conditions of six selected LAB strains is presented in Table III. In general, six selected strains exhibited a good tolerance to artificial simulated gastrointestinal conditions. After treatment with artificial simulated gastric juice for 3 h, the survival rate of six LAB strains decreased with the growth reduction ranging from 18.66% to 29.06%. WDS-3 showed the best tolerance to artificial simulated gastric juice with an 81.34 ± 4.41 survival rate. Subsequently, after treatment with artificial simulated intestinal juice for 5 h, the growth reduction ranged from 29.44% to 39.96%. WDS-3 also displayed the best tolerance to artificial simulated intestinal juice, showing a 70.56 ± 4.49% survival rate. Due to their high tolerance to artificial simulated gastrointestinal conditions, WDS-3, WDS-4, WDS-7, WDS-9, WDS-10, and WDS-18 were chosen for further testing.

Table II
Adhesion rate to HT-29 cells of 11 isolated LAB strains.

Strains	Adhesion capacity to HT-29 Cell (%)
WDS-2	3.79 ± 0.72^{a}
WDS-3	9.65 ± 1.50^{bcd}
WDS-4	11.50 ± 1.22^{e}
WDS-7	10.74 ± 0.99^{cde}
WDS-8	2.47 ± 0.46^{a}
WDS-9	9.43 ± 0.93^{bc}
WDS-10	8.99 ± 1.05^{b}
WDS-11	3.32 ± 0.50^{a}
WDS-15	2.92 ± 0.80^{a}
WDS-18	11.23 ± 0.71^{de}
WDS-20	4.16 ± 0.37^{a}
LGG	15.56 ± 1.31^{f}

Results are expressed as the mean ± SD.
[a-f] – different letters along the column represent statistical significance ($p < 0.05$)

Table III
Tolerance to artificial simulated gastrointestinal conditions of six isolated LAB strains.

Strains	Artificial simulated gastric juice, 3 h, survival rate (%)	Artificial simulated intestinal juice, 5 h survival rate (%)
WDS-3	81.34 ± 4.40^{b}	70.56 ± 4.49^{b}
WDS-4	77.45 ± 3.66^{ab}	62.36 ± 3.06^{ab}
WDS-7	78.99 ± 3.98^{ab}	67.81 ± 3.57^{ab}
WDS-9	76.95 ± 2.03^{ab}	66.19 ± 3.48^{ab}
WDS-10	70.94 ± 4.27^{a}	60.04 ± 1.25^{a}
WDS-18	71.29 ± 2.13^{a}	60.75 ± 5.17^{a}
LGG	84.72 ± 4.96^{b}	77.75 ± 4.25^{c}

Results are expressed as the mean ± SD.
[a-c] – different letters along the column represent statistical significance ($p < 0.05$)

Table IV
Antioxidant capacities of six isolated LAB strains.

Strains	Antioxidant capacities (%)		
	DPPH scavenging activity	$ABTS^{+}$ scavenging activity	Superoxide anion scavenging activity
WDS-3	19.32 ± 1.24^{ab}	52.35 ± 2.47^{b}	41.31 ± 2.64^{a}
WDS-4	31.04 ± 1.71^{c}	65.20 ± 2.38^{d}	49.72 ± 1.63^{b}
WDS-7	30.15 ± 2.24^{c}	68.31 ± 1.50^{d}	48.92 ± 1.36^{b}
WDS-9	18.09 ± 1.78^{a}	40.78 ± 1.43^{a}	40.77 ± 1.85^{a}
WDS-10	22.08 ± 1.61^{b}	49.01 ± 1.72^{b}	41.94 ± 1.69^{a}
WDS-18	32.00 ± 2.71^{c}	56.97 ± 1.68^{c}	48.68 ± 1.33^{b}
LGG	21.32 ± 1.13^{ab}	51.94 ± 2.04^{b}	40.12 ± 1.07^{a}

Results are expressed as the mean ± SD.
[a-d] – different letters along the column represent statistical significance ($p < 0.05$)

Antioxidant capacities. Antioxidant activities including DPPH radical scavenging, $ABTS^{+}$ radical scavenging, and superoxide anion scavenging were assayed in this study. The results are presented in Table IV. In general, WDS-4, WDS-7, and WDS-18 exhibited prominent antioxidant activities with significantly higher DPPH, $ABTS^{+}$, and superoxide anion scavenging activities than those of the reference strain LGG ($p < 0.05$). WDS-18, WDS-7, and WDS-4 showed the highest DPPH radical scavenging activity of 32.00 ± 2.71%, the highest $ABTS^{+}$ radial scavenging activity of 68.31 ± 1.50%, and the highest superoxide anion scavenging activity of 49.72 ± 1.63%, respectively.

Antibacterial activity. The inhibition activity of three isolated strains against four pathogens including *E. coli*, *S. aureus*, *S. enterica*, and *S. sonnei* is shown in Fig. 1. Overall, all isolated strains displayed certain inhibition activities against indicator pathogens, with the diameter of the clear zones ranging from 7.2 to 22.0 mm. Among the three isolates, WDS-7 exhibited the highest inhibition effect on four pathogens. It is worth noting that *S. aureus* was the most inhibited bacteria by all three isolated LAB strains compared to the other tested pathogens. Due to the remarkable antibacterial activity, WDS-7 was screened for further analysis.

Molecular identification. Due to the efficient adhesion to HT-29 cells, prominent antioxidant activity, and remarkable antimicrobial activity, the WDS-7

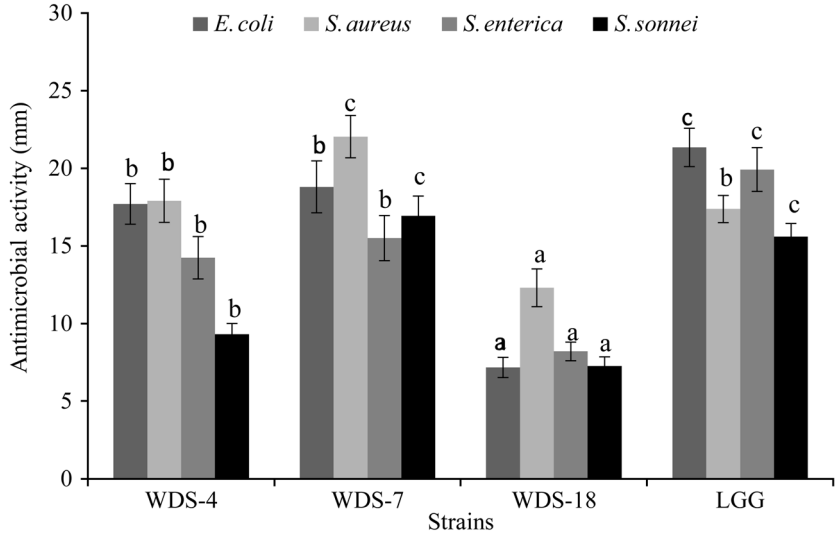

Fig. 1. Antibacterial activity of six isolated LAB strains. a–c) Different letters represent statistical significance ($p < 0.05$).

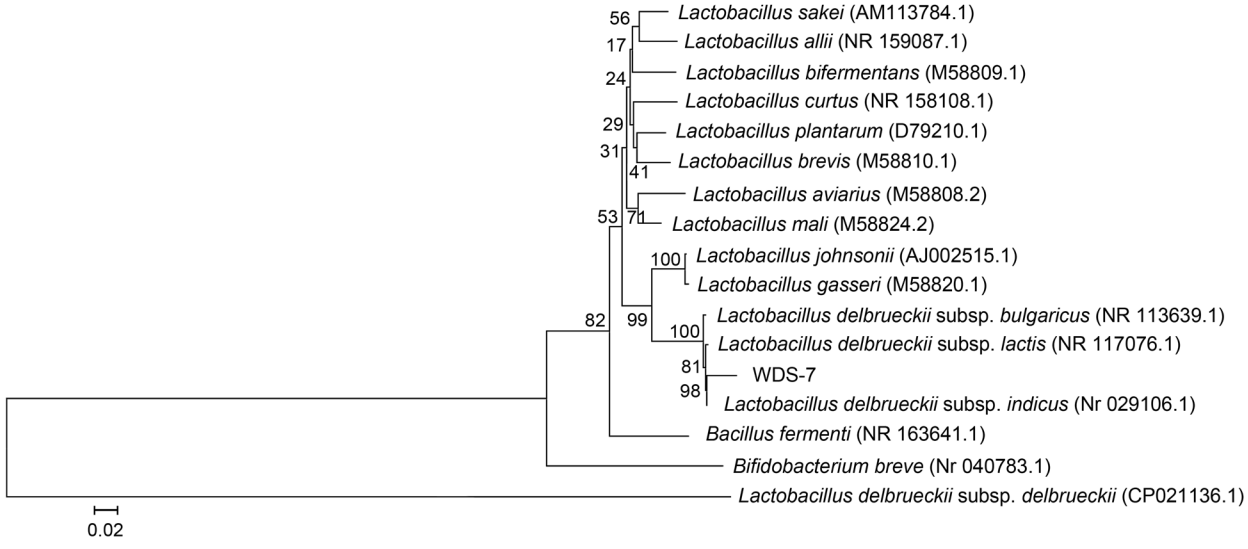

Fig. 2. Phylogenetic tree constructed based on the 16S rRNA gene sequence of *Lactobacillus delbrueckii* ssp. *indicus* WDS-7 strain.

strain was selected to be identified by the 16S rRNA gene sequencing. The 16S rRNA gene sequence of the WDS-7 strain was uploaded to NCBI (the accession No. MN 759441), and the sequence similarity comparison was performed with BLAST. The phylogenetic tree of strain is shown in Fig. 2. The homology of WDS-7 and *L. delbrueckii* ssp. *indicus* 16S rRNA gene sequence reached 98%. Therefore, based on the 16S rRNA gene sequencing and the results of colony morphology, Gram staining, physiological, and biochemical identification described above, the WDS-7 strain was identified as *L. delbrueckii* ssp. *indicus* and simply named as *L. delbrueckii* ssp. *indicus* WDS-7.

Morphological observation on WDS-7. The micrographs of *L. delbrueckii* ssp. *indicus* WDS-7 strain under SEM are shown in Fig. 3. The colonies of WDS-7 were large, rod-shaped, and smooth on the surface, neat edge, opaque, paired, or linked. The strain was Gram-positive, and it was non-motile. No flagella or polar fibers were observed under SEM imaging.

Growth curve and acid production curve. The growth curve of *L. delbrueckii* ssp. *indicus* WDS-7 strain was drawn and analyzed, as shown in Fig. 4. The strain was in the latency phase from 0 to 2 h with a low growth, but the biomass of the strain increased in the fastest rate from 2 to 12 h, when the cell growth was in the exponential phase. The biomass reached the peak value at 18 h, and the maximum absorbance value at 600 nm was about 1.71. Then the absorbance value decreased slowly; the bacterial cells entered the stable growth phase. The pH value of the initial culture of strain WDS-7 was about 6.2 (Fig. 4). However, with the increase of culture time and biomass, the pH value decreased rapidly after 2 h. After 20 h of incubation, the

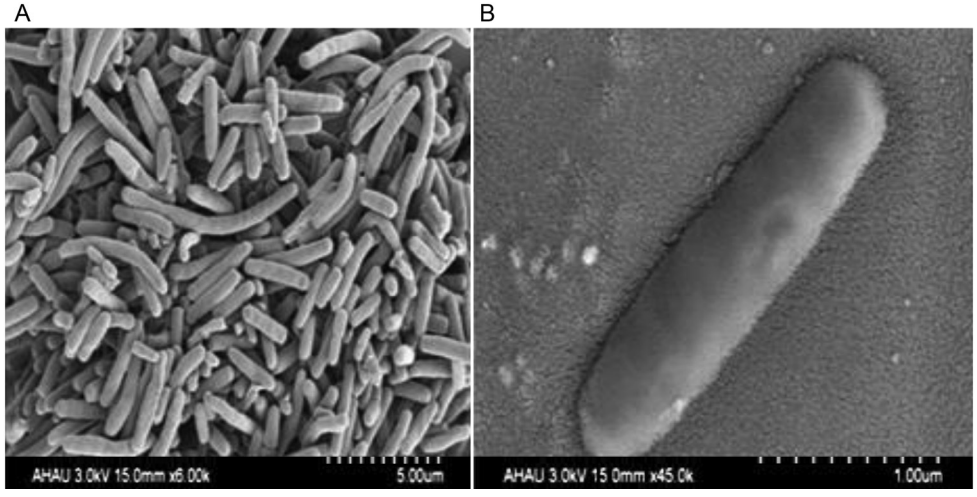

Fig. 3. SEM images of *Lactobacillus delbrueckii* ssp. *indicus* WDS-7 strain. A) SEM image of numerous bacteria located at random, B) SEM image of a single cell.

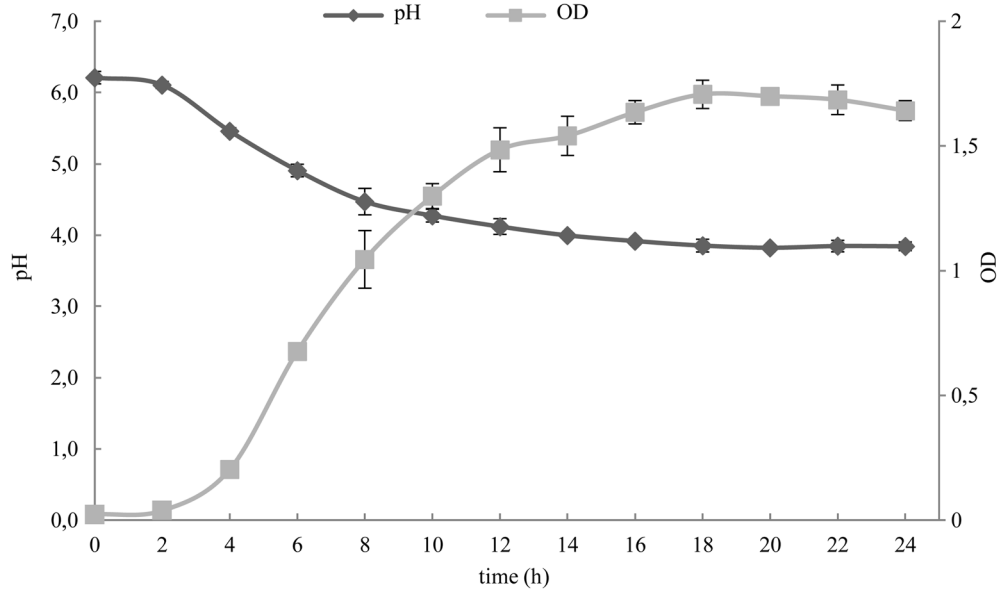

Fig. 4. Growth curve and acid production curve of *Lactobacillus delbrueckii* ssp. *indicus* WDS-7 strain.

pH value reached the lowest, i.e., 3.82, and remained under 4.0 in the stable growth phase.

Heat resistance. The heat resistance of *L. delbrueckii* ssp. *indicus* WDS-7 strain was investigated, and the results are presented in Table V. In general, WDS-7 strain was inactivated after treatment in water bath at 70°C and 80°C for 3 min, and the survival rate was about 0%. When exposed to 60°C the survival rate of the strain was 12.38 ± 2.33%. After treatment at 50°C for 3 minutes, the survival rate of the WDS-7 strain was higher than that of LGG and was 91.81 ± 7.43%.

Table V
The cell survival rates after heat treatment of *Lactobacillus delbrueckii* ssp. *indicus* WDS-7 strain.

Strains	Temperature (°C)			
	50	60	70	80
WDS-7	91.81 ± 7.43%	12.38 ± 2.33%*	≈ 0%	0%
LGG	83.38 ± 7.32%	4.79 ± 0.93%	≈ 0%	0%

Results are expressed as the mean ± SD.
* – along the column represent statistical significance ($p < 0.05$)

BSH activity. The BSH activity of *L. delbrueckii* ssp. *indicus* WDS-7 strain was assessed qualitatively in our study. In contrast to the control MRS agar plate, precipitation around the colonies on MRS agar plate with TDCA indicated that the WDS-7 strain possessed the BSH activity.

Biofilm formation. The ability of *L. delbrueckii* ssp. *indicus* WDS-7 to form biofilm on glass and plastic surfaces was evaluated with the crystal violet method. The results are illustrated in TableVI. LGG was a mild

Table VI
Biofilm formation by *Lactobacillus delbrueckii* ssp. *indicus* WDS-7 strain.

Strains	Biofilm formation	
	Glass	Plastic
WDS-7	+	++
LGG	+	+

+ and ++ represent mild biofilm producer ($0.1 < OD_{590} < 0.2$) and strong biofilm producer ($OD_{590} > 0.2$), respectively

biofilm producer ($0.1 < OD_{590} < 0.2$) due to its weak ability of biofilm formation on glass and plastic surfaces. Similarly, WDS-7 also displayed a weak biofilm formation ability on the glass surface, whereas strong on plastic surface ($OD_{590} > 0.2$). WDS-7 was the better biofilm producer on plastic surface compared to LGG.

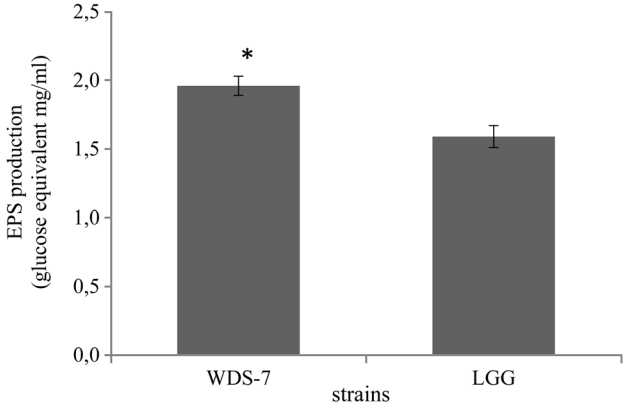

Fig. 5. EPS production activity of *Lactobacillus delbrueckii* ssp. *indicus* WDS-7 strain.
* – represents the statistical significance ($p < 0.05$).

EPS production. The EPS production by *L. delbrueckii* ssp. *indicus* WDS-7 is shown in Fig. 5. Compared to LGG, WDS-7 exhibited a significantly higher ($p < 0.05$) ability to produce EPS (1.94 mg/ml as a glucose equivalent).

Table VII
Antibiotic susceptibility of *Lactobacillus delbrueckii* ssp. *indicus* WDS-7 strain.

Strains	Antibiotic susceptibility											
	Metronidazole	Chloramphenicol	Streptomycin	Kanamycin	Ampicillin	Gentamicin	Tetracycline	Erythromycin	Rifampicin	Ciprofloxacin	Doxycycline	Vancomycin
WDS-7	R	S	R	R	S	R	I	S	S	I	S	R
LGG	R	S	R	R	S	R	S	S	S	S	S	R

R, I, and S represent resistance, intermediate susceptibility, and susceptibility to the antibiotic, respectively

Table VIII
Enzyme production by *Lactobacillus delbrueckii* ssp. *indicus* WDS-7 strain.

Enzyme	Strains	
	LGG	WDS-7
Alkaline phosphatase	1	1
Esterase	2	1
Esterase lipase	1	2
Lipase	0	0
Leucine arylamidase	2	4
Valine arylamidase	3	2
Cystine arylamidase	1	0
Trypsin	0	0
α-Chymotrypsin	0	0
Acid phosphatase	2	0
Naphthol-AS-BI-phosphohydrolase	2	2
α-Galactosidase	1	3
β-Galactosidase	2	4
β-Glucuronidase	0	0
α-Glucosidase	2	2
β-Glucosidase	1	0
N-Acetyl-b-glucosaminidase	0	0
α-Mannosidase	0	0
α-Fucosidase	1	0
Control	0	0

0: 0 nmol, 1: 5 nmol, 2: 10 nmol, 3: 20 nmol, 4: 30 nmol, 5: 40 nmol

Antibiotic susceptibility, hemolysis and production of biogenic amines. The antibiotic susceptibility of *L. delbrueckii* ssp. *indicus* WDS-7 is presented in Table VII. WDS-7 strain showed similar antibiotic resistance patterns compared to LGG, and was sensitive to most antibiotics tested, including chloramphenicol, ampicillin, tetracycline, erythromycin, rifampicin, ciprofloxacin, and doxycycline. It was resistant tometronidazole, streptomycin, kanamycin, gentamicin, and vancomycin. Moreover, any hemolytic activity and production of biogenic amines was observed for WDS-7 strain.

Enzyme production. The enzyme production by *L. delbrueckii* ssp. *indicus* WDS-7 is shown in Table VIII. Compared to LGG, the WDS-7 strain exhibited a similar enzyme production pattern. *L. delbrueckii* ssp. *indicus* WDS-7 possessed various enzyme activities, viz., alkaline phosphatase, esterase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, valine arylamidase, α-galactosidase, lipase, β-galactosidase and α-glucosidase. In contrast, the production of the α-chymotrypsin, cystine arylamidase, N-acetyl-β-glucosaminidase, β-glucuronidase, lipase, acid phosphatase, β-glucosidase, trypsin, α-fucosidase, and α-mannosidase was not observed.

Discussion

Adhesion to the intestinal mucosa is vital for cultivating probiotics, as it is a prerequisite for colonization in the gastrointestinal tract (Papadimitriou et al. 2015). Georgalaki et al. (2017) reported that the adhesion rate of *Lactobacillus plantarum* ACA-DC 805 to HT-29 cells reached up to 9.5 ± 1.3. The above results are following our results. Cao et al. (2018) found that *L. plantarum* STM6-1 showed the highest adhesion rate to HT-29 (27.2%), followed by *L. plantarum* STM6-2 (17.6%). The results obtained in our work indicated a lower adhesion rate than the reports mentioned by Cao et al. (2018). All 11 LAB strains selected in this research displayed different degrees of adhesion to HT-29 cells. The intestinal adhesion ability of probiotics varies by strain and species. The composition and structure of bacterial cell membrane and the production of secreted proteins may participate in bacterial adhesion to intestinal epithelial cells (Khan and Kang 2016).

The candidate selected as probiotic should survive under high acid conditions and maintain high cell concentration within 2–3 hours of transit in the stomach. Similarly, a potential probiotic is considered is considered to show tolerance to bile salt in the human gut (Kandylis et al. 2016). Son et al. (2017) found that *L. plantarum* Ln4 and G72 had apparent resistance to low pH after 24 h of incubation in the artificial gastric juice. The survival of *L. brevis* strain LSe isolated from an Iranian traditional dairy product was not significantly changed due to drop in pH of the simulated gastric juice from 6 to 3, and after 3 h, 6 h and 24 h of incubation (Shakibaie et al. 2017). Bao et al. (2010) reported that 11 LAB strains of *L. fermentum* originated from traditional dairy products represented survival above 80% after 3 h incubation in simulated gastric juice of pH 2.5. The result of the present study illustrated that six LAB strains showed tolerance to artificial simulated gastrointestinal conditions, which were similar to those mentioned above. It may be due to the production of organic acids by LAB in the process of fermentation, which can reduce the pH of the environment surroundings. Moreover, the traditional fermented buffalo milk is stored in a closed and cryogenic environment, resulting in the organic acid not being easy to volatilize, which maintains and reduces the pH value further. This treatment method on buffalo milk could give these LAB strains the ability to tolerate the extreme living environment, such as low pH value.

Probiotics with antioxidant activity benefit the host by destroying and neutralizing free radicals (Talebi et al. 2018). At present, DPPH and $ABTS^+$ free radical scavenging activities are important tools to evaluate the antioxidant activity of probiotics. Das et al. (2020) investigated the antioxidant activities of eight *Lactobacillus* spp. strains isolated from the traditional fermented foods of Meghalaya, India, and the $ABTS^+$ radical scavenging activity of *L. fermentum* K7 reached 80.78 ± 0.78%. Kaya Ozdgan et al. (2012) reported that the DPPH scavenging capacity of *Lactobacillus lactis* LL27 strain was 75 ± 3%. The antioxidant capacities obtained in our work were lower than in the two reports mentioned above, indicating that the antioxidant activity of *L. lactis* may be dependent on the source and strain. Cao et al. (2018) reported that DPPH radial scavenging activities of *L. plantarum* ST and STDA10 strains isolated from Yunnan De'ang Pickled Tea were almost 30%. Our findings were comparable to the above results reported by Cao et al. (2018). WDS-4, WDS-7, and WDS-18 had notable antioxidant activities in the current research.

Antimicrobial activity is an important criterion when screening potential probiotics, as antimicrobial activity prevents potentially harmful intestinal microorganisms from colonizing the host's intestinal mucosa (Gheziel et al. 2019). Edalati et al. (2019) discovered that *L. plantarum* strain CAU2522 isolated from raw camel's milk from three districts of Kerman province had antagonistic properties on *S. aureus*. Prabhurajeshwara and Chandrakanth (2019) reported that 13 *Lactobacillus* spp. strains isolated from commercial yoghurt showed antagonistic effects against seven indicator microorganisms tested, but the degree of antagonism varied among the *Lactobacillus* spp. strains. Y9, Y10 and Y13 isolates were the most effective in inhibiting pathogens. It was also reported that *L. plantarum* YS5 isolated from yogurt showed excellent antibacterial effect against *E. coli*, *S. aureus* and *Shigella flexneri*, and the inhibition zone of *S. flexneri* was the largest (31.5 ± 0.5 mm) (Nami et al. 2019). In this work, antimicrobial activity varying between strains isolated might be attributed to the differences between species and strains. Some research illustrates that antimicrobial activity is species- and strain-dependent (El-Jeni et al. 2016; Das et al. 2016).

A microbial growth curve can provide useful helpful information for understanding microbial growth trends and selecting the optimal growth stage (Yang et al. 2018). Rapid growth and low pH of the culture may constitute an important feature for the industrial production of a potential probiotic strain. Yang et al. (2021) described that eight *Lactobacillus* spp. strains isolated from soybeans showed the same growth trend, the lag period was short (0–2 h), and the logarithmic growth period was 2–8 h. Pellegrino et al. (2019) also reported three selected *Lactobacillus* spp. strains including *L. lactis* CRL1655, *Lactobacillus perolens* CRL1724, and *L. plantarum* CRL1716 that showed an incubation period of 2 h and an exponential period of growth lasting up to 8 h. In our study, the kinetic growth of

L. delbrueckii ssp. *indicus* WDS-7 strain was consistent with the above results reported. WDS-7 strain had the short latency phase and the pH value of culture reached to 3.82 after 20 h of incubation. It demonstrated that WDS-7 strain had strong capacity for acid production.

Potential probiotic must be able to withstand the harsh conditions e.g., heat, often encountered in food processing, to be successfully applied in functional foods. Ren et al. (2018) found most *Lactobacillus* spp. strains isolated from homemade fermented foods including *L. plantarum*, *Lactobacillus pentosus*, and *Lactobacillus paracasei* with antibacterial activities were highly resistant to heat (65–121°C). The above results were much better than the results of the current research. Wang et al. (2021) also reported that no strains survived incubation at 50°C, while *L. rhamnosus* ZX691 strain grew well at 45°C. The results of this study were superior to the results reported by Wang et al. (2021). In heat resistance test, WDS-7 strain had reasonable growth at 50°C and good thermal stability, making it a potential probiotic selection for food application.

The positive results showing the presence of precipitation around the colonies on MRS agar plate containing TDCA by LAB are advantageous for probiotics as it can help to detoxify bile salt by producing BSH activity (Sharma et al. 2021). It was observed that *L. plantarum* cam 15 from camel milk could combine the bile salts with BSH activity (Sharma et al. 2019). Saliba et al. (2021) reported that all *Lactobacillus* spp. strains isolated from Lebanese Baladi goat milk exhibited a partial BSH activity. The above results were in agreement with our findings. Recent works have found that intestinal probiotics, such as *Lactobacillus* spp., are resistant to the decontamination of bile salts. One of the mechanisms for the resistance is the deconjugation of bile salts through BSH (Prete et al. 2020).

The ability to form biofilm is another desirable characteristic of probiotics. Biofilms are complex multi-species communities that are closely linked to the surface. Therefore, screening of potential probiotic strains usually involves the determination of its biofilm formation capacity (Muruzović et al. 2018). Among five LAB strains isolated from Algerian infant feces, *L. plantarum* LSC3 and LSC22 were the best suited for producing biofilms on plastic and glass surfaces (Gheziel et al. 2019). The results of the present work were consistent with the above results. In this research, WDS-7 isolated from Chinese traditional fermented buffalo milk showed strong ability of biofilm formation, so it certainly has potential for further investigation as probiotics. Our results revealed some variability in biofilm formation capacity, which was consistent with previous work on *Lactobacillus reuteri* (Mackenzie et al. 2010).

Probiotics possessing the capability of EPS production are considered an advantage (Bermúdez-Humarán and Langella 2011). Comparatively with the rest of the cultures, *L. rhamnosus* K4E had the highest EPS production with 950 ± 0.256 mg/l, followed by *L. plantarum* RD7 (710 ± 0.388 mg/l) (Das et al. 2020). The result of EPS production reported above was slightly lower than that of our study. Similarly, Abouloifa et al. (2020) found all the *Lactobacillus* spp. strains isolated from traditional fermenting green olives showed an EPS production capacity. Furthermore, Sharma et al. (2019) revealed that *L. plantarum* K90, *L. fermentum* K75, and *L. fermentum* K78 strains isolated from traditionally fermented wheat flour dough known as "babroo", could be used as potential probiotic candidates with EPS production. EPS improves the colonization of probiotics on the surface of gastrointestinal mucosa by increasing the autoaggregation ability of probiotics (Kanmani et al. 2013). The capability of EPS production of these LAB strains may explain the high adhesion capacity obtained in this work.

In terms of antibiotic resistance, potential probiotic exhibiting sensitivity to antibiotics is preferable for application. Our findings were similar to the antibiotic resistance of *Lactobacillus* spp. isolates originated from traditional dairy products in East Azerbaijan Province in Iran (Faghfoori et al. 2017), and relatively better than the antibiotic resistance of *Lactobacillus curvatus* P99 isolated from fermented oat dairy beverage (Funck et al. 2019). However, LAB strains are known to be inherently sensitive to β-lactam, tetracycline, and macrolides antibiotics but resistant to aminoglycosides antibiotics (Kumar and Kumar 2015). *L. delbrueckii* ssp. *indicus* WDS-7 was resistant to metronidazole, streptomycin, kanamycin, gentamicin, and vancomycin, a cell wall synthesis inhibitor. Jatmiko et al. (2017) reported that *Lactobacilli* are naturally resistant to vancomycin, but vancomycin resistance is encoded by chromosome and cannot be transferred to other microbial species. Lack of hemolytic activity is one of the safety prerequisites for screening probiotics (FAO/WHO 2002). Our conclusion agreed with the known hemolysis of *L. fermentum* isolated from fermented dairy milks (Thirabunyanon et al. 2009).

The absence of biogenic amines production in *Lactobacillus* spp. was already expected an essential criterion of food safety for probiotic candidate strains (Casarotti et al. 2017). Colombo et al. (2020) reported 11 *Lactobacillus* spp. strains previously isolated from a dairy environment showed no *in vitro* detection of biogenic amines production. Yüceer and Özden Tuncer (2015) also described *Lactobacillus sakei* ssp. *carnosus* and *L. sakei* ssp. *sakei* strains isolated from fermented Turkish sausage did not decarboxylate histidine, lysine, or ornithine. The results obtained in this study agreed

with those mentioned above. In our study, no color change of the indicator was observed, it was proved that the WDS-7 strain did not produce biogenic amines, and *L. delbrueckii* ssp. *indicus* WDS-7 strain was safe in food application.

The lack of harmful activities, such as β-glucuronidase activity, must also be included in the safety assessment. In present study, *L. delbrueckii* ssp. *indicus* WDS-7 did not have β-glucuronidase, α-chymotrypsin, and N-acetyl-β-glucosaminidase activities, but showed higher production of β-glucosidase than LGG. *L. plantarum* SK1305 strain isolated from Korean green chili pickled pepper did not produce β-glucuronidase but produced α-and β-glucosidase, α- and β-galactosidase, naphthol-AS-BI-phosphohydrolase, acid phosphatase, and N-acetyl-β-glucosaminidase (Niu et al. 2019). *Lactobacillus* spp. strains, which had previously been isolated from traditional fermenting green olives, also produced several enzymes, such as naphthol-AS-BI-phosphohydrolase and β-galactosidase but did not produce β-glucuronidase, α-chymotrypsin, and other harmful enzymes (Abouloifa et al. 2020). The above conclusions were consistent with the results of this work. The absence of harmful enzymes and the production of valuable enzymes suggested the reliable security of the strain and the possibility of using the strain in the production of fermented milk products.

Conclusions

In this work, *L. delbrueckii* ssp. *indicus* WDS-7 strain was isolated. It fulfilled the probiotic criteria *in vitro* by exhibiting preferable adhesion capacity to HT-29 cells, good tolerance to artificial simulated gastrointestinal conditions, excellent antioxidant capacities, stronger antibacterial activity, and safety in use. Moreover, it also possessed good probiotic activities, including heat resistance, BSH activity, biofilm formation, and EPS production. This study demonstrated the probiotic potential of *L. delbrueckii* ssp. *indicus* WDS-7 strain would be a novel probiotic strain for application in fermented dairy products. For the first time, a probiotic strain isolated from Chinese traditional fermented buffalo milk displayed excellent probiotic properties, and it could be a promising candidate to produce functional probiotic food with potential health benefits.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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