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Research Article

Evaluation of the Antibacterial Activity and Probiotic Potential of *Lactobacillus plantarum* Isolated from Chinese Homemade Pickles

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This study investigated the antipathogenic activity and probiotic potential of indigenous lactic acid bacteria (LAB) isolated from Chinese homemade pickles. In total, 27 samples were collected from different sites in China. Fifty-nine yielded pure colonies were identified by 16S rRNA gene sequencing as LAB and were initially evaluated for the antibacterial activity in vitro. Initial screening yielded *Lactobacillus plantarum* GS083, GS086, and GS090, which showed a broad-spectrum antibacterial activity against food-borne pathogens, especially multidrug-resistant pathogens. Meanwhile, organic acids were mainly responsible for the antimicrobial activity of the LAB strains, and the most abundant of these was lactic acid (19.32 ± 0.95 to 24.79 ± 0.40 g/l). Additionally, three *L. plantarum* strains demonstrated several basic probiotic characteristics including cell surface hydrophobicity, autoaggregation, and survival under gastrointestinal (GI) tract conditions. The safety of these isolates was also evaluated based on their antibiotic susceptibility, hemolytic risk, bile salt hydrolase activity, and existence of virulence or antibiotic resistance genes. All strains were safe at both the genomic and phenotypic levels. Therefore, *L. plantarum* GS083, GS086, and GS090 are fairly promising probiotic candidates and may be favorable for use as preservatives in the food industry.

1. Introduction

Fermentation is a traditional method used to prolong the shelf life and improve the flavors of food [1]. Fermented foods such as pickles are widely utilized by most families in China and have been continually consumed for thousands of years. A variety of lactic acid bacteria (LAB) strains such as *Lactobacillus*, *Leuconostoc*, *Lactococcus*, and *Pediococcus* are involved in the pickle fermentation phase and have crucial health-improving effects [2]. Currently, LAB are classified as "generally recognized as safe" microorganisms and are widely used in the food industry [3]. In addition to their probiotic functions, LAB can improve food flavor and nutritional value by generating aromatic compounds and converting isoflavone glucosides into aglycones [4, 5]. Therefore, screening probiotic LAB from fermented food has gained increasing attention in the recent years.

Microbiologic contamination is one of the most important reasons of food spoilage and/or reductions in its shelf life. Food contamination with food-borne pathogens such as *Escherichia* and *Salmonella* has led to severe infections, which can sometimes even be fatal [6, 7]. Moreover, antibiotics are commonly used to reduce the harm caused by microbial contamination. However, one problem associated

with the excessive use of antibiotics is the ongoing occurrence of multidrug-resistant pathogens [8]. These organisms cause persistent risks for the whole chain in the food industry, given that cannot easily be inactivated by chemical or physical methods. As a result, there is an urgent need to find favorable biological preservatives as promising alternatives to antibiotics.

LAB, not only as antagonists of pathogenic microorganisms but also producers of antimicrobial metabolites, have attracted much attention. Several studies have proven that LAB can inhibit the growth of pathogenic microorganisms via multiple mechanisms, including competitively inhibiting pathogen binding, enhancing the host immune system, and producing pathogen growth-inhibitory compounds such as organic acids, bacteriocins, and hydrogen peroxide [9]. Therefore, LAB could be candidate biological preservatives in the food industry. However, not all of these bacteria can be applied to the control of food-borne pathogens in the food industry as they might produce unfavorable flavors in food. However, LAB strains isolated from traditional fermented food are more likely to be accepted by customers.

Before LAB strains are potentially used as probiotics, their probiotic characteristics and safety should be considered [10]. Furthermore, a potent probiotic isolate should possess certain characteristics such as survival and colonization ability in different environments [11]. Furthermore, they should be able to withstand bile salts and the low pH of gastric juice and have adhesion ability, which could be helpful in colonizing the human host [12]. According to the FAO/WHO guidelines, probiotic microorganisms should be safe for humans, with the most important concerns being potential virulence and antibiotic resistance [10, 13]. Hence, the utility of LAB should be evaluated at both phenotypic and genomic levels.

Thus, this work investigated the antibacterial activity of indigenous LAB strains obtained from Chinese homemade pickles against food-borne and multidrug-resistant pathogens, combined with the characterization of the antibacterial metabolites produced by them, to reveal their probiotic potential.

2. Materials and Methods

2.1. Isolation and Characterization of LAB Strains. A total of 27 samples of traditional homemade pickles with different fermentation methods were collected around China. LAB were isolated according to the methods described by Yi et al. [14]. LAB species were confirmed by 16S rRNA sequencing, using the universal primers 27F : AGAGTTTGATCCTGGCTCAG and 1492R : ACGGCTACCTTGTTACGACTT [15], and evolution analysis was performed by the neighbor-joining method (MEGA X version 10.1.7) and visualized with iTOL (https://itol.embl.de/itol.cgi).

2.2. Antibacterial Activity of LAB Strains against Food-borne and Multidrug-Resistant Pathogens

2.2.1. Preparation of the Cell-Free Culture Supernatant. The cell-free culture supernatant (CFS) of LAB was prepared according to the method described by Muthusamy et al. [16]. LAB were statically cultured at 37°C for 24 h. Cell suspensions were centrifuged at 3,100 × g at 4°C for 15 min, and the supernatants were filtered through a sterilized 0.22 μm filter. The CFS samples were stored at 4°C before use.

2.2.2. Information on Food-Borne Pathogens and Culture Preparation. Eight common food-borne pathogens were selected as indicators in this study, including *Listeria monocytogenes* ATCC 19117, *Bacillus cereus* ATCC 14579, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 8739, *Salmonella typhimurium* ATCC 14028, *Cronobacter sakazakii* ATCC 29544, and *Pseudomonas aeruginosa* ATCC 15442. Moreover, six multidrug-resistant bacteria were also selected as indicators including *L. monocytogenes* 1846–1, *B. cereus* 3311-2A, *S. aureus*117-2, *E. coli* 2624–2, *S. typhimurium* 54–9, and *C. sakazakii* cro 300A [6, 17–21]. All indicators were cultured overnight in LB broth at 37°C.

2.2.3. Antibacterial Spectrum of LAB Strains. The inhibitory activity of CFS produced by LAB was measured by the Oxford cup agar diffusion method [22]. Overnight cultures of indicator bacteria were diluted and spread onto nutrient agar plates. Then, 100 μl of CFS was added to sterile Oxford cups on the plates for coculture at 37°C for 24 h. Then, the diameter of the inhibition zone was measured by using a pair of Vernier calipers.

2.3. Antibacterial Metabolites Produced by L. plantarum Strains

2.3.1. Sensitivity of Antibacterial Metabolites to pH and Enzymes. To verify the pH sensitivity of the LAB strains, the pH of CFS was adjusted to 5.5 using 1.0 M NaOH. Similarly, CFS samples were inactivated by catalase, trypsin, pepsin, and proteinase K (2 mg/ml) at 37°C for 2 h. Residual antibacterial activity of the treated CFS was determined against *S. typhimurium* ATCC14028 (representative of Gram-negative bacteria [G^-]) and *L. monocytogenes* ATCC19117 (representative of Gram-positive bacteria [G^+]).

2.3.2. Quantification of Organic Acids by HPLC. Six types of organic acids in the CFS were measured by HPLC (Agilent, USA) according to Upreti et al. [23]. The data were processed using OpenLAB CDS ChemStation Edition TM software. The obtained peaks were compared with standards (purity ≥ 99%).

2.4. Probiotic Characteristics of L. plantarum Strains

2.4.1. Carbohydrate Fermentation Patterns. Fermentation patterns of LAB were tested with an API 50 CHL test based on 49 selected carbohydrate sources. Briefly, overnight cultures were suspended in 10 ml of the API 50 CHL medium, and each sample was applied onto cupels containing different carbohydrates on an API 50 CH test strip.

Fermentation patterns were determined after incubation for 24–48 h at 37°C.

2.4.2. Testing Tolerance to Gastrointestinal Tract Conditions. The gastric and pancreatic juices, used to simulate the digestive environment, were prepared according to the method described by Katarzyna and Alina [24]. Simulated gastric juice was prepared by dissolving 0.35% (w/v) pepsin in PBS, which was acidified to a pH of 2.0. Simulated pancreatic juice (pH 8.0) was composed of 1.1% (w/v) $NaHCO_3$ and 0.1% (w/v) trypsin. The simulated gastric and pancreatic juices were filtered through a sterilized 0.22 μm filter.

LAB cells were suspended in simulated gastric juice and incubated at 37°C for 3 h. Their viability was, then, determined by the flat colony counting method. The collected cells from the gastric phase were suspended in simulated pancreatic juice for 24 h, and then, the bacterial survival rate was estimated by the DeMan Rogosa Sharpe (MRS) agar plate enumeration method.

2.4.3. Cell Adhesion Activity. Autoaggregation was analyzed using a modified method reported by Ogunremi et al. [25]. The suspensions were mixed and incubated at room temperature for 4 h and, then, measured based on their absorbance at 600 nm. Calculations were based on equation (1) in the main text, where A = the absorbance at 0 h and A_t = the absorbance at 4 h.

$$x = \frac{A - A_t}{A} \times 100. \quad (1)$$

The cell surface hydrophobicity of LAB strains was evaluated by measuring the bacterial cell adhesion to the hydrocarbon xylene according to the method described by Rokana et al. [26]. The LAB cells were cultured overnight and collected by centrifugation at 12,400 × g at 4°C for 10 min. Cells were resuspended in PBS, and their absorbances were detected at 600 nm. Then, a 3 ml cell suspension sample was mixed with 1 ml of hydrocarbon xylene. After incubation at 37°C for 1 h, the absorbance of the obtained aqueous layer was determined at 600 nm. The percent hydrophobicity was measured based on the decrease in absorbance. Calculations were performed using equation (2) in the main text, where A = the absorbance at 0 h and A^* = the absorbance at 1 h.

$$x = \frac{A - A^*}{A} \times 100. \quad (2)$$

2.5. Safety Evaluation of L. plantarum Strains

2.5.1. Antibiotic Susceptibility Testing. The antibiotic susceptibility of LAB was determined by the broth microdilution method [27]. Nine types of antibiotics were tested, including chloramphenicol, erythromycin, rifampicin, tetracycline, gentamycin, clindamycin, imipenem, ampicillin, and vancomycin. They were dissolved in the respective diluents and prepared at different concentrations (from 0.5 to 1,024 μg/ml). Susceptible and resistant strains were defined according to the standards reported by EFSA [28].

2.5.2. Hemolytic and Bile Salt Hydrolase (BSH) Activity. The LAB were streaked on blood agar plates to evaluate hemolysis activity according to Lee [29]. The BSH activities of LAB were checked by culturing the bacteria on MRS agar containing 0.5% taurodeoxycholic acid under anaerobic conditions for 48 h. The area of bacterial colonies showing white precipitates was scored as bile salt hydrolase positive [30].

2.5.3. Whole-Genome Sequencing and Bioinformatic Analyses. Whole-genome sequencing was performed according to the method described by Pang [31]. High-quality reads were assembled using SPAdes v. 3.6.2 program, and putative open reading frames were predicted with Prokka 1.1.3. Functional annotation was performed by rescreening BLASTp against the Nonredundant Protein Database of the NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The cyclic images and comparative genomic analyses were generated using the BLAST Ring Image Generator (BRIG), in which the sequence of *L. plantarum* WCFS1 was downloaded from the NCBI.

The presence of virulence factors in the genome of LAB was identified using the virulence factor database (VFDB, http://www.mgc.ac.cn/VFs/main.htm). The genetic determinants conferring antimicrobial resistance (AMR) in the genome were searched using two publicly available databases, namely, the Comprehensive Antibiotic Resistance Database (CARD, http://arpcard.mcmaster.ca) and ResFinder (https://cge.cbs.dtu.dk/services/ResFinder/).

2.6. Statistical Analysis. Statistical analysis was performed using GraphPad Prism version 8.01 software. All data are shown as the mean values ± standard deviations from triplicate samples. Differences with $p < 0.05$ were considered statistically significant.

3. Results

3.1. Isolation and Identification of LAB Strains. In total, 59 LAB strains were isolated from 27 samples of pickles with different fermentation methods from around China (Figure 1). The results of 16S rRNA gene sequencing and homology searching using BLAST confirmed that these strains included *Lactobacillus* (42), *Lactococcus* (6), *Weissella* (5), *Enterococcus* (3), *Pediococcus* (2), and *Leuconostoc* (1).

3.2. Antibacterial Activity of L. plantarum Strains against Food-borne and Multidrug-Resistant Pathogens. As shown in Table S1, nine isolates showed antimicrobial activity against eight food-borne pathogens, including G^- and G^+ bacteria. These strains were screened, and their inhibitory activity against six multidrug-resistant bacteria was also evaluated.

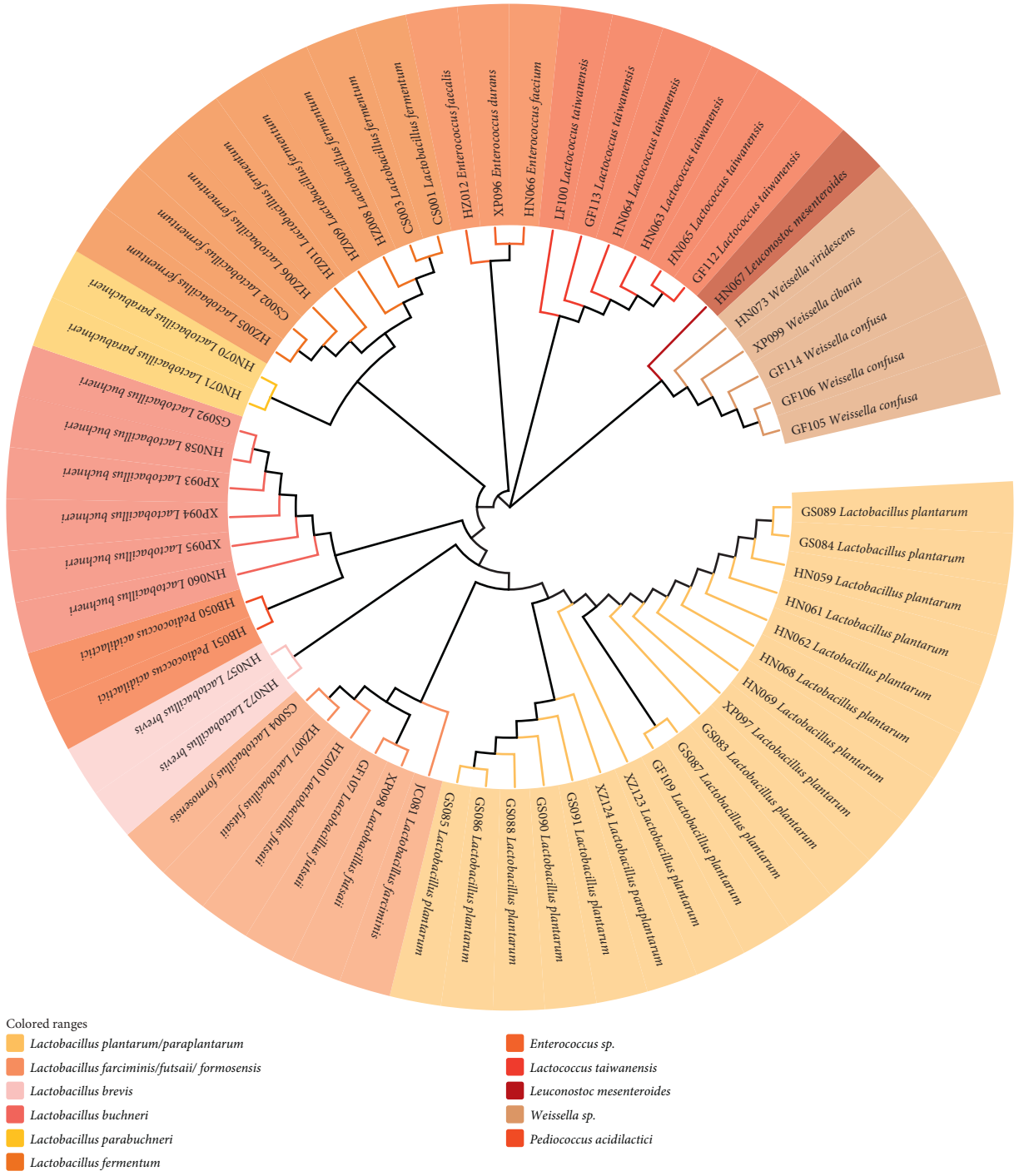

Figure 1: Circular phylogenetic tree based on the neighbor-joining method of 16S rRNA gene sequences of the isolated LAB. The scale bar represents 0.010-nucleotide substitutions per position.

Among them, three strains of LAB including *L. plantarum* GS083, GS086, and GS090 showed active resistance to all multidrug-resistant pathogens; these were subjected to further analyses (Figure 2).

3.3. Sensitivity to pH and Enzymes. The antibacterial activity of CFS samples of different *L. plantarum* strains only disappeared when they were neutralized at pH 5.5 (Table 1). In addition, the inhibitory effect of CFS after protease treatment was almost the same as that before treatment, suggesting that the antibacterial substances are not proteinaceous.

3.4. Organic Acids Produced by L. plantarum Strains. The organic acids in the CFS were detected, among which the most abundant was lactic acid, with concentrations ranging

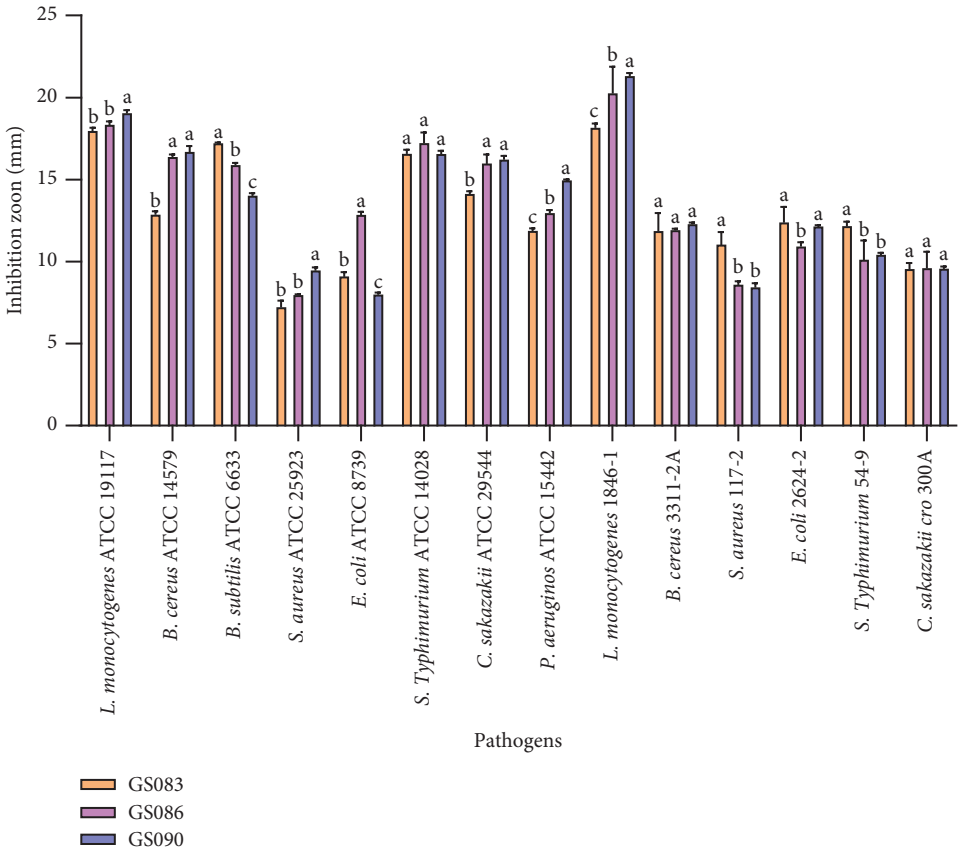

Figure 2: Antibacterial activity of *L. plantarum* GS083, GS086, and GS090 against 14 pathogen indicators. The zone of inhibition is expressed as the mean ± SD of three replicates.

Table 1: Characterization of antibacterial compounds from three *L. plantarum* strains.

Treatment	Residual inhibitory activity*					
	S. typhimurium ATCC14028			*L. monocytogenes* ATCC19117		
	GS083	GS086	GS090	GS083	GS086	GS090
Control	+++	+++	+++	++++	++++	++++
5.5	—	—	—	—	—	—
Catalase	+++	+++	+++	++++	++++	++++
Trypsin	+++	+++	+++	++++	++++	++++
Pepsin	+++	+++	+++	++++	++++	++++
Proteinase K	+++	+++	+++	++++	++++	++++

*The diameter of the inhibition zone (mm): − < 6, 6 < + < 10, 10 < ++ < 14, 14 < +++ < 18, ++++ > 18.

Table 2: Quantitative detection of organic acids by HPLC (g/l).

Organic acid	*L. plantarum* GS083	*L. plantarum* GS086	*L. plantarum* GS090
Formic acid	0.53 ± 0.10^{Ac}	0.43 ± 0.24^{Ac}	ND^{*Bc}
Malic acid	0.36 ± 0.09^{Ac}	0.82 ± 0.11^{Ac}	0.49 ± 0.15^{Ac}
Lactic acid	19.32 ± 0.95^{Ba}	24.79 ± 0.40^{Aa}	19.85 ± 0.43^{Ba}
Acetic acid	ND^{Bc}	2.00 ± 0.71^{Ab}	2.15 ± 0.27^{Ab}
Citric acid	1.06 ± 0.38^{Ab}	0.55 ± 0.08^{Ac}	0.64 ± 0.20^{Ac}
Succinic acid	1.54 ± 0.15^{Ab}	1.29 ± 0.26^{Ab}	0.69 ± 0.01^{Bb}

*Not detected. A-C: different superscript small letters in the same row denote differences ($p < 0.05$). a-c: different superscript small letters in the same column denote differences ($p < 0.05$).

Table 3: Carbohydrate fermentation patterns of *L. plantarum* GS083, GS086, and GS090.

Carbohydrates	GS083	GS086	GS090
Glycerol	w	w	w
Erythritol	–	–	–
D-arabinose	–	–	–
L-arabinose	+	+	+
Ribose	+	+	+
D-xylose	–	–	–
L-xylose	–	–	–
Adonitol	–	–	–
Methyl-βD-xylopyranoside	–	–	–
Galactose	+	+	+
Glucose	+	+	+
Fructose	+	+	+
Mannose	+	+	+
Sorbose	–	–	–
Rhamnose	+	+	+
Dulcitol	–	–	–
Inositol	–	–	–
Mannitol	+	+	+
Sorbitol	+	+	+
Methyl-αD-mannopyranoside	+	+	+
Methyl-αD-glucopyranoside	–	–	–
N-acetylglucosamine	+	+	+
Amygdalin	+	+	+
Arbutin	+	+	+
Esculin	+	+	+
Salicin	+	+	+
Cellobiose	+	+	+
Maltose	+	+	+
Lactose	+	+	+
Melibiose	+	+	+
Sucrose	+	+	+
Trehalose	+	+	+
Inulin	–	–	–
Melezitose	+	+	+
Raffinose	+	+	+
Amidon	w	–	w
Glycogen	–	–	–
Xylitol	–	–	–
Gentiobiose	+	+	+
Turanose	+	–	w
Lyxose	–	–	–
Tagatose	–	–	–
D-fucose	–	–	–
L-fucose	–	–	–
D-arabitol	–	–	w
L-arabitol	–	–	–
Gluconate	+	+	+
2-Keto-gluconate	–	–	–
5-Keto-gluconate	–	–	–

Fermentation results are indicated as follows: +, positive; w, weak positive; –, negative.

from 19.32 ± 0.95 to 24.79 ± 0.40 g/l (Table 2). Furthermore, formic acid was detected only in the CFS of *L. plantarum* GS083 and *L. plantarum* GS086. The CFS samples of all tested strains contained acetic acid except for that of *L. plantarum* GS086.

3.5. Carbohydrate Fermentation Patterns of L. plantarum Strains. Fermentation patterns of carbohydrate sources by each *L. plantarum* strain are summarized in Table 3. Differences in fermentation capability were observed as follows: GS083 weakly fermented amidon and did not ferment D-arabitol, while only fermenting turanose; GS090 weakly fermented amidon, turanose, and D-arabitol, resulting in a color transition from green to blue in the API indicator medium instead of yellow, whereas GS086 yielded completely negative results. Based on the patterns identified through the APIWEB database of BioMérieux, the identities (%) of GS083, GS086, and GS090 were 99.9% compared to *L. plantarum* group 1.

3.6. Tolerance of L. plantarum Strains to Gastrointestinal Tract Conditions. Each *L. plantarum* strain was tested for colonization of the GI tract by evaluating their survival in simulated gastric and pancreatic digestion environments (Table 4). All the isolates examined survived in both gastric and pancreatic digestion, which helps in colonizing the intestines. The population of *L. plantarum* strains was superior to 6.8 ± 0.20 lg cfu/ml at the end of these phases.

3.7. Cell Adhesion Activity of L. plantarum Strains. Different *L. plantarum* strains exhibited a high percentage of autoaggregation, ranging from $85.20 \pm 1.07\%$ to $88.01 \pm 1.40\%$ after 4 h incubation (Table 4). Meanwhile, these strains were tested for their cell surface hydrophobicity to estimate their adhesion ability. As shown in Table 4, the tested isolates showed different hydrophobicities.

3.8. Safety of L. plantarum Strains. All three *L. plantarum* strains met the requirements of MIC cutoff values suggested by the EFSA guideline on the antibiotic susceptibility of LAB (Figure 3). Three strains were susceptible to all analyzed antimicrobial agents (including chloramphenicol, erythromycin, rifampicin, tetracycline, gentamycin, clindamycin, imipenem, and ampicillin) with the exception of vancomycin, as an MIC of 512 mg/ml was observed for vancomycin.

The tested LAB strains did not exhibit any hemolytic effect on the blood agar (γ-hemolysis), supporting their safety in vivo (Table 4). Moreover, probiotics with BSH activity showed enhanced tolerance to the bile salts, accordingly lowering blood cholesterol and preventing hypercholesterolemia [32]. The qualitative assessment of bile salt hydrolase activity, indicated by the presence of white precipitation around the colonies in the three LAB strains studied, showed that all strains were positive for BSH activity (Table 4).

The genome assembly and annotation statistics are shown in Table 5. The genomic sequences of *L. plantarum* GS083, GS086, and GS090, respectively, had an identity of 99.18%, 99.16%, and 99.16% with the type genome of *L. plantarum* WCFS1 based on average nucleotide identity (ANI) [33]. CDS sequences of *L. plantarum* GS083, GS086, GS090, and WCFS1 were compared and mapped to the genome of *L. plantarum* WCFS1 (Figure 4). The result revealed that *L. plantarum* GS083 had more genes orthologous with those of *L. plantarum* WCFS1.

Table 4: Probiotic properties, hemolytic, and bile salt hydrolase activity of the selected strains.

Strain		GS083	GS086	GS090
Resistance to gastric and pancreatic juices*	0 h	9.9 ± 0.06	10.1 ± 0.21	9.9 ± 0.07
	3 h	7.5 ± 0.05	8.5 ± 0.12	8.2 ± 0.06
	24 h	6.8 ± 0.20	6.9 ± 0.04	7.3 ± 0.09
Cell surface hydrophobicity (%)		14.86 ± 1.05[b]	21.03 ± 2.01[a]	21.88 ± 1.44[a]
Autoaggregation (%)		85.20 ± 1.07[a]	87.49 ± 3.08[a]	88.01 ± 1.40[a]
Hemolytic activity		−	−	−
Bile salt hydrolase activity		+	+	+

*0 h- viability at the beginning of the assay, 3 h- gastric phase viability after simulation of gastric conditions, 24 h- pancreatic phase viability after simulation of pancreatic condition. a-b: different superscript small letters in the same row denote differences ($p < 0.05$). +: positive, −: negative.

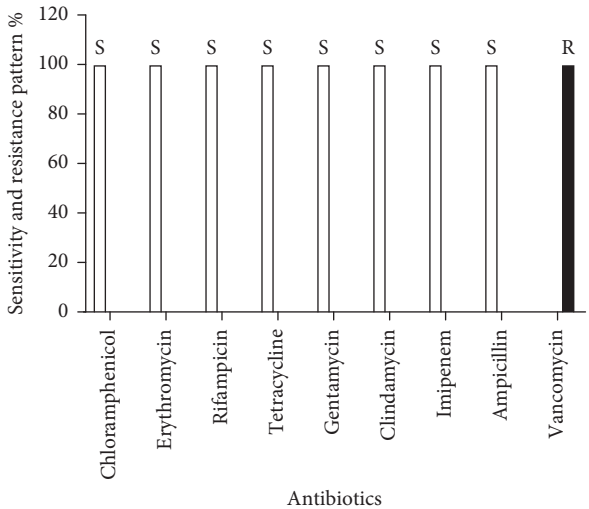

Figure 3: Antibiotic resistance of the selected *L. plantarum* strains. S represents susceptible; *R* represents resistance.

Table 5: Summary of the assembly and annotation statistics of three *L. plantarum* strains.

Strain	GS083	GS086	GS090
Genome size (bp)	3296019	3206156	3220543
GC content (%)	44.26	44.44	44.43
No. of coding sequences	3162	3097	3070
No. of rRNAs	8	8	8
No. of tRNAs	60	59	59

No virulence genes were found under the stringent criteria of >80% identity and >60% coverage. Using the default settings (perfect/strict option for CARD; 90% threshold and 60% minimum length for ResFinder) to search two AMR databases, CARD and ResFinder, no hits were obtained for AMR genes among the genomes of the three LAB strains, suggesting the safety of these isolates.

4. Discussion

Pickles have abundant microbiota and could be utilized as a source for obtaining novel probiotic strains [2, 34, 35]. Additionally, LAB isolated from pickles have many beneficial health effects, such as antibacterial [14] and immunomodulatory [35] activity. In this study, 59 LAB strains were isolated from Chinese pickles including those from the genera *Lactobacillus*, *Lactococcus*, *Weissella*, *Enterococcus*, *Pediococcus*, and *Leuconostoc*, and their antibacterial activity and probiotic potential were further investigated.

The antipathogen potency of CFS produced by LAB was tested. Here, 98.3% of the isolates had antibacterial activity against, at least, one food-borne pathogen, and 91.5% of LAB could inhibit the growth of both G^- and G^+ bacteria. Cervantes-Elizarrarás et al. found that 60% of isolates from aguamiel and pulque (10 strains) inhibited the growth of *E. coli* (G^-) and *S. aureus* (G^+) [36]. An interesting phenomenon was discovered, i.e., *L. monocytogenes* among all tested pathogens was the most sensitive to the CFS produced by LAB. This result was consistent with the findings of Ayala et al. [37]. The prevalence of multidrug-resistant strains of common bacterial pathogens is increasing worldwide [38]. Moreover, infections caused by resistant bacteria might lead to an increase in morbidity and mortality [39]. Some LAB can inhibit the growth of multidrug-resistant pathogens by producing antimicrobial compounds. For example, hydrogen peroxide and lactic acid produced by *L. fermentum* 3872 prevent infections caused by multidrug-resistant *Campylobacter* strains [40]. With this study here, *L. plantarum* GS083, GS086, and GS090, three newly identified LAB, showed prominent antibacterial activity against food-borne and multidrug-resistant pathogens.

LAB usually produce antimicrobial compounds comprising organic acids, hydrogen peroxide, and bacteriocin, among others [41]. Sensitivity tests suggested that the CFS samples from our isolates did not contain any compounds of a proteinaceous nature [42]. It is reasonable to infer that the antibacterial activity of the studied CFS samples might be attributed to organic acids which can also play a role during growth in GI tract conditions. This phenomenon was consistent with the results of previous studies. For example, Barbara et al. reported that organic acids produced by *L. plantarum* CRL 759 inhibit the growth of methicillin-resistant *S. aureus* and *P. aeruginosa* [43]. Furthermore, organic acids can penetrate the cell membrane, thereby affecting cell functions by acidifying the cytoplasm and inhibiting the activity of acid-sensitive enzymes [44]. To verify organic acid production by the three *L. plantarum* strains, the CFS samples were analyzed by HPLC; organic acids including lactic acid, formic acid, malic acid, acetic acid, citric acid, and succinic acid were found to be produced. It is worth mentioning that the highest amount of lactic acid was produced by the three *L. plantarum* strains,

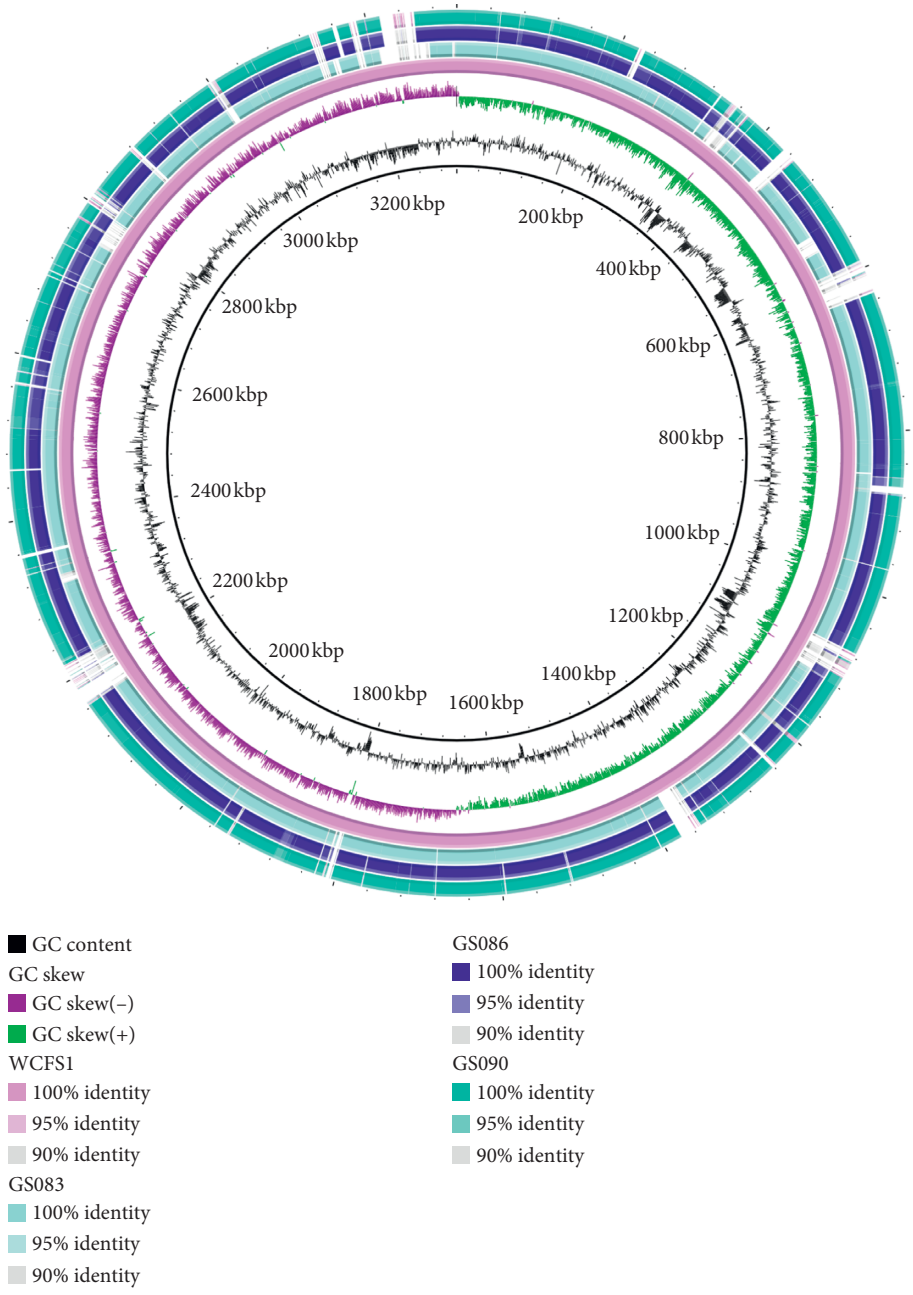

Figure 4: BLAST rings of CDS sequences of *L. plantarum* GS083, GS086, GS090, and WCFS1 mapped onto the genome of the *L. plantarum* WCFS1.

which is consistent with the finding of a study by Muthusamy et al. Lactic acid is one of the major metabolites produced by LAB and is usually produced at a higher level by LAB than other organic acids [16].

To better evaluate the probiotic potential of the three *L. plantarum* strains, several characteristics were tested. The secretion of gastric acid and transit through the stomach constitute a primary defense mechanism that all ingested microorganisms must overcome, including probiotics [45]. A simulated gastric and pancreatic digestion environment was generated to test the survival of three *L. plantarum* isolates under the harsh conditions present in the GI tract. The counts of viable LAB cells were within the range of regulations (6 lg to 10 lg per day) [46], and the three *L. plantarum* strains were deemed adequate to exert probiotic effects in vivo. The capacity of probiotic microorganisms to autoaggregate plays a pivotal role in the colonization of the host epithelia, a prerequisite that aids the host defense mechanisms against gut and skin infections [47]. Autoaggregation of higher than 40% is required for a strain to be a potential probiotic [48]. The percent autoaggregation for the three *L. plantarum* strains after 4 h was greater than 85%, indicating their good adhesion ability. In addition, the surface adhesion ability of bacteria also depends on their hydrophobicity [49]. The results showed that all isolates had hydrophobicity ranging from 14% to 25%, with GS090

having the highest level. Significant differences existed among the investigated strains, which might be attributed to differences in hydrophobic and hydrophilic extensions in the cell wall [31]. In addition, the probiotic properties of the three *L. plantarum* strains were illuminated based on their complete genome sequences.

The FAO/WHO recommended that as a safety measure, the antibiotic resistance profile of a proposed probiotic should also be evaluated [10]. *L. plantarum* GS083, *L. plantarum* GS086, and *L. plantarum* GS090 were only resistant to vancomycin. However, glycopeptide (vancomycin) resistance has been reported in LAB, which is not transferable to pathogens and is rather associated (in most cases) with their innate resistance resulting from the impermeability of their membrane, presumably through a resistance efflux mechanism [50]. Meanwhile, three *L. plantarum* strains showed nonhemolytic and BSH activities, which were recommended as safety characteristics for probiotic selection [51]. According to the whole-genome sequence analysis, the three *L. plantarum* strains lack virulence genes and antibiotic resistance genes. Taken together, these three strains are safe to be used as probiotics. Pickle is a traditional fermented vegetable food with long shelf life, and LAB play a key role in its fermentation.

5. Conclusions

In this study, LAB showed different antimicrobial activities against food-borne and multidrug-resistant pathogens isolated from Chinese homemade pickles, including *Lactobacillus*, *Lactococcus*, *Weissella*, *Enterococcus*, *Pediococcus*, and *Leuconostoc*. The three LAB isolates *L. plantarum* GS083, *L. plantarum* GS086, and *L. plantarum* GS090 were found to have a broad-spectrum antibacterial activity against all the tested pathogens. Furthermore, the three *L. plantarum* strains produced organic acids, including lactic acid, formic acid, malic acid, acetic acid, citric acid, and succinic acid, which are the major metabolites exerting negative effects on the growth of pathogens. Moreover, properties of gastrointestinal tolerance, cell adhesion, BSH, and the lack of multidrug resistance, hemolysis, virulence genes, and antibiotic resistance genes could also contribute to the probiotic potential of these three *L. plantarum* strains. The results ultimately indicate that *L. plantarum* GS083, GS086, and GS090 have potential for application as biological preservatives in the food industry.

Data Availability

All the data supporting the findings are incorporated within the article. Raw data can be presented by the principal investigator upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Y. Zeng and Y. Li contributed equally to the research.

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Supplementary Materials

Supplementary Table 1: antibacterial spectrum of LAB strains against eight food-borne pathogens. Supplementary Table 2: antibacterial spectrum of LAB strains against six multidrug-resistant bacteria. The diameter of the inhibition zone (mm): - < 6, 6 < + < 10, 10 < ++ < 14, 14 < +++ < 18, ++++ > 18. (*Supplementary Materials*)

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