Research Article

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In vitro evaluation of lactic acid bacteria with probiotic activity isolated from local pickled leaf mustard from Wuwei in Anhui as substitutes for chemical synthetic additives

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Abstract: The extensive abuse of chemical synthetic additives has raised increased attention to food safety. As substitutes, probiotics play an important role in human health as they balance the intestinal microbes in host. This study was aimed to isolate and evaluate the potential probiotic activities of lactic acid bacteria (LAB) from a local pickled leaf mustard (PLM) from Wuwei city in Anhui province through *in vitro* experiments. A total of 17 LAB strains were obtained as probiotics. All the isolates were sensitive to chloramphenicol, tetracycline, erythromycin, and doxycycline but exhibited resistance to antibiotics (e.g., streptomycin, kanamycin, gentamicin, and vancomycin). Out of the 17 strains, 9 were sensitive to most of the antibiotics and had no cytotoxic activity on human colorectal adenocarcinoma cell line (HT-29) cells. The isolated AWP4 exhibited antibacterial activity against four indicator pathogen strains (ATCC8099: *Escherichia coli*, ATCC6538: *Staphylococcus aureus*, ATCC9120: *Salmonella enteric*, and BNCC192105: *Shigella sonnei*). Based on the phylogenetic analysis of the 16S rRNA gene, AWP4 belonged to *Lactiplantibacillus plantarum*. This study indicated that the Wuwei local PLM could be a potential resource to isolate beneficial LAB as probiotics. The data provide theoretical guidance for further animal experiments to estimate the probiotic effect and safety of *Lpb. plantarum* AWP4 *in vivo*.

Keywords: antibiotic susceptibility, chemical synthetic additive, food consumption, intestinal microflora, probiotic activity

1 Introduction

The growing concern of consumers for food safety due to the extensive abuse of chemical synthetic additives has prompted the search for green and secure food additives to replace the chemical synthetic ones [1]. Increased attention has been paid to the development of probiotic foods because they could prevent diseases. Probiotics are different kinds of living bacteria that have beneficial health effects on humans [2,3]. At present, probiotics are not only used as antibiotic substitutes to prevent or heal several diseases [4] but also applied as additives in food products to inhibit the growth of pathogenic microorganisms [5,6]. Most bacteria considered as probiotics belong to lactic acid bacteria (LAB), such as *Bifidobacterium* and *Lactiplantibacillus* [7]. LAB could produce many kinds of active metabolites, and they are widely used in healthcare, food fermentation, and industrial additives [8–11]. Dairy products are the most convenient and suitable carriers for probiotics to provide consumers with health benefits [12]. However, high sugar and cholesterol and lactose intolerance in dairy products are the major bottlenecks that restrict the development of dairy-derived probiotic products. Screening of probiotic LAB strains from plant-derived foods is a potential method to overcome these

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drawbacks [13]. Therefore, new probiotics are worth isolating from the relatively undeveloped niche.

Pickled leaf mustard (PLM) is a classic representative of traditional fermented vegetable food in South China, and it has a huge number of consumers because of its unique flavor, crisp taste, rich nutrition, and healthcare functions. Pickles are a natural storehouse of LAB, such as *Lactiplantibacillus* spp., *Leuconostoc* spp., and *Weissella* spp., which are all potential probiotic microbes [14–16]. For instance, *W. Ciabia* JW15 strain isolated from kimchi exhibited probiotic, immune stimulation, and antioxidant activities [17]. However, studies on the isolation of LAB as potential probiotics from a local PLM in Wuwei city of Anhui province have not been reported yet.

Probiotics are specific bacterial strains that promote health functions. However, the bacterial strains used as probiotics must be tested for their safety before formal application, and their probiotic functions should be evaluated subsequently. Safety assays include fast and precise identification, antibiotic sensitivity, and nonpathogenicity [18,19]. Detection of functions includes intestinal adhesion and resistance to gastrointestinal environment [20]. Moreover, prebiotic evaluation includes antibacterial activity, cholesterol assimilation, and inhibition of α-amylase activity [21]. The resistance of gastrointestinal environment and intestinal adhesion capacity are considered the most critical prerequisites for probiotic LAB [22]. Oxidative stress is one of the main risk factors for diseases. Excessive production of reactive oxygen species could cause chronic diseases, such as nervous system or cardiovascular diseases and rheumatoid arthritis [23]. Therefore, the potential probiotic activities of LAB are important indicators of their application in food products.

Taken together, further work should be performed to obtain and identify new LAB for their application in food products. Therefore, this study was aimed to isolate and evaluate the potential probiotic activities of LAB strains derived from Wuwei city's local PLM in Anhui province. Moreover, further tests, including antibacterial activity, adhesion and antioxidant capacities, cholesterol assimilation, and α-amylase inhibition, were carried out. This study provided new information on how to obtain more beneficial LAB strains, which could be further used in the food industry as additives.

2 Materials and methods

2.1 Isolation of LAB and growth condition

PLM was selected from Wucheng town, Wuwei city (E 117°28′, N 30°56′), Anhui province, China. The samples were mixed under sterile condition and diluted to 10^{-8}–10^{-9}. One hundred microliters of solution (10^{-9}) were plated on De-man, Rogosa, and Sharpe (MRS) agar medium (Qingdao Hope Bio-technology Co., Ltd.; containing 1% $CaCO_3$) and incubated at 37°C for 48 h in anaerobic environment. The criteria used were according to the reported methods [22,23]. The morphological characteristics of the colonies and Gram staining were observed, and catalases and biochemical reactions were evaluated using a commercial kit (Qingdao Hope Bio-Technology Co., Ltd). Single white colonies that were Gram-positive and had calcium dissolving circle were selected and purified by streaking on MRS agar more than three times. The purified strains were preserved in MRS broth with 20% (v/v) glycerol and kept at −80°C. The strains were activated in MRS broth at 37°C for 48 h before use. One model strain *Lactiplantibacillus rhamnosus* GG ATCC53103 (LGG) was used as the control.

2.2 Sequencing of 16S rRNA gene

The genomic DNA of AWP4 was extracted using a bacterial DNA kit (Tiangen Biotech Co., Ltd.) in accordance with the manufacturer's instructions. Primers 27f (5′-AGA GTTGATCCTGGCTCAG-3′) and 1492r (5′-GGTATCCTTGTTA CTACTT-3′) were used [24]. Fifty microliters of mixed solution (1.0 μL Taq DNA polymer, 5.0 μL 10× PCR buffer containing 2.5 mM Mg^{2+}, 1.0 μL 10 mM dNTPs, and 39.0 μL ddH_2O, 1.0 μL DNA template, and 1.5 μL primer) was used. The PCR parameters were set as follows: initial denaturation at 95°C for 5 min, followed by 35 cycles at 95°C for 30 s, at 58°C for 30 s, and at 72°C for 90 s, and a final elongation at 72°C for 7 min. The PCR amplification fragments were visualized using 1% agarose. The PCR products were sequenced by Shanghai Personal Gene Technology Co., Ltd. The 16S rRNA gene sequence was compared and matched with the sequences in NCBI GenBank. The sequenced genes were analyzed, and a phylogenetic tree was constructed using MEGA 6.0 with 1,000 bootstrap replications.

2.3 Hemolysis test, antibiotic susceptibility, and cytotoxic activity assays

Hemolysis test was performed as previously described [25]. The LAB strains were incubated on blood agar plate with 5% (v/v) sterilized defibrinated sheep blood for 48 h at 37°C. The strains displaying β-hemolysis were considered as having no probiotic property [26]. All LAB strains

that displayed negative results were subjected to subsequent assays. Antibiotic susceptibility was assessed using disc agar diffusion method [27] with minor modifications. Approximately 100 μL of cells of LAB (10^7 CFU mL^{-1}) were plated on MRS agar plate. The discs containing antibiotics were placed on the surface of the medium, and the plates were incubated at 37°C for 24–48 h. The concentrations of antibiotics were set as follows: 5 μg mL^{-1} for metronidazole, rifampicin, and ciprofloxacin; 10 μg mL^{-1} for streptomycin, ampicillin, and gentamicin; 15 μg mL^{-1} for erythromycin; and 30 μg mL^{-1} for chloramphenicol, tetracycline, doxycycline, kanamycin, and vancomycin. The inhibition diameter of the circle was measured using the method reported by Clinical and Laboratory Standards Institute [28]. The cytotoxic activity of LAB strains on HT-29 was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [29] with few modifications. In brief, the HT-29 cells were transferred into a 24-well plate (1×10^4 cells per well) in Dulbecco's modified Eagle: F12 medium (DMEM/F12; Gibco, USA) with 10% fetal bovine serum and maintained under a humidified atmosphere of 5% CO_2 at 37°C for 24 h. Subsequently, the cells were washed with phosphate buffer saline (PBS) solution three times and then treated with 200 mL of MTT solution (0.5 mg mL^{-1}) for 3.5 h. Cell viability was measured using the following formula: cell viability rate (%) = $A_t/A_c \times 100$, where A_c is the absorbance of the control reaction at 540 nm and A_t is the absorbance in the presence of bacterial supernatant at 540 nm.

2.4 Evaluation of probiotic properties

2.4.1 Resistance to artificially simulated gastrointestinal juices

The resistance to simulated gastrointestinal juices was evaluated with few modifications [30,31]. In brief, the LAB strains were incubated in MRS broth supplemented with 0.1% (w/v) ascorbic acid at 37°C for 48 h. The pellets were washed twice with PBS (pH 7.4). A total of 4 mL of artificially simulated gastric juice (125 mM NaCl, 7 mM KCl, 45 mM $NaHCO_3$, and 3 g L^{-1} pepsin; pH 2.0; Shanghai Lanji Technology Development Co., Ltd.) were used. The bacterial cells (10^8 CFU mL^{-1}) were kept in a water bath at 37°C for 3 h. The cells were obtained by centrifugation at 10,000×*g* for 10 min, and the pellets were washed twice with PBS. Four milliliters of artificially simulated intestinal juice (22 mM NaCl, 3.2 mM KCl, 7.6 mM $NaHCO_3$, 1 g L^{-1} pancreatin [Shanghai Lanji Technology Development Co., Ltd.], and 3 g L^{-1} bovine bile salts [pH 8.0; Beijing Solarbio Science and Technology Co., Ltd]) were used to suspend the cells in a water bath at 37°C for 5 h. A concentration (10^{-9}) of cells was plated on the MRS agar medium. The number of viable bacteria was observed by colony counting.

2.4.2 Antibacterial activity assay

Fresh LAB bacterial strains were inoculated in MRS broth at 2% inoculation amount at 37°C for 48 h. The fermentation liquid was collected with centrifugation at 4°C and 8,000 rpm for 10 min, filtrated using a 0.22 μm filter membrane, and stored at 4°C for further use. The inhibitory effects of the LAB strains on the indicator pathogens were investigated using the disc diffusion method [24] with few modifications. Four pathogenic strains (ATCC8099: *Escherichia coli*, ATCC6538: *Staphylococcus aureus*, ATCC9120: *Salmonella enteric*, and BNCC192105: *Shigella sonnei*) were used as indicators and preserved by the Institute of Microbiology, Anhui Academy of Medical Sciences, China. Approximately 100 μL of indicator pathogenic strains (10^7 CFU mL^{-1}) was plated on Luria–Bertani agar plates. The inhibition circle was measured to assess the antibacterial activity of the LAB strains.

2.4.3 Inhibition of intestinal adherence on pathogenic bacteria

The inhibition of intestinal adherence on pathogenic bacteria was estimated as previously described [22] with slight modifications. The HT-29 cells (Hunan Fenghui Biotechnology Co., Ltd.) were added to the sterilized cell culture bottle and maintained in DMEM/F12 supplemented with 10% (v/v) fetal bovine serum (Zhengjiang Tianhang Biotechnology Co., Ltd.) under a humidified atmosphere of 5% CO_2 at 37°C. The HT-29 cells were harvested after being incubated to 80–90% confluences. Subsequently, the cells were added into the 24-well plate (2×10^6 cells per well) and maintained in DMEM/F12 with 10% fetal bovine serum for 48 h under the condition of 5% CO_2 at 37°C. The medium was refreshed every day. Equal volumes of LAB (1×10^8 CFU mL^{-1}) and pathogenic bacteria (1×10^8 CFU mL^{-1}) were added into the wells and incubated at 37°C for 2 h. The following formula was used: anti-adhesion rate (%) = $(1 - N_1/N_0) \times 100$, where N_1 is the number of pathogen bacteria cells adhered and N_0 is the number of pathogen bacteria cells added.

2.5 Adhesion capacity test

2.5.1 Determination of cell-surface hydrophobicity

Cell-surface hydrophobicity was determined using the protocol described by Mohanty et al. [32] with minor modifications. The culture of the LAB was centrifuged at 10,000 rpm for 10 min, the supernatant was removed, and the cell precipitate was washed twice with phosphate urea magnesium buffer. The LAB suspension was adjusted to 0.7 at 600 nm. One-third volume of *n*-hexadecane was added into the LAB suspension and incubated for 1 h at 37°C. The aqueous phase was carefully separated to measure its absorbance value (600 nm). The hydrophobicity was calculated as follows: hydrophobicity ratio (%) = $100 \times (A_0 - A_1)/A_0$, where A_0 is the initial OD_{600} value and A_1 is the final OD_{600} value.

2.5.2 Auto-aggregation and co-aggregation assays

Auto-aggregation was evaluated with few modifications [33]. The LAB strains were cultivated overnight at 37°C on anaerobic conditions and then the cells were centrifuged at 12,000×*g* for 10 min at 4°C. The cell pellets were washed three times with PBS and suspended to a concentration of 10^8 CFU mL^{-1}. The incubation condition was 37°C for 4 h. The absorbance value at 600 nm was determined as follows: auto-aggregation ratio (%) = $[1 - (A_1 - A_0)] \times 100$, where A_1 is the OD_{600} value after 4 h of culture and A_0 is the OD_{600} value at 0 h. Co-aggregation assay was performed following the method described previously by Niu et al. [34] with minor modifications. A volume of 1.5 mL suspension of LAB (10^8 CFU mL^{-1} in PBS) was added to 1.5 mL suspension of pathogenic bacteria (10^8 CFU mL^{-1} in PBS). The mixture was incubated at 37°C for 4 h after vortexing for 10 s. The absorbance value at 600 nm was determined. Three milliliters of LAB suspension in PBS and 3 mL suspension of pathogenic bacteria in PBS were used as the control. The following formula was used: Co-aggregation ratio (%) = $[1 - A_{mix}/(A_{probiotic} + A_{pathogen})/2] \times 100$, where A_{mix}, $A_{probiotic}$, and $A_{pathogen}$ represent the OD_{600} values of the mixture (probiotic + pathogen), probiotic, and pathogen, respectively.

2.5.3 Mucin binding test

The procedure used for mucin binding assay was as described previously [35]. One hundred microliters of 10 mg mL^{-1} pig mucin solution prepared using PBS (pH = 7.2) were added to the microtitration holes in the 96-well plate (Corning, USA) and incubated overnight at 4°C. The holes were washed twice with PBS and saturated for 4 h at 4°C with 200 mL of 2% (w/v) bovine serum protein (Beijing Solarbio Science and Technology Co., Ltd). The cell cultures were set to a final concentration of 1×10^8 CFU mL^{-1}. The aliquots of the 100 mL suspension above was added into each well and incubated for 1 h at 37°C. The holes were washed 12 times with 1 mL of PBS solution and then 200 mL of 0.5% (v/v) Triton X-100 solution was added into the holes and incubated at 25°C for 2 h with regular vibration to release the adhered bacteria. The suspension of appropriate concentration was coated on MRS plates and incubated at 37°C for 24 h under anaerobic conditions. PBS was used as the negative control. The mucin binding rate was calculated using the following formula: binding ratio (%) = $N_1/N_0 \times 100$, where N_0 is the initial cell number of LAB cells added to the holes and N_1 is the number of attached LAB cells.

2.6 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and (2,2-azinodi-3-ethylbenzthiazoline sulfonate) ABTS$^+$ radical and hydroxyl scavenging activities

The DPPH radical scavenging activity was assessed in accordance with the reported method [17] with few modifications. A volume of 0.2 mL of LAB cell suspension (1×10^9 CFU mL^{-1}) or PBS solution was added into 1 mL of DPPH solution in methanol (100 mM), vortexed for 30 s, and incubated in the dark at 37°C for 20 min, followed by centrifugation at 8,000×*g* for 5 min. The absorbance value at 517 nm was determined. The PBS solution was used as the blank control. The DPPH radical scavenging activity was calculated as follows: DPPH radical scavenging rate (%) = $(1 - A_{sample}/A_{control}) \times 100$, where A_{sample} and $A_{control}$ represent the OD_{517} values of the sample and control, respectively. In addition, the ABTS cation radical scavenging activity was evaluated in accordance with the described protocols [30] with few modifications. In brief, 7 mM of ABTS$^+$ solution was prepared with 2.45 mM potassium persulfate solution. After incubation was performed in the dark at room temperature for 12 h, the absorbance value of the ABTS$^+$ solution at 734 nm was adjusted to 0.7, with deionized water as the ABTS$^+$ working solution. A volume of 0.1 mL of LAB suspension (1×10^9 CFU mL^{-1}) or PBS solution was added into 1 mL of ABTS$^+$ solution and incubated in the dark at 37°C

for 20 min. The cells were collected via centrifugation at 8,000×*g* for 5 min. The absorbance value at OD_{734} nm was determined. The PBS solution was used as the control. The $ABTS^+$ radical scavenging activity was calculated using the following formula: $ABTS^+$ radical scavenging rate (%) = $(1 - A_{sample}/A_{control}) \times 100$, where A_{sample} and $A_{control}$ represent the OD_{734} values of the sample and control, respectively. The hydroxyl radical scavenging activity was determined in accordance with the previous method [36] with few modifications. A total of 2 mL of 5 mM ferrous sulfate solution was mixed with 2 mL of 5 mM salicylic acid ethanol solution and then 2 mL of 3 mM hydrogen peroxide solution was added into the mixture for reaction, followed by the addition of 2 mL of LAB suspension (10^9 CFU mL^{-1}) or PBS solution, incubation in a water bath at 37°C for 20 min, and centrifugation at 8,000×*g* for 5 min. The absorbance value at 510 nm was determined. The PBS solution was used as the blank control. In reference to the scavenging activities of Trolox on DPPH radical, $ABTS^+$ radical, and hydroxyl, the results were reported in Trolox equivalent. The hydroxyl radical scavenging activity was calculated as follows: hydroxyl radical scavenging rate (%) = $(1 - A_{sample}/A_{control}) \times 100$, where A_{sample} and $A_{control}$ represent the OD_{510} values of the sample and control, respectively.

2.7 Cholesterol assimilation assay and α-amylase inhibition activity

Cholesterol assimilation was determined using the previous method [37] with few modifications. In brief, the LAB strains were inoculated in MRS broth medium at 2%, with an OD_{600} nm of 0.5. Then 150 μg mL^{-1} of water-soluble cholesterol (Beijing Solarbio Science and Technology Co., Ltd.) and 0.3% bile (w/v) were added into the culture and cultivated overnight at 37°C. Then the residual cholesterol in the cultivation solution was determined. The absorbance value was determined at 540 nm. The PBS solution was used as the control. The cholesterol assimilation rate was calculated as follows: cholesterol assimilation rate (%) = $(1 - A_{sample}/A_{control}) \times 100$, where A_{sample} and $A_{control}$ are the OD_{540} values of the sample and control, respectively. The inhibition of α-amylase activity was determined following the protocol [38] with few modifications. Two milliliters of α-amylase solution (2.0 mg mL^{-1}) were prepared with PBS buffer solution (pH 7.0), and 2 mL of LAB cells was added to the α-amylase solution and reacted at 37°C for 30 min. The absorbance value at 660 nm was determined. MRS broth and PBS were used as blank controls. The α-amylase inhibition ratio was calculated as follows:

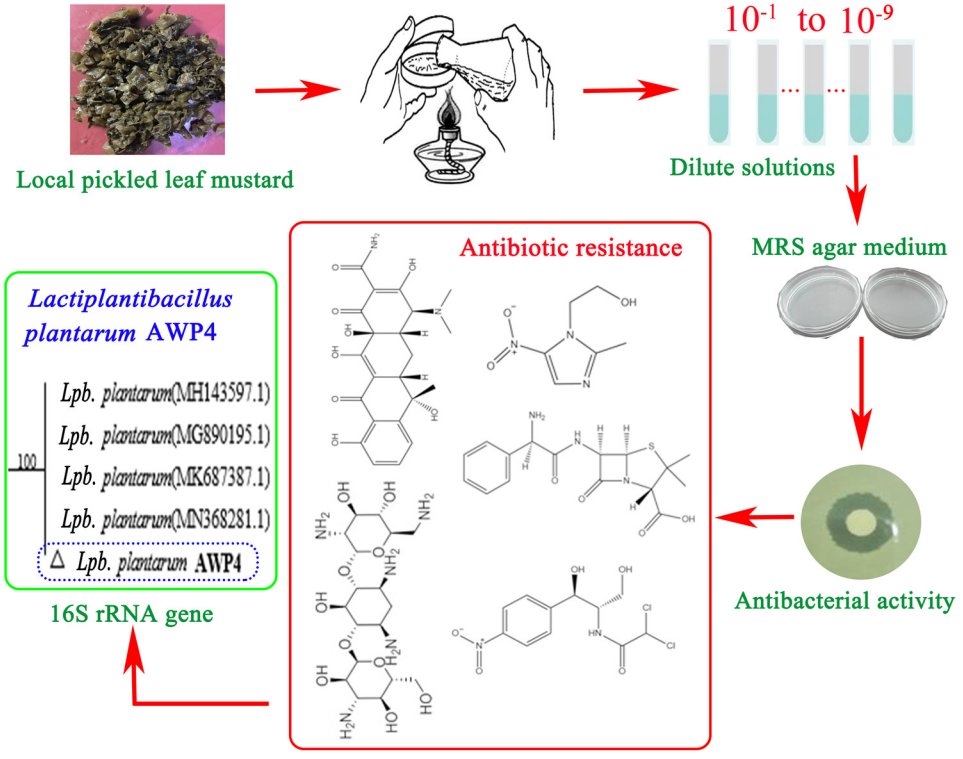


Figure 1: Schematic of LAB screening processes.

α-amylase activity inhibition ratio (%) = $(A_1 - A_2)/A_3 \times 100$, where A_1 is the OD_{660} value of cell cultures, A_2 is the OD_{660} value of MRS broth medium, and A_3 is the OD_{660} value of PBS solution.

2.8 Statistical analysis

Experiments were carried in triplicate, and all data were calculated as mean values and standard deviations. Data analysis was performed on SPSS version 23.0 (IBM, USA). The difference was estimated by one-way ANOVA with Duncan's multiple range test, and statistical significances were calculated at $P < 0.05$ level.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results

3.1 Physiological and biochemical characteristics of isolates

A total of 24 isolates were obtained after the preliminary physiological and biochemical experiments. A schematic of the LAB screening processes is shown in Figure 1. The morphological and phenotypic characteristics of the isolated colonies showed the characteristics of smooth surface, round, and porcelain white. The results revealed that all the 24 isolates were Gram-positive, long rod-shaped, or rod-shaped. Combined with the physiological and biochemical identification of standard *Lactiplantibacillus*, 17 out of 24 isolates were putatively identified as LAB (Table 1).

Table 1: Physiological and biochemical identification results of 17 LAB strains

Tested item	Strain numbers[a]	Strain AWP4
Glucose gas production	–	–
Gelatin	5	–
Catalase	–	–
6.5% Nacl	8	–
18% Nacl	3	–
15°C growth test	+	+
45°C growth test	+	+
Arabinose	+	+
Cellobiose	11	+
Esculin	13	+
Fructose	10	+
Gluconate	13	+
Lactose	9	+
Mannose	8	+
Mannitol	11	+
Sorbitol	10	+
Melezitose	+	+
Melibiose	9	+
Raffinose	7	+
Rhamnose	5	–
Salicin	7	+
Glucose	14	+
Sucrose	10	+
Trehalose	9	+
Xylose	–	–
Inulin	6	+
Maltose	11	+

16 isolates were listed (AWP1, AWP3, AWP5, AWP6, AWP7, AWP8, AWP9, AWP10, AWP11, AWP13, AWP16, AWP17, AWP18, AWP20, AWP21, and AWP22).
+: positive or weakly positive reaction, –: negative reaction.
Number: the number of positive reaction.
[a] Number of positive strain in this item.

3.2 Safety evaluation of LAB strains

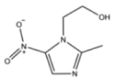
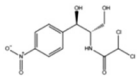
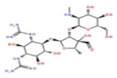
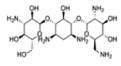
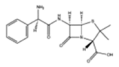
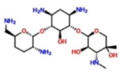
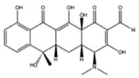
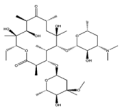
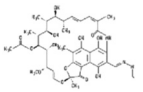
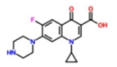
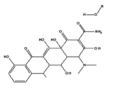
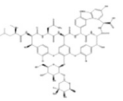
The results of 17 LAB strains showed negative hemolysis test, indicating that all the strains had no hemolysis and were safe for further use. All the 17 isolates were sensitive to chloramphenicol, tetracycline, erythromycin, and doxycycline and resistant to metronidazole, streptomycin, kanamycin, gentamicin, vancomycin, and ciprofloxacin. Among them, 12 were sensitive to ampicillin, while the remaining 5 strains were resistant to it. Eleven strains were sensitive to rifampicin, and the remaining six were resistant to it (Table 2). Out of the 17 isolates, 9 (including AWP4, AWP7, AWP8, AWP10, AWP11, AWP13, AWP17, AWP18, and AWP21) were sensitive to most antibiotics, and they were selected for further analysis.

MTT method was used to determine the cytotoxic activities of the nine isolated LAB strains on HT-29 cell line (Figure 2a). The cell viability rates of the nine isolates were still at a high level, ranging from 90.26% to 91.92%, and their cytotoxic activities on HT-29 cells were all less than 10%. Compared with the control strain (*Lactiplantibacillus rhamnosus* GG ATCC53103, LGG), the nine LAB strains had no cytotoxic activities on HT-29 cells.

3.3 Resistance to artificially simulated gastrointestinal juices

Out of the 17 LAB strains, 9 were selected to test their resistance to artificially simulated gastrointestinal juices

Table 2: Antibiotic susceptibility results of isolated LAB strains

Strains	Antibiotics											
	Metronidazole	Chloramphenicol	Streptomycin	Kanamycin	Ampicillin	Gentamicin	Tetracycline	Erythromycin	Rifampicin	Ciprofloxacin	Doxycycline	Vancomycin
												
AWP1	R	S	R	R	S	R	S	S	R	R	S	R
AWP3	R	S	R	R	R	R	S	S	S	R	S	R
AWP4	R	S	R	R	S	R	S	I	S	R	S	R
AWP5	R	S	R	R	S	R	S	S	R	R	S	R
AWP6	R	S	R	R	S	R	S	S	R	R	S	R
AWP7	R	S	R	R	S	R	S	S	S	R	S	R
AWP8	R	S	R	R	S	R	S	S	S	R	S	R
AWP9	R	S	R	R	R	R	S	S	R	R	S	R
AWP10	R	S	R	R	S	R	I	S	S	R	S	R
AWP11	R	I	R	R	S	R	S	S	S	R	S	R
AWP13	R	S	R	R	S	R	S	I	S	R	S	R
AWP16	R	S	R	R	R	R	S	S	R	R	S	R
AWP17	R	S	R	R	S	R	S	S	S	R	S	R
AWP18	R	S	R	R	S	R	S	S	S	R	S	R
AWP20	R	S	R	R	R	R	S	I	R	R	S	R
AWP21	R	S	R	R	S	R	S	S	S	R	S	R
AWP22	R	I	R	R	R	R	S	I	S	R	S	R
LGG	R	S	R	R	R	R	R	R	S	R	S	R

R: resistant to antibiotics, I: intermediately sensitive to antibiotics, S: sensitive to antibiotics.
LGG: *Lactiplantibacillus rhamnosus* GG ATCC53103.

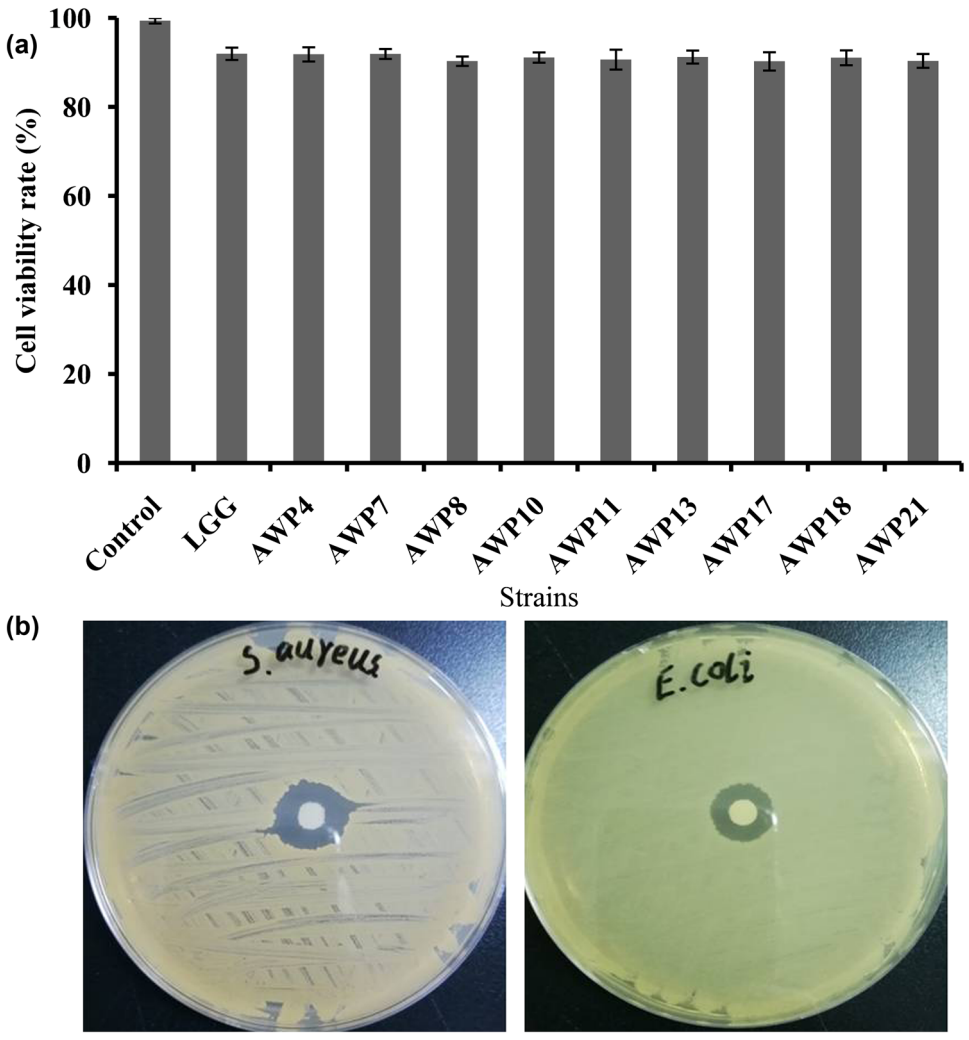


Figure 2: Cell viability of isolated LAB strains (a) and inhibition of indicator strains (b). Error bars represent deviations of mean values (n = 3).

(Table 3). Most of the selected strains exhibited a strong resistance. Among them, seven retained high viability, but the viable counts of AWP8 and AWP18 declined sharply. After treatment with artificially simulated intestinal juice was subsequently conducted for 5 h, six strains displayed high cell survival level, whereas three (AWP8, AWP13, and AWP18) showed a low survival level. The referenced strain LGG showed low tolerance to artificially simulated gastrointestinal juices. Given their high resistance to gastrointestinal juices, AWP4, AWP7, AWP10, AWP11, AWP17, and AWP21 were chosen for testing their antibacterial activities.

3.4 Antibacterial activity of LAB strains

Disc diffusion method was performed to determine the antibacterial activities of the six strains against *E. coli*, *S. aureus*, *S. enterica*, and *S. sonnei* (Table 4). Among them, three exhibited a certain inhibition on the growth of four indicator pathogens, which had a broad spectrum of bacteriostasis. The proliferation of *E. coli* and *S. enterica* could be inhibited by all the six LAB strains. Among them, two strains (AWP4 and AWP17) had the strongest antibacterial effect on *E. coli*, with over 18 mm of inhibition zone in diameter (Figure 2b). AWP4 also displayed the strongest bacteriostatic effect on the growth of *S. enterica*. However, AWP10 and AWP21 showed the weakest antibacterial effect on *E. coli* and *S. enterica*. The growth of *S. aureus* and *S. sonnei* was inhibited by five and three LAB strains, respectively. The growth of *S. aureus* could not be inhibited by AWP21, while the inhibition effect of AWP10, AWP17, and AWP21 on *S. sonnei* was not detected. LGG exhibited excellent antibacterial activities on the four selected indicator pathogens. Three strains, namely, AWP4, AWP7, and AWP11, which all had antibacterial

Table 3: Survival of LAB cells simulated by artificial gastrointestinal juices (log 10 CFU mL^{-1})

Strains	Artificially simulated gastric juice	Artificially simulated intestinal juice
AWP4	7.88 ± 0.30^{d}	7.87 ± 0.27^{c}
AWP7	8.04 ± 0.22^{d}	8.01 ± 0.17^{c}
AWP8	$<4 \pm 0.00^{a}$	$<4 \pm 0.00^{a}$
AWP10	7.92 ± 0.17^{d}	7.90 ± 0.22^{c}
AWP11	8.10 ± 0.14^{d}	8.03 ± 0.13^{c}
AWP13	7.04 ± 0.24^{c}	$<4 \pm 0.00^{a}$
AWP17	7.87 ± 0.25^{d}	7.85 ± 0.28^{c}
AWP18	$<4 \pm 0.00^{a}$	$<4 \pm 0.00^{a}$
AWP21	7.94 ± 0.17^{d}	7.91 ± 0.14^{c}
LGG	6.12 ± 0.15^{b}	4.85 ± 0.21^{b}

Data are presented as mean ± SD in triplicate. a–d: different superscript lowercase letters in the same column indicate significant difference ($P < 0.05$).

activities on the four selected indicator pathogens, were selected for the analysis of the inhibition effect on the intestinal adherence of pathogenic bacteria.

3.5 Inhibition effect on intestinal adherence of pathogenic bacteria

The three LAB strains showed excellent inhibition abilities on intestinal adherence of the four indicator pathogens. The inhibition rate ranged from 46.92 to 63.67% (Table 5). Among them, AWP4 exhibited the highest inhibition rate of 63.67% on *E. coli*, significantly higher than LGG (49.49%). AWP7 showed the lowest inhibition effect on *E. coli*, with an inhibition rate of 51.05%, also higher than LGG. However, the difference was not significant. AWP11 possessed the highest inhibition rate of 60.84% on *S. enterica*, while AWP7 had the lowest at 58.32%. However, no significant difference in the inhibition effect on *S. enterica* was observed among the three LAB strains. The intestinal adherence of *S. aureus* and *S. sonnei* was inhibited by AWP11, with the highest inhibition rates of 56.95 and 53.53%, respectively. These rates were significantly higher than those of LGG. Given that AWP4, AWP7, and AWP11 exhibited greater inhibition effect on the intestinal adherence of pathogenic bacteria, they were selected to evaluate their adhesion activities.

Table 4: Antimicrobial activity of LAB strains isolated from Anhui Wuwei local PLM

Strains	Diameter of inhibit zone (mm)			
	E. coli	*S. aureus*	*S. enterica*	*S. sonnei*
AWP4	19.31 ± 1.09^{c}	14.92 ± 1.24^{b}	19.44 ± 1.22^{c}	11.50 ± 0.85^{a}
AWP7	16.23 ± 1.25^{b}	13.35 ± 0.62^{ab}	14.69 ± 0.81^{b}	18.27 ± 1.52^{c}
AWP10	9.18 ± 0.88^{a}	12.13 ± 0.98^{a}	9.54 ± 0.79^{a}	—
AWP11	15.70 ± 0.90^{b}	22.12 ± 1.82^{d}	15.57 ± 1.04^{b}	19.78 ± 1.58^{c}
AWP17	18.29 ± 1.17^{c}	12.66 ± 1.29^{ab}	14.46 ± 1.27^{b}	—
AWP21	10.58 ± 0.94^{a}	—	9.16 ± 0.37^{a}	—
LGG	21.34 ± 1.23^{d}	17.37 ± 0.87^{c}	19.92 ± 1.41^{c}	15.59 ± 0.85^{b}

Data are presented as mean ± SD in triplicate. a–d: different superscript lowercase letters in the same column indicate significant difference ($P < 0.05$); —: no inhibition zone.

Table 5: Inhibition effect of intestinal adherence on pathogenic bacteria of LAB strains

Strains	Inhibition ratio of intestinal adherence (%)			
	E. coli	*S. aureus*	*S. enterica*	*S. sonnei*
AWP4	63.67 ± 6.31^{b}	47.98 ± 5.73^{ab}	59.87 ± 6.23^{a}	46.92 ± 4.88^{ab}
AWP7	51.05 ± 4.36^{a}	47.26 ± 4.11^{ab}	58.32 ± 5.50^{a}	48.60 ± 5.40^{ab}
AWP11	57.72 ± 7.76^{ab}	56.95 ± 5.82^{b}	60.84 ± 2.35^{a}	53.53 ± 4.33^{b}
LGG	49.49 ± 4.93^{a}	44.60 ± 4.67^{a}	58.17 ± 5.22^{a}	42.48 ± 3.47^{a}

Data are presented as the mean ± SD in triplicate. a and b: different letters in the same column indicate significant difference ($P < 0.05$).

Table 6: Adhesion capacities of LAB strains

Strains	Adhesion capacities (%)		
	Cell surface hydrophobicity	Auto-aggregation	Mucin binding
AWP4	43.84 ± 3.35^{a}	44.56 ± 2.64^{b}	49.70 ± 3.98^{a}
AWP7	54.27 ± 2.99^{b}	48.78 ± 4.06^{b}	63.81 ± 5.95^{b}
AWP11	40.80 ± 3.08^{a}	30.39 ± 5.08^{a}	53.16 ± 4.40^{a}
LGG	51.35 ± 3.54^{b}	34.53 ± 3.87^{a}	65.16 ± 5.18^{b}

Data are presented as mean ± SD in triplicate. a and b: different superscript lowercase letters in the same column indicate significant difference ($P < 0.05$).

3.6 Adhesion capacities of LAB strains

The cell-surface hydrophobicity to *n*-hexadecane and auto-aggregation; the co-aggregation with four pathogens, including *E. coli*, *S. aureus*, *S. enterica*, and *S. sonnei*; and the mucin binding of the three LAB strains were evaluated (Tables 6 and 7). All the three selected LAB strains exhibited a certain cell-surface hydrophobicity. The ratio of cell-surface hydrophobicity ranged from 40.80% to 54.27%. AWP7 displayed the highest hydrophobicity ratio among the three strains, but compared with the control strain LGG (51.35%), no significant difference in the hydrophobicity ratio was observed. AWP11 showed the lowest hydrophobicity ratio. As shown in Table 6, the auto-aggregation ratios exhibited by the three strains ranged from high to low arrangement as follows: 48.78% (AWP7), 44.56% (AWP4), and 30.39% (AWP11). Out of the three LAB strains, AWP7 showed significantly higher auto-aggregation ratio than LGG (34.53%). The co-aggregation of the three LAB strains with the four pathogens was also estimated (Table 7). The co-aggregation ratio of AWP11 with *E. coli* was the highest at 33.13%, while that of AWP4 with *S. sonnei* was the lowest at 1.56%. The mucin binding characteristics of the three LAB strains were determined in the last part of adhesion capabilities measurement. The results are shown in Table 6. All the three LAB

Table 7: Co-aggregation property of LAB strains

Strains	Co-aggregation (%)			
	E. coli	*S. aureus*	*S. enterica*	*S. sonnei*
AWP4	16.46 ± 3.34^{a}	9.36 ± 3.07^{a}	26.95 ± 5.38^{bc}	1.56 ± 0.58^{a}
AWP7	18.51 ± 2.56^{a}	23.84 ± 2.85^{b}	17.27 ± 2.64^{a}	5.91 ± 1.66^{b}
AWP11	33.13 ± 2.53^{b}	31.66 ± 5.71^{c}	23.20 ± 3.69^{ab}	9.77 ± 0.75^{c}
LGG	31.95 ± 3.20^{b}	13.88 ± 2.97^{a}	31.85 ± 3.11^{c}	18.03 ± 3.10^{d}

Data are presented as mean ± SD in triplicate. a–d: different superscript lowercase letters in the same column indicate significant difference ($P < 0.05$).

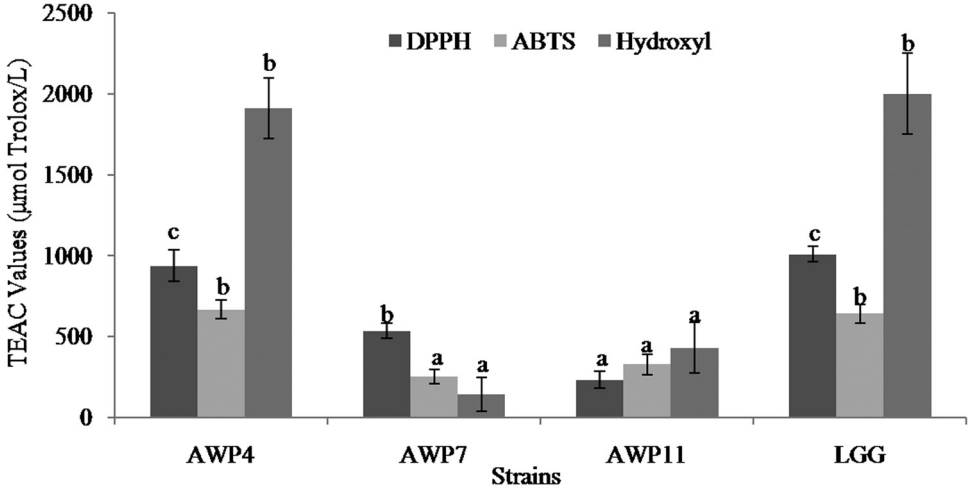


Figure 3: Antioxidant activity of LAB strains. Error bars represent deviations of mean values ($n = 3$). Values followed by different superscript letters indicate significant difference ($P < 0.05$).

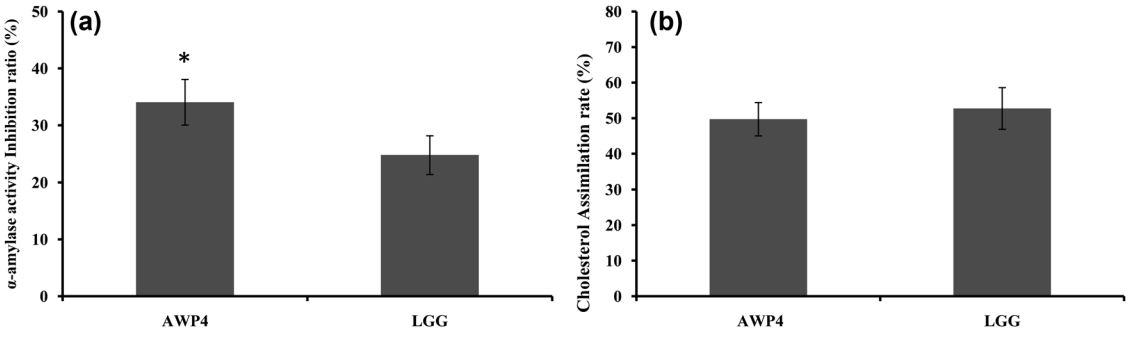


Figure 4: α-Amylase activity inhibition (a) and cholesterol assimilation ability (b) of AWP4. Error bars represent deviations of mean values (n = 3). *Significant difference ($P < 0.05$).

strains were able to bind to mucin within the range of 49.70–63.81%. Their mucin binding ratio was lower than that of LGG (65.16%). AWP7 possessed the highest binding ratio, though not significantly different from that of LGG.

3.7 *In vitro* antioxidant activity of LAB strains

The antioxidant activities of the three LAB strains were measured, including DPPH radial scavenging activity, $ABTS^+$ radial scavenging activity, and hydroxyl radial scavenging activity (Figure 3). The DPPH radial scavenging activities were in the range of 233.15–936.82 μmol L^{-1}. AWP4 exhibited significantly higher DPPH radial scavenging activity than the others, but it was lower than that of the reference strain LGG. AWP4, AWP7, and AWP11 showed $ABTS^+$ radial scavenging activities of 666.92, 252.03, and 327.24 μmol L^{-1}, respectively. The $ABTS^+$ radial scavenging activity of AWP4 was significantly higher than that of AWP7 and AWP11. AWP4 displayed the highest hydroxyl radial scavenging activity of

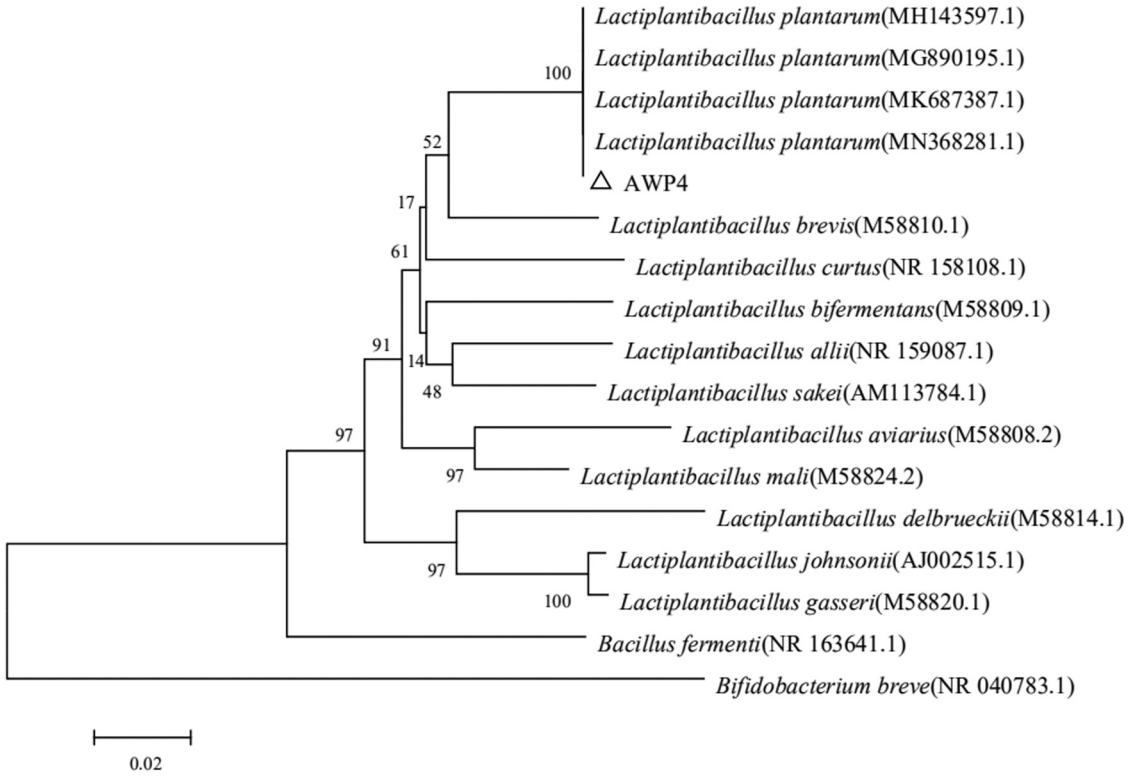


Figure 5: Phylogenetic tree constructed based on 16S rRNA gene sequence of AWP4. Sequenced genes were analyzed, and a phylogenetic tree was constructed using MEGA 6.0 with 1,000 bootstrap replications.

1909.90 μmol L^{-1}, while AWP7 showed the lowest hydroxyl radial scavenging activity of 140.97 μmol L^{-1}. AWP4 exhibited significantly better antioxidant activities, including DPPH radial scavenging activity, $ABTS^{+}$ radial scavenging activity, and hydroxyl radial scavenging activity, than the two other strains. Given its excellent antioxidant activity, AWP4 was further selected for 16S rRNA gene identification.

3.8 Sequencing of 16S rRNA gene, cholesterol assimilation ability, and α-amylase activity

The inhibition ratio on the α-amylase activity of AWP4 was 34.05% (Figure 4a), which was significantly higher than that of LGG (24.78%). The cholesterol assimilation ability of AWP4 was also determined. The cholesterol assimilation rates of AWP4 and LGG were 49.72 and 52.74% (Figure 4b), respectively. The cholesterol assimilation rate of LGG was slightly higher than that of AWP4, but no significant difference was observed. AWP4 was identified as *Lpb. plantarum* (Figure 5). The 16S rRNA gene sequence of AWP4 was deposited to NCBI (accession number: MN 759440).

4 Discussion

In this study, 17 isolates were obtained and identified as *Lpb. plantarum* by physiological and biochemical experiments *in vitro* from Wuwei city's local PLM in Anhui province. All the strains were safe for further use because no hemolysis ability was detected. The safety of LAB strains should be considered in the screening of probiotics, including identity and non-harmfulness activities. A previous study has illustrated that most of the LAB strains isolated from food showed γ-hemolysis [39]. The potential resistance gene to antibiotic is well-known to be genetically transferable [40]. Antibiotic resistance is an important issue to be considered in the process of isolating potential probiotics for safe application. All strains displayed multidrug resistance, which was considered to be the intrinsic, dependent, and endogenous resistance of LAB. Most LAB isolates were sensitive to erythromycin, but they were resistant to vancomycin due to their dependent and endogenous resistance to this drug [41]. The inherent resistance of *Lpb. rhamnosus*, *Lpb. casei*, *Lpb. salivarius*, and *Lpb. plantarum* to vancomycin was also reported [42]. The resistance to aminoglycoside antibiotics, such as streptomycin, kanamycin and gentamicin, is regarded to be an inherent characteristic of *Lactiplantibacillus*, as the absorption of antibiotic by lactobacilli is prevented by the absence of cytochrome-mediated electron transfer, leading to the resistance of LAB [30]. Among the 17 isolates in the present study, 9 exhibited sensitivity to most antibiotics. The results were in agreement with those of studies on the antibiotic sensitivity of LAB strains [30,34]. The development or selection of new beneficial microbes and their absence of cytotoxicity should be assessed. Although some LAB strains have been previously used for their technological properties or as probiotics for humans and animals, a case-by-case-evaluation is still needed [43]. Colorimetric analysis is based on the ability of viable cells to reduce a soluble yellow tetrazolium salt (MTT) to blue formazan crystals. In the present study, nine strains showed absence of cytotoxic activity on HT-29 cells, comparable with the cytotoxic activities as previously reported by Baccouri et al. [44] and Talebi et al. [29].

The adaptation of LAB strains to the environment with low pH and heavy bile salt concentration in the gastrointestinal tract and further survival are the preconditions for LAB [45]. At present, probiotics have been widely used in food and health industry, and their resistance to gastric acid and intestinal bile salt is very important for their application in this industry [46]. *Lpb. plantarum* Ln4 and G72 demonstrated obvious resistance to low pH environment after 24 h of incubation under the condition of artificial gastric juice [14]. The *W. cibaria* strain D29 displayed the highest resistance to artificial gastric juice and bile salt, with survival rates of 79.37 and 91.64% [47], respectively. Moreover, 18 strains survived after treatment with artificial gastrointestinal juices, and no significant difference in the number of viable cells was observed [30]. The results of the present study illustrated that six LAB strains showed enhanced resistance to artificially simulated gastrointestinal juices, similar to the conclusions on the tolerance to artificially simulated gastrointestinal juices of LAB strains as above. This finding may be attributed to the organic acid produced by LAB isolated from PLM during the fermentation process, and this organic acid could reduce the pH value of the growth environment. PLM is stored under a closed and cold environment to make the organic acid not easy to volatilize and maintain a stable pH value. The LAB isolates obtained from low pH condition in this study indicated that they could adapt to low pH condition and survive under extreme circumstances.

Antibacterial activity is an important selection criterion because of its prevention from potentially harmful

intestinal microorganisms to guard against their colonization on the host intestinal mucosa [48,49]. Previous studies have confirmed that LAB have a wide spectrum of inhibition against pathogenic microbes, such as *Salmonella*, *E. coli*, *Listeria*, and *Streptococcus* [50]. The LAB from kimchi had a strong inhibitory effect on representative food-borne pathogens, such as *E. coli*, *S. aureus*, and *S. enteric* [47]. Moreover, *Lpb. plantarum* YS5, which was isolated from yogurt, exhibited excellent antibacterial ability on *E. coli*, *S. aureus*, and *S. flexneri* [37]. The present study showed that out of the six LAB strains, three possessed antibacterial activity against four indicator pathogens. Probiotics have been considered to exhibit their antibacterial activity by colonizing in the gastrointestinal tract and producing antibacterial substances that could inhibit pathogens [51]. These antimicrobial substances belong to different metabolites produced by LAB strains, including organic acids, hydrogen peroxide, and bacteriocins [52].

One of the key steps to determine the pathogenicity of intestinal pathogens is the ability to attach to the surface of intestinal epithelial cells through their fimbriae or pili, which grows on the surface of the pathogenic bacterial cells [53]. By co-culture with LAB, the colonization of intestinal pathogens on the surface of the intestinal epithelial cells could be reduced to prevent invasion of pathogens to the host. *Lpb. plantarum* DM69 displayed excellent ability to inhibit the intestinal adherence of pathogenic bacteria, and its inhibition rate on *S. enterica* was as high as 75% [32]. In addition, three probiotics, namely, *Lactococcus lactis* KC24, *L. reuteri*, and *L. rhamnosus* 3698, had the ability to effectively inhibit the intestinal adherence of *Campylobacter jejuni* and *Helicobacter pylori* [54]. Liu et al. [55] have reported that six LAB isolates strongly inhibited the intestinal adhesion of *E. coli*, and CCFM 233, with the highest adhesion ability, had the best inhibition rate on this intestinal adhesion. The results of the present study were basically consistent with the above conclusions about the inhibition of LAB on the intestinal adherence of pathogenic bacteria. Competitive rejection of pathogen adhesion to host cells by LAB has been considered as one of the important factors for LAB to inhibit pathogens [56]. The results suggested that AWP4, AWP7, and AWP11 possessed excellent intestinal adherence inhibition on the four indicator pathogens. Among them, AWP4 exhibited the highest intestinal adherence inhibition and the strongest antimicrobial effect on *E. coli*. Besides, AWP11 had not only the highest intestinal adherence inhibition but also the strongest antimicrobial ability on *S. aureus* and *S. enterica*, indicating that the antibacterial ability of this LAB strain basically had a positive correlation with the inhibition of intestinal adherence on the same pathogen in this work. However, further research should be employed to deeply explore the relationship and the underlying mechanism between the antibacterial activity and inhibition of intestinal adherence of LAB isolates.

The adhesion and colonization ability of LAB to the human intestinal wall is an important characteristic on the screening of probiotic strains, as the adhesion ability could contribute to prolonging the residence time of probiotic strains in the intestinal tract, which could enable them to play their own probiotic functions [57]. The adhesion capacity of microorganisms is a complex process of physical and chemical interaction between the surface of the microbe and the intestinal mucosa, including electrostatic interaction and cell-surface hydrophobicity [58,59]. Low co-aggregation of probiotics with pathogens may play an important role in preventing biofilm formation and reducing pathogen colonization. For instance, the *Siamensis* strain B44v derived from Thai pickled vegetables showed high auto-aggregation and hydrophobicity [60]. The mucin binding rate of B44v reached 88.7%. The cell-surface hydrophobicity to *n*-hexadecane and xylene of LAB strains, including PUFSTP 35, PUFSTP 38, PUFSTP 44, and PUFSTP 74, isolated from the traditional brine pickle was high [61]; the hydrophobicity to *n*-hexadecane of LAB originating from traditional pickles was the strongest at 97.96, 82.41, 67.29, and 62.36% [62], respectively. Moreover, the hydrophobicity difference was significant at the species level. All the three selected LAB strains in the present study displayed different degrees of hydrophobicity (40.80–54.27%), auto-aggregation (30.39–48.78%), co-aggregation (1.56–33.13%), and mucin binding ability (49.70–63.81%). The binding to host intestinal mucin is helpful for the colonization of LAB in the intestine [63]. The different degrees of interaction between mucin and the glycochain molecule was the main reason for the different mucin binding abilities of LAB [64].

The oxygen radicals produced in the body damage the biological macromolecules, and probiotics with antioxidant activity could protect the host from peroxidation by neutralizing the radicals [29]. It also contributes to the prevention of cardiovascular disease, diabetes, and gastrointestinal ulcer [65]. Determining the DPPH radical scavenging ability is one of the important methods to investigate the antioxidant activity of probiotics. $ABTS^{+}$ belongs to a kind of colorless radical that could dissolve in organic or water phase and react with an antioxidant [66]. Hydroxyl radical, with the strongest chemical activity among the active oxygen radicals, could cause peroxide damage on proteins, lipids, DNA, and other

macromolecules. Therefore, hydroxyl radical scavenging may be one of the most effective measures to resist the invasion of various diseases [67]. Yang et al. [68] determined the scavenging ability of strain JR14 isolated from Chinese traditional fermented pickles to have a hydroxyl radical of 1535.18 μmol L^{-1} in terms of Trolox equivalents. The results of other research studies presented that PUFSTP71, PUFSTP 39, and PUFSTP81 exhibited high DPPH scavenging ability (517.55 μmol L^{-1}), excellent $ABTS^{+}$ radical scavenging ability (655.54 μmol L^{-1}), and good hydroxyl radical scavenging ability (1689.00 μmol L^{-1}) [66], respectively. The DPPH radical scavenging rate of *Lpb. plantarum* Ln4 screened from kimchi reached 662.39 μmol L^{-1} [14]. The scavenging ability to hydroxyl and DPPH radical of *Lpb. plantarum* DM5 was 1800.44 and 912.03 μmol L^{-1} in terms of Trolox equivalents [69], respectively. The present study showed that AWP4 also possessed antioxidant activity similar to the above results. AWP4 displayed better antioxidant activity than the other two strains, which may be attributed to its excellent protection mechanism from the oxidative damage of hydroxyl radicals and other ROS [69]. Probiotic fermentation is also a good antioxidant method compared with scavenging hydroxyl radical by chelating metal ions with some antioxidants [70].

Combined with the colony morphological characteristics and phylogenetic analysis of 16S rRNA gene, AWP4 was identified as *Lpb. plantarum*. Diabetes mellitus is a kind of metabolic disease characterized by hyperglycemia, which has already become a worldwide medical problem. If not properly managed, it could lead to chronic damage and dysfunction of various tissues, especially eyes, kidney, heart, blood vessels, and nerves. Using α-amylase inhibitor as a hypoglycemic agent to slow the digestion and absorption of starch and reduce postprandial hyperglycemia could treat diabetes mellitus [71]. However, various side effects, such as diarrhea, abdominal distention, and flatulence, have been reported with the use of synthetase inhibitors [72]. Screening safe and healthy enzyme inhibitors without side effects or with fewer negative effects has become a new direction of research on enzyme inhibitors. Therefore, the inhibition of α-amylase activity is one of the important indices to assess the potential probiotic activity. In the present study, the α-amylase activity inhibition ratio of AWP4 was significantly higher than that of LGG.

The cholesterol assimilation ability of LAB is an important parameter in screening probiotic strains with healthcare functions [50]. *Lpb. plantarum* strain YS5 showed a strong effect on lowering sera cholesterol level with high cholesterol scavenging capacity [37]. *Lpb. plantarum* FB003 possessed the highest cholesterol clearance rate of 43.1% [50]. Sharma et al. [63] have reported that the cholesterol clearance rate of *Lpb. plantarum* K90 was up to 75%. In the present study, AWP4 also displayed better cholesterol assimilation ability than that in previous studies. The cholesterol scavenging ability of probiotics may be due to the assimilation and binding of cholesterol on the surface of probiotic cells, eventually resulting in the coprecipitation of cholesterol [73]. In addition, the combination of cholesterol with the cell wall of probiotics or the physiological function of the end products of short chain fatty acid fermentation was related to the cholesterol assimilation ability of probiotics [74]. Lambert et al. [75] have proposed that the cholesterol assimilation ability of probiotics was related to the bile salt hydrolytic enzyme (BSH) of probiotics, especially the interference of intestinal absorption micelle formation and BSH activity.

5 Conclusion

This study was the first to isolate potential probiotic candidates from Wuwei city's local PLM in Anhui province and further evaluate their potential probiotic activities as substitutes for chemical synthetic additives. A total of 17 strains were obtained from PLM and tested for safety, good resistance to artificial simulated gastrointestinal juices, antibacterial activity on common gastrointestinal pathogens, inhibition of intestinal adherence of pathogen bacteria, and adhesion capacities. AWP4 (*Lpb. plantarum*) exhibited remarkable antioxidant activity. It also possessed good probiotic and healthcare functions, including inhibition of α-amylase activity and cholesterol assimilation ability. The data illustrated that AWP4 is a satisfactory probiotic candidate, and it could be used not only in food fermentation but also in industrial additives. However, further animal experiments should be carried out to evaluate the probiotic effect and safety of AWP4 *in vivo*.

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Conflict of interest: All authors declare no competing interests.

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