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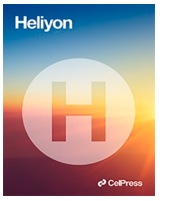

Research article

Isolation and evaluation of *Pediococcus acidilactici* YH-15 from cat milk: Potential probiotic effects and antimicrobial properties

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ABSTRACT

The study aimed to screen for the possible presence of lactic acid bacteria (LAB) in cat milk in order to evaluate their probiotic properties. The isolates were characterized by biochemical identification, morphological tests and 16S rDNA sequencing. Afterward, gastrointestinal passage, *in vitro* safety and probiotic properties were evaluated. The results showed that the isolates had 10 strains of *Pediococcus acidilactici* permitted in the feed additive catalog. The high survival rate in the acid and bile salt resistance test reflected the good strain tolerance of the isolates to the simulated gastrointestinal conditions of the host *in vitro*. The mean inhibitory diameters of the 10 strains against chloramphenicol and tetracycline were 23.6 mm and 17.4 mm, respectively; none of the hemolytic tests showed α/β hemolytic ring. The bacteriostatic test showed that *P. acidilactici* YH-9, YH-14 and YH-15 had inhibitory effects on four common pathogenic bacteria, including *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Streptococcus*. The adhesion test showed that *P. acidilactici* YH-15 had good adhesion to HT-29 cells. Based on these results, we concluded that *P. acidilactici* YH-15 extracted from cat milk has potential application as a clinical probiotic therapy and health care product.

1. Introduction

For a long time, it was widely believed that breast milk was sterile and any microorganisms present were thought to result from exogenous contamination. However, recent advancements in sequencing technology have provided new insights into the microbiota of breast milk. Studies have now confirmed the presence of bacteria in breast milk, including species such as *Staphylococcus epidermidis*, *Lactobacillus gasseri*, *Bifidobacterium breve*, and *Streptococcus salivarius*, which have been identified in both human colostrum and normal milk [1]. The 7 strains of LAB isolated from breast milk and the probiotic properties of the strains were studied [2]. Through the study of 66 breast milk samples in Germany and Austria, the probiotics in breast milk accounted for 92 percent of all the bacteria in breast milk was found [3]. The bacteria that infants acquire from breast milk play a crucial role in the establishment of their early gut microbiota [4].We hypothesize that a similar phenomenon may occur in kittens.

There is a significant difference in lactose content between cat milk, cow milk, and goat milk [5,6]. When using cow milk or goat

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milk as a cat milk substitute, due to the small amount or low activity of lactase secreted in the small intestine of kittens, lactose cannot be completely decomposed into absorbable monosaccharides, causing easy diarrhea due to lactose intolerance [7,8]. Some studies have shown that LAB can break down lactose [9]. The use of LAB probiotics in kittens may reduce the probability of kittens developing diarrhea due to lactose intolerance. Moreover, probiotics derived from feline milk are theoretically safer for kittens.

However, due to the difficulty of obtaining cat milk, few studies on probiotics in cat milk have been found [10]. Therefore, the purpose of our research is to isolate, screen and identify probiotics from cat milk and then select strains with better probiotic performance so as to provide new candidate strains for kitten milk health care and treatment.

2. Materials and methods

2.1. Cat milk sample collection

The cat milk samples were obtained from 4 healthy nursing female cats at the Beijing University of Agricultural Pet Hospital, from August to December 2023. Disposable medical gloves were worn and the nipples along with the surrounding skin were gently wiped with alcohol-soaked cotton for at least 30 s. Gauze soaked in warm saline was then used to remove any residual alcohol. After discarding the first 2–3 drops of milk, samples were aseptically collected into 2 mL sterile centrifuge tubes and immediately placed in an ice box for preservation, Sent samples to the Veterinary Clinical Disease Diagnosis and Treatment Laboratory of Beijing University of Agriculture for further processing within 2 h [11]. Animal research was carried out following the ARRIVE guidelines [12]. Project that all procedures were under Faculty of the Ethics Committee of Beijing University of Agriculture supervision (Ethics review approval number: BUA2023087). The data collected related to the patient and its publication was under the owner's consent.

2.2. Bacterial strains

Escherichia coli (ATCC25922), *Salmonella typhimurium* (ATCC14028), *Staphylococcus aureus* (ATCC29740), *Streptococcus agalactiae*, and *Lactobacillus plantarum* were purchased from the Guangdong Microbial Culture Collection Center and preserved by the Bacterial Disease Diagnosis Laboratory, College of Animal Science and Technology, Beijing University of Agriculture.

2.3. Isolation, purification and preservation of LAB from cat milk

The fresh cat milk sample (0.5 ml) was taken and added to 9.5 mL of MRS (De Man, Rogosa, and Sharpe medium)Broth. The mixture was then incubated in a 37 °C incubator for 48 h. After incubation, the bacterial solution was diluted with sterile Phosphate-buffered saline (PBS) (jetbiofil, Guangzhou, China). An appropriate dilution was streaked onto MRS agar medium and anaerobic culture was performed at 37 °C for 24–48 h. Several single colonies with smooth surfaces and a white or milky white color were selected and purified using the streak plate method on MRS agar medium. The isolates underwent various biochemical assays according to "Bergey's manual of determinative bacteriology ", including gram staining, assessment of colony morphology, catalase activity, and gas formation, to identify lactic acid bacteria (LAB) [13]. For strain preservation, purified strains were transferred into MRS broth (Hopebio, Qingdao, China) and cultured anaerobically at 37 °C for 12–24 h. The cultures were then mixed with 50 percent Sterile Glycerin Solution (Sangon Biotech, Shanghai, China) in a 1:1 ratio and stored at −80 °C. Prior to use, strains underwent at least three activation cycles.

2.4. Identification of the selected isolates by 16S rRNA gene sequencing

Bacterial genomic DNA extraction kit (Solarbio, Beijing, China) was used to extract DNA from test bacteria; 16S rDNA PCR (polymerase chain reaction) amplification used bacterial universal primers (Sangon Biotech, Shanghai, China); 27F: GAGAGTTTGATCCTGGCTCAG; 1492R: TACGGCTACCTTGTTACGAC. Fifty μL PCR reaction system was used, Taq Reaction Buffer (10 ×) 5 μL, PCR Dye 5 μL, dNPTs 1 μL, TaqDymerase 0.5 μL, DNA template: 2 μL, 27F: 2 μL, 1492R: 2 μL and added dd water (double distilled water) to make up to 50 μL.

The PCR amplification protocol proceeded as follows: initially, a pre-denaturation step was conducted at 95 °C for 5 min. This was followed by 30 cycles, each comprising denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 90 s. Subsequently, there was a final extension step at 72 °C for 7 min, followed by cooling to 20 °C for 3 min. The resulting PCR products were analyzed using 1 percent agarose gel electrophoresis to visualize the bands. The PCR products were then submitted to the Shanghai Sangon Beijing Branch for sequencing. The sequenced gene sequences were compared against the NCBI (National Center for Biotechnology Information) database to assess their homology.

2.5. Gastrointestinal passability test

2.5.1. Acid resistance test of strains

The acid and bile tolerance of the isolates was assessed by following the approach of Yin [14]. The test strain was cultured in MRS liquid medium for 3 generations under sterile conditions, and the third activated bacterial solution was counted by plate counting method, and then the concentration of the bacterial solution was adjusted to 1×10^8 CFU/mL (colony-forming units/mL). Hundred μL of the above bacterial solution was inoculated into 9.9 mL of hydrochloric acid acid-acidified MRS liquid medium (pH 3.0), and the

un-acidified MRS medium was used as a control, and placed in an anaerobic incubator at 37 °C for anaerobic cultivation. The culture was incubated anaerobically at 37 °C in an incubator.

At 0 h and 2 h of incubation, 100 μL of each test bacterial solution was taken and added into 0.9 mL of sterile saline for gradient dilution (10^{-1}∼10^{-6}), 200 μL of each concentration of bacterial solution was taken and evenly coated on the MRS solid medium, and 3 plates of each dilution were repeated, and then incubated anaerobically at a constant temperature of 37 °C for 24 h [15]. The number of colonies was counted, and the number of bacteria in the sample was converted from the number of dilutions and the amount of inoculum sampled. In order to clearly state the results of plate colony counts, it has been preferred to use CFU rather than absolute colony counts to express the viable bacterial content of a sample.

According to the survival rate of the bacteria after 2 h in the acidified medium, the acid tolerance ability of the bacteria was judged. The survival rate was calculated as follows:

Survival rate (%) = A_1/A_0

Note 1: A_1 is the number of live bacteria cultured for 2 h in the acidified (pH 3.0) MRS medium of the test bacteria; A_0 is the number of viable bacteria in the MRS medium cultured for 0 h after the test bacteria are acidified (pH 3.0).

2.5.2. Bile resistance test of strains

According to the method in 2.5.1, inoculated the test bacteria in the MRS liquid medium containing 0.3 percent (w/v) bile salts (Solarbio, Beijing, China) and cultivate them for 4 h, calculated the bile salt tolerance of the test bacteria. The formula for calculating the bile salt survival rate is:

Survival rate (%) = B_1/B_0

Note 2: B_1 is the number of viable bacteria of the test bacteria after being cultured for 4 h in the MRS medium containing 0.3 percent bile salt; B_0 is the number of viable bacteria of the test bacteria after being cultured for 0 h in the MRS medium containing 0.3 percent (w/v) bile salt.

2.6. Safety assessment of screened strains

2.6.1. Determination of antibiotic tolerance

The test strains were continuously cultured in MRS liquid medium for 3 generations under sterile conditions, with an estimation of viable bacteria by standard plate count and then the concentration of the bacterial liquid was adjusted to 10^8 CFU/mL, taken it 0.2 mL spread the MRS agar plate evenly. The antibiotic susceptibility of isolated bacteria was determined using the Kirby-Bauer (K-B) method. Each experiment was performed three times and the average value was taken.

2.6.2. Hemolytic test

Glycerol-preserved *P. acidilactici* was reactivated for 3 generations. The bacterial solution was streaked on a Columbia agar with a 5 percent sheep blood-composition plate (Thermo Fisher, Beijing, China); the positive control group was the *E. coli* group. After culturing in a constant temperature incubator at 37 °C for 24 h, judge whether the strain has hemolysis according to whether it occurs (α hemolysis, β hemolysis and γ hemolysis).

2.7. Evaluation of probiotic properties of screened strains

2.7.1. Bacteriostatic test of supernatant

The antibacterial effect of the isolates was evaluated according to the method of Hu [16]. The test strain was continuously cultured under sterile conditions in an MRS liquid culture medium for 3 generations. After the third activation, count using the standard plate count method. The bacterial liquid concentration was adjusted to 10^6 CFU/mL, respectively, inoculated in MRS broth, incubated at 37 °C for 24–48 h at a constant temperature and then centrifuged at 10000 r/min for 10 min. The supernatant was taken and filtered in an μL traclean table using a 0.22 μm filter membrane. The sterile supernatant was placed on a refrigerator at −4 °C for standby.

The diameter of the inhibition zone was determined as follows: The antibacterial activity of the tested bacteria was assessed using the agar diffusion method. After activating 10 types of tested bacteria for 3 generations, the bacterial solution was adjusted to a concentration of 10^6 CFU/mL. Subsequently, 200 μL of the mixed bacterial solution was evenly spread onto ordinary nutrient agar plates (Hopebio, Qingdao, China) and left to stand on an μLtra-clean bench for 30 min. Three sterile Oxford cups were then placed on each agar plate, into which 200 μL of filter-sterilized LAB supernatant was added. The plates were refrigerated at 4 °C for 3 h and then transferred to a constant temperature incubator set at 37 °C for 24 h. The diameter of the inhibition zone was measured and recorded, with each experimental group repeated three times.

2.7.2. Bacterial growth curve

Activated the strain 3 times; added the third-generation bacterial solution to the sterile MRS medium at a ratio of 3 percent. Starting from 0 h, measure the OD (optical density) 600 value sequentially at intervals of 2 h and draw the growth curve.

2.7.3. Adhesion test

The HT-29 cells were resuscitated and passaged for three generations and inoculated into six-well culture plates pre-covered with sterile coverslips. Placed them in a cell culture incubator and continue to incubate until the cells grow into a dense monolayer. Two concentrations of test group bacterial suspensions were prepared at 1×10^7 CFU/mL and 1×10^8 CFU/mL 1 mL of antibiotic-free DMEM culture medium and 1 mL of the above prepared bacterial suspensions were added to the cell culture wells to form test group 1 and test group 2. A blank and a positive control group were set up, and the test group bacteria were substituted by sterile PBS and Lactobacillus plantarum, respectively. suspension, respectively. The experiment was repeated 3 times for each test strain. Incubate in a cell incubator for 2 h. After 2 h, remove the six-well plate and gently wash the cells 5 times using sterile PBS buffer to remove unadhered bacteria. The cells were then fixed using anhydrous ethanol for 20 min. Cell slides were removed and Gram stained. Twenty fields of view were randomly selected under the microscope and the number of lactic acid bacteria adhering to the cells in each field of view was counted.

2.8. Data analysis

Experimental data was recorded as the mean ± standard deviation of triplicates. The data were statistically analyzed through Graphpad Prism 9.5 software. Significant differences were determined through an ordinary one-way ANOVA test set at $P < 0.05$.

3. Results and discussion

3.1. Isolation and identification of LAB in cat milk

Among 78 cat milk samples collected from 4 healthy female cats, a total of 100 strains of LAB were screened out by selecting smooth colonies, white or milky white colonies, gram staining and microscopic examination. The catalase contact test of these strains was negative and did not produce gas. Morphology and microscope images of some strains screened (Figs. 1 and 2).

3.2. Identification of 16S rRNA gene sequence

The bacterial genomic DNA of a single colony strain after screening and purification was extracted, the target band of about 1500 bp was obtained by PCR amplification (Fig. 3) and sent to the Shanghai Sangong Beijing Branch for sequencing. The comparison of the sequencing results with NCBI showed that most of the strains were *S. aureus, Staphylococcus epidermidis, Streptococcus lutetiensis* and *Streptococcus equinus;* these strains accounted for 90 percent of the isolated strains. In all strains, there are 10 strains of *P. Acidilactici* (Table 1). We have deposited the sequences of 10 bacterial strains in GenBank and received accession numbers OQ730535-OQ730544. It complies with the feed additive catalog of the Ministry of Agriculture and Rural Affairs of China, which allows the addition of probiotics. Number 10 strains of *P. acidilactici* (YH-5, YH-7, YH-8, YH-9, YH-10, YH-11, YH-13, YH-14, YH-15, YH-19) for further research.

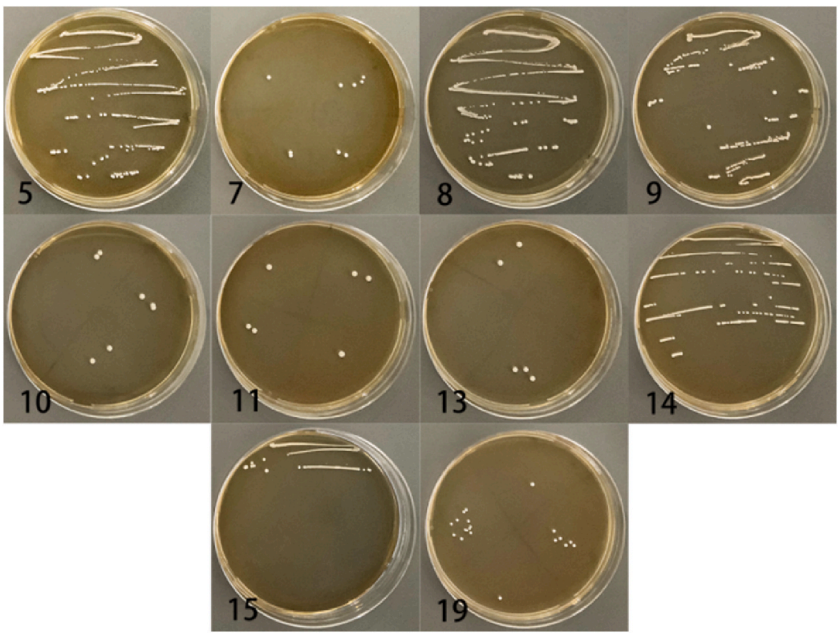

Fig. 1. Morphological manifestations of the major colonies.

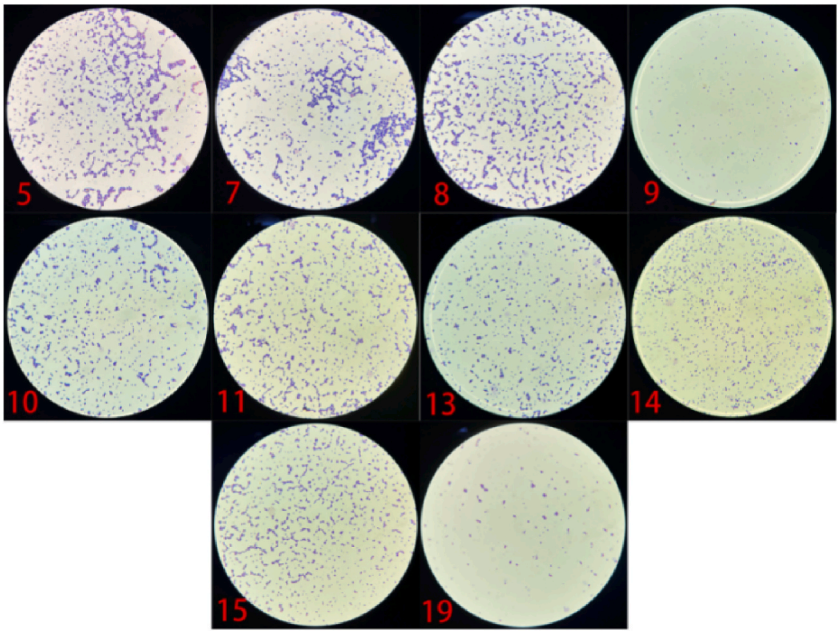

Fig. 2. Microscopic morphology observations of the 10 test strains.

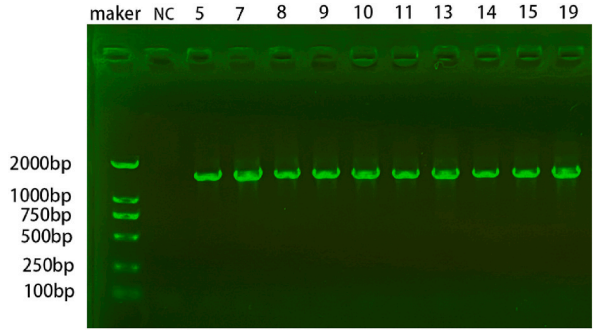

Fig. 3. Electrophoresis of PCR products of strains.

3.3. Phylogenetic tree based on 16S rRNA

The 16S rRNA gene is a sequence present in the genome of any bacterium. The construction of a phylogenetic tree based on 16S rRNA has important significance for Identifying and classifying microorganisms. Since 16S rRNA sequences are highly conserved and species-specific in bacteria, the classification and identification of microorganisms can be determined through comparison of 16S rRNA sequences; moreover, the phylogenetic position of unknown microorganisms can be determined. It is used to explore the evolution and evolution process of microorganisms and to understand the relationship.

Compared the genes of the experimental strains with the genes in the NCBI database and the parameter was set to identify >95. Sequences with high similarity to the 20 16S rRNAs were then selected, and a phylogenetic tree was constructed after sequence comparison using MEGA 11 software.

3.4. Gastrointestinal passability test

3.4.1. Acid resistance test of strains

Under acidic conditions (pH 3.0), Ten species of *P. acidilactici* isolated from the samples showed different degrees of growth tolerance (Fig. 5).

Note 3: The same letter in the data column means no significant difference ($P > 0.05$) and no same letter means a significant difference ($P < 0.05$). Legend letters in Figs. 6 and 10 have the same meaning.

The 10 test strains were cultured at pH 3.0 for 2 h; the survival rates of strains YH-5, YH-9, YH-11, YH-13, YH-14 and YH-15 exceeded 100 percent, those were respectively 186 percent, 164 percent, 125 percent, 150 percent, 131 percent and 214 percent.

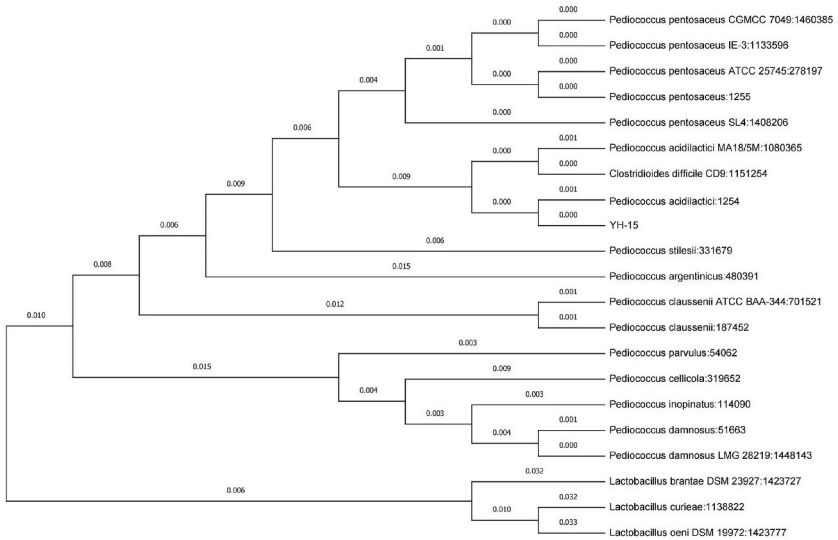

Fig. 4. Phylogenetic tree of YH-15 based on 16S rRNA Phylogenetic tree results showed that YH-15 was adjacent to *P. acidilactici:* 1254 (Fig. 4).

Table 1
Homologous alignment results of 16S rDNA sequences.

Strain ID	Identification result	Gene sequence similarity(%)
YH-5	*P. acidilactici*	99.79
YH-7	*P. acidilactici*	99.86
YH-8	*P. acidilactici*	100.00
YH-9	*P. acidilactici*	99.80
YH-10	*P. acidilactici*	99.86
YH-11	*P. acidilactici*	99.86
YH-13	*P. acidilactici*	99.86
YH-14	*P. acidilactici*	99.93
YH-15	*P. acidilactici*	99.86
YH-19	*P. acidilactici*	99.86

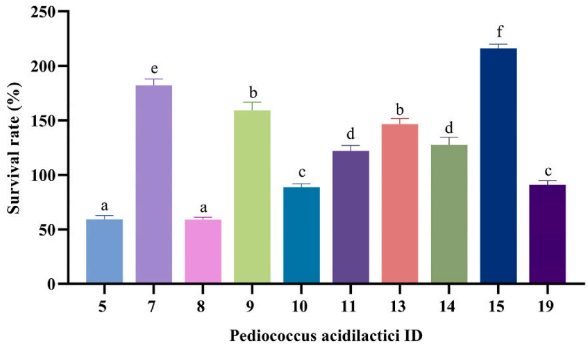

Fig. 5. Assessment of acid resistance in 10 *P. acidilactici* strains.

3.4.2. Bile resistance test of strains

The survival rate of different strains co-cultured with 0.3 percent bile salt for 4 h (Fig. 6).

The growth tolerance of probiotics to bile salts is also specific for different strains [17,18]. The bile salt tolerance test of the tested strains was repeated many times; the results showed that all the strains were sensitive to 0.3 percent bile salts and the survival rates of the strains were all low after co-culturing for 4 h. The survival rate of the 10 test strains decreased significantly after being cultured in the MRS medium containing 0.3 percent bile salt for 4 h; the survival rate of the highest 3 strains was 18 percent, 14 percent and 16 percent, respectively.

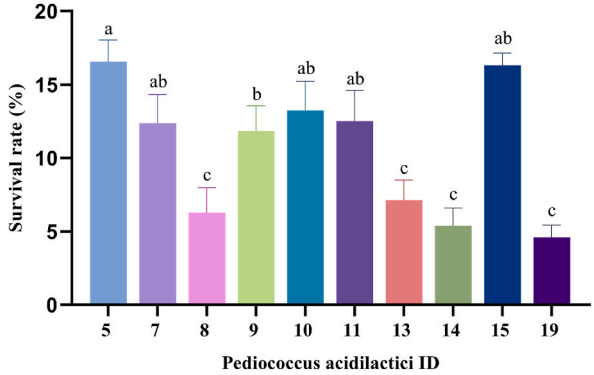

Fig. 6. Evaluation of bile salt tolerant performance of 10 *P. acidilactici* strains.

3.5. Safety assessment

3.5.1. Antibiotic tolerance

The 10 strains showed the strongest sensitivity to chloramphenicol, with an average inhibition diameter of 23.6 ± 2.54 mm; the second is the sensitivity to tetracycline, with an average inhibition diameter of 17.4 ± 3.45 mm; the sensitivity to penicillin and ampicillin is basically the same, with an average inhibition diameter of 9.42 ± 1.62 mm and 9.60 ± 2.44 mm, respectively; most strains showed resistance to cephradine.

3.5.2. Hemolytic test

Using E. coli as a control group, as can be seen in the figure(Fig. 7), no translucent or translucent hemolytic ring was observed on the blood plates of the 10 isolated strains, thus all LAB strains are non-hemolytic (γ-hemolysis).

3.6. Potential for Prebiotic properties

3.6.1. Bacteriostatic test of supernatant

In this experiment, four common pathogenic bacteria, viz., *E. coli* (ATCC25922), *S. Typhimurium* (ATCC14028), *S. aureus* (ATCC29740) and *S. agalactiae,* were used as the test objects and the antibacterial performance of the supernatant of the test strains was evaluated (Table 2 and Fig. 8). The strains with the strongest antibacterial effect on *E. coli* were strains YH-14 and YH-15; the antibacterial diameters were all above 19.5 mm, which was 20.89 ± 0.89 mm and 19.99 ± 0.41 mm respectively; the strains with the

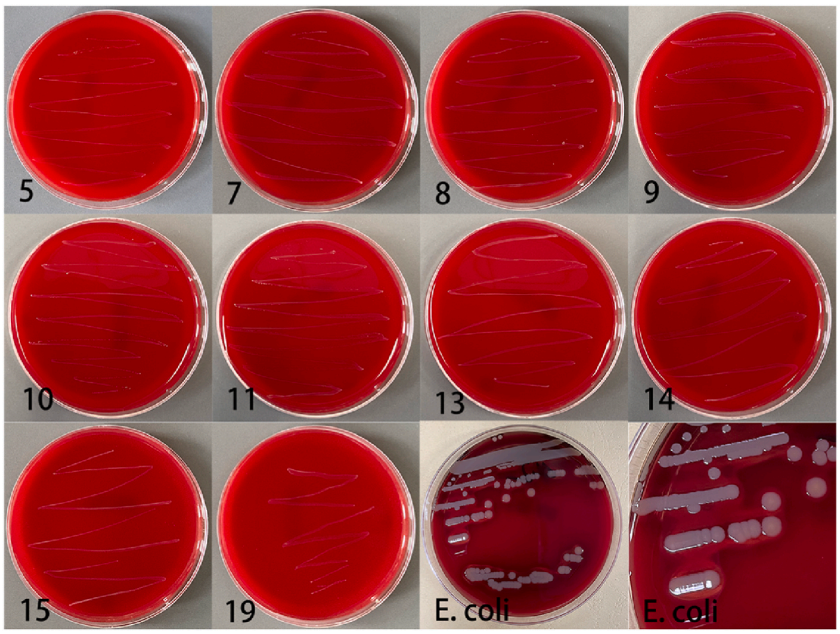

Fig. 7. Hemolytic test of selected screened strains and control group.

strongest antibacterial effect on *S. Typhimurium* were strains YH-9, YH-11 and YH-15, the antibacterial diameters were all above 20 mm, which were 23.71 ± 2.8 mm, 21.48 ± 1.18 mm and 26.05 ± 0.65 mm respectively; the strains with the strongest antibacterial effect on *S. Typhimurium* were strains YH-8, YH-9, YH-14 and YH-19, all of which had an inhibitory diameter of more than 20 mm, which were 24.16 ± 0.31 mm, 20.38 ± 0.49 mm, 21.07 ± 0.75 mm and 22.54 ± 0.25 mm, respectively. Combined with the results of previous experiments, after comprehensive data analysis, strain YH-15 was finally selected (inhibitory diameters against *Streptococcus, Salmonella, S. Typhimurium and E. coli* were 16.15 ± 0.14 mm, 26.05 ± 0.65 mm, 19.19 ± 0.25 mm and 19.99 ± 3.41 mm, respectively) for the next test.

Note 4: In the table (Fig. 8A–D), the same lowercase letters in the sa me row indicate no significant difference ($P > 0.05$) and different lowercase letters indicate significant differences ($P < 0.05$). Inhibition diameter of common pathogenic bacteria by the supernatant of test strains (mm).

3.6.2. Bacterial growth curve

The bacterial concentration (OD600 value) of YH-15 gradually increased over time. Its growth curve is shown in the figure(Fig. 9).

3.6.3. Adhesion test

The number of adherent bacteria in the adhesion test of YH-15 is shown inthe figure(Fig. 10). There are large differences in the adhesion of YH-15 bacteria solution to cells at different concentrations; the adhesion ability of *L. plantarum* (27.25 cells/cell) is significantly higher at the same concentration than that of YH-15 (6.85 cells/cell). When the concentration of YH-15 was increased to 10^8 CFU/mL, the number of adhered cells was 68.6 per cell.

4. Discussion

4.1. Isolation and identification of LAB in cat milk

A large number of LABs, including *Bifidobacterium, Streptococcus, Lactobacillus, Enterococcus and Pediococcus*, were isolated from breast milk by traditional isolation methods [19–21]. Although many experiments have found bacteria in breast milk, the types of bacteria found are not the same. For example, Pengyu Hu screened 22 strains of *Lactobacillus* and 3 strains of *Bifidobacteria* from breast milk [16]. According to the results of a study in Taiwan, the most frequently occurring bacterial species in the samples was *Staphylococcus*, which indicates that skin may be an important source of breast milk microbiota composition; that is, skin pollution may affect the breast milk microbiota composition in an important way [22]. Although in this experiment we had performed related aseptic operations before milk collection, most of the strains found were *S. Typhimurium, Staphylococcus epidermidis, Streptococcus luteum and Streptococcus equi*; these strains accounted for 90 percent of all isolates. This result is similar to that of Chen P W. But in addition, we found 10 strains of *P. acidilactici*, which is considered to be a beneficial type of colony. It's also a case of approval of *P. acidilactici* as a feed additive in the European Union [23].

4.2. Gastrointestinal passability test

LABs are a normal physiological flora in the animal body and only when they reach a certain amount in the intestinal tract can they have a positive impact on the health of the host [24]. Therefore, the primary consideration in the development of probiotics is to screen for LAB that has high viability and can survive in the intestinal tract. Because the pH of gastric juice is usually around 2.0–3.0, this environmental condition will cause most of the microorganisms entering the stomach to lose their vitality [25]. Generally, food stays in

Table 2
Antibiotic sensitivity of each isolated strain.

Antimicrobials	Mean diameter of inhibition zone/mm Strains ID									
	YH-5	YH-7	YH-8	YH-9	YH-10	YH-11	YH-13	YH-14	YH-15	YH-19
Chloramphenicol	24.90 ± 0.46	28.40 ± 3.08	26.67 ± 1.58	22.64 ± 0.44	22.59 ± 0.14	21.03 ± 0.53	21.68 ± 1.83	20.32 ± 0.45	23.56 ± 1.58	24.46 ± 0.11
Penicillin	8.76 ± 0.46	9.97 ± 0.59	10.27 ± 0.13	11.82 ± 1.17	9.61 ± 2.72	7.33 ± 0.62	6.63 ± 0.10	8.02 ± 1.24	10.39 ± 0.46	11.40 ± 2.70
Cefradine	0.00	0.00	0.00	0.00	6.70 ± 0.07	0.00	6.50 ± 0.10	6.70 ± 0.12	6.70 ± 0.00	0.00
Tetracycline	17.66 ± 0.10	21.21 ± 0.30	25.28 ± 3.58	18.57 ± 0.23	16.92 ± 1.18	12.41 ± 1.79	15.22 ± 1.59	14.83 ± 0.35	15.49 ± 2.30	16.39 ± 0.11
Ciprofloxacin	6.20 ± 0.09	0.00	0.00	0.00	6.80 ± 0.12	0.00	6.70 ± 0.17	0.00	6.60 ± 0.11	0.00
Streptomycin	6.50 ± 0.11	0.00	0.00	0.00	6.60 ± 0.08	0.00	6.60 ± 0.23	0.00	6.80 ± 0.16	0.00
Ampicillin	10.5 ± 0.31	7.82 ± 1.50	8.71 ± 1.98	9.07 ± 2.03	7.23 ± 0.48	8.59 ± 0.07	7.51 ± 0.16	8.26 ± 0.74	13.72 ± 0.08	14.59 ± 0.57

The data are expressed as the means ± SE.

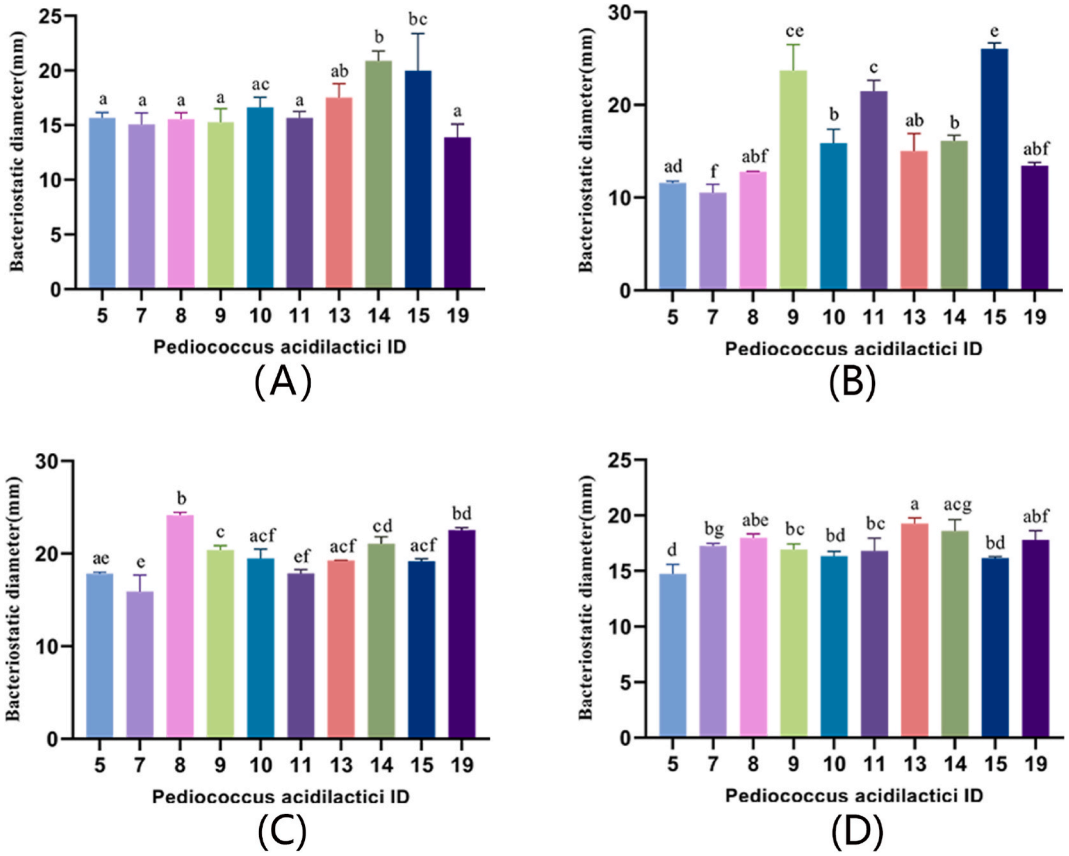

Fig. 8. Inhibition diameter of common pathogenic bacteria by the supernatant of test strains (mm) (Fig. 8A). *Escherichia coli* ATCC25922 (Fig. 8B); *Salmonella typhimurium* ATCC14028 (Fig. 8C); *Staphylococcus aureus* ATCC29740; and (Fig. 8D) *Streptococcus agalactiae*.

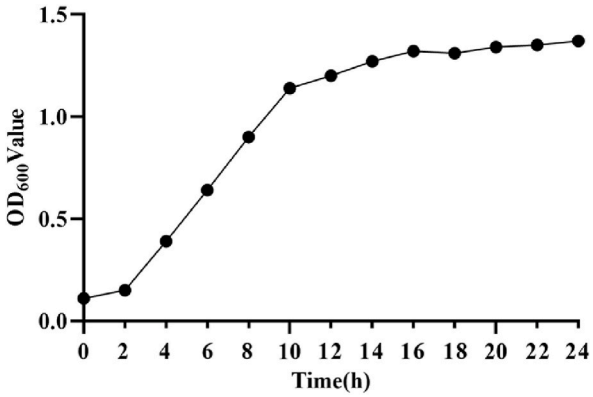

Fig. 9. Growth curve of YH-15.

the stomach for no more than 2 h, so this test simulates the stomach environment by adding the target strain to the medium at pH 3.0 for 2 h. After food is digested by the gastric juice, it enters the duodenum, where it mixes with bile salts. Bile salts can change the permeability of cell membranes and cause cell death by destroying the integrity of cell membranes [26]. Some studies have shown that the content of bile salts in the small intestine is around 0.3 percent and generally, it takes 1–4 h for food to pass through the small intestine [27,28]. Therefore, in this experiment, the target strain was added to a medium with 0.3 percent bile salts and cultured for 4 h to simulate the intestinal environment. The strains were then counted with standard plates to assess their resistance to acid and bile salts.

If the strain can survive and reproduce in a simulated environment with a pH 3.0 and 0.3 percent bile salt, it indicates that the strain has a certain tolerance in the acid-base environment of simulated gastric juice and intestinal juice *in vitro*. In this study, the survival

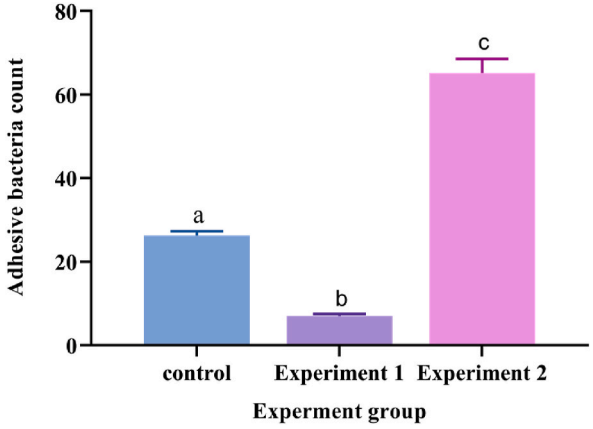

Fig. 10. The number of adherent bacteria in the adhesion test of YH-15.

rates of strains YH-5, YH-9, YH-11, YH-13, YH-14 and YH-15 exceeded 100 percent at pH 3.0. It shows that these strains have good acid resistance and can grow in an acidic environment of pH 3.0. The survival rate of 10 test strains decreased significantly after being cultured in 0.3 percent bile salt MRS medium for 4 h; the highest survival rate was for YH-5, YH-15 and YH-10 strains (the survival rate is only 16.56 percent, 16.32 percent and 13.24 percent), this result is similar to Wei Na's research [29]. This limits its performance as a probiotic to some extent. Despite this, He Ting's research shows that porcine-derived *Lactobacillus johnsonii L531* and *C21* exhibit poor tolerance to bile salts, but they can still enter the gastrointestinal tract of piglets alive and have a beneficial effect on the intestinal flora of piglets [30].

4.3. Safety assessment

Resistance assessment is an important aspect when evaluating the safety of probiotics [31]. Drug-resistant genes can be exchanged in the gut microbial community through multiple pathways, thereby conferring drug resistance to sensitive pathogenic bacteria and causing harm to the host's health [32]. But at the same time, the drug resistance of probiotic strains also has advantages, such as the ability to survive in the intestinal tract when taking antibiotics, which is conducive to intestinal colonization to help restore the homeostasis of intestinal flora and alleviate the problem of gastrointestinal flora imbalance caused by antibiotics [33,34]. Therefore, it is very important to do drug susceptibility tests on probiotics to know the drug resistance of different strains. In this paper, the antibiotic susceptibility of *P. acidilactici* from cat milk was detected, which provided a theoretical basis for the rational use of isolated antibiotics. When faced with different kinds of antibiotics, 10 different *P. acidilactici* strains showed different susceptibilities. In addition, all LAB strains are non-hemolytic (γ-hemolysis). This demonstrates the safety of the screened strains to a certain extent.

4.4. Evaluation of probiotic properties

In order for probiotics to play a probiotic role, they usually need to adhere to the cell surface and form a defensive barrier in the intestine to prevent the colonization and proliferation of pathogenic microorganisms and maintain a healthy balance of the host's microbial environment [35].The results of the preliminary tests showed that strains YH-9, 13, 14, and 15 all had good antibacterial properties.At the same time, they were combined with the previous gastrointestinal passability test and the safety evaluation test. Strain YH-15 was selected for the next step of the bacterial adhesion test.

Our research results showed that different concentrations of YH-15 bacterial fluid had great differences in cell adhesion. At the same concentration, the adhesion ability of *L. plantarum* (27.25 bacteria/cell) was significantly higher than that of YH-15 (6.85 bacteria/cell). When the concentration of YH-15 was increased to 10^8 CFU/mL, the number of adhesions was 68.6 Bacteria/cell. The reason may be the difference in structure (for example, pili, cell wall, outer membrane protein, capsule) or the content of bacterial components (for example, protein, peptide, sugar on the surface) [36]. More in-depth research is yet to be conducted.This experiment proves that YH-15 has epithelial cell adhesion, laying a good foundation for exerting probiotic effects.

5. Conclusion

Overall, we isolated a strain of *P. acidilactici* from cat milk and named it YH-15. YH-15 demonstrated good tolerance to acidic and alkaline environments, as well as strong cell adhesion ability. It also exhibited antibacterial activity against 4 common pathogens and showed specific sensitivity to 7 antibiotics. As a *P. acidilactici* strain derived from cat milk, we believe that YH-15 has the potential to play an important role in the future treatment and health care of cats.

CRediT authorship contribution statement

Yahui Li: Writing – original draft, Validation, Methodology. **Yiwen Zhang:** Writing – review & editing, Conceptualization. **Junxin Zhao:** Writing – review & editing, Resources, Conceptualization. **Xuan Zhang:** Formal analysis, Data curation. **Shiwei Liu:** Resources, Methodology, Investigation. **Hanmeng Qi:** Resources, Methodology, Investigation. **Fuqiang Qiao:** Visualization, Investigation. **Hua Yao:** Supervision, Conceptualization.

Ethics statement

Project that all procedures were under Faculty of the Ethics Committee of Beijing University of Agriculture supervision (Ethics review approval number: BUA2023087). The data collected related to the patient and its publication was under the owner's consent.

Data availability statement

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

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Declaration of competing interest

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

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