Vietnam Journal of Food Control

A Scientific Journal of National Institute for Food Control

Journal homepage: http://vjfc.vn/

Research Article

# Screening and identification of probiotic lactic acid bacteria isolated from meconium

**Pham Van Kien[1*], Hoa Thi Minh Tu[2], Nguyen Thi Lam Doan[3]**

[1]*Department of Food Engineering, School of Chemistry and Life Sciences, Hanoi University of Science and Technology, Hanoi, Vietnam*

[2]*Institute of Biotechnology, Vietnam Academy of Science and Technology, Hanoi, Vietnam*

[3]*Faculty of Environmental Sciences, University of Science, Vietnam National University, Hanoi, Vietnam*

*(Received: 02 Apr 2026, Revised: 12 Jun 2026, Accepted: 15 Jun 2026)*

***Abstract***

This study aimed to identify potential probiotic lactic acid bacteria (LAB) strains isolated from meconium. Based on evaluations of antimicrobial activity, acid and bile salt tolerance, antibiotic resistance, and auto-aggregation ability, two strains (K24 and K36) were identified as promising probiotic candidates. The strains exhibited strong inhibitory activity against several pathogenic bacteria, including *Escherichia coli* ATCC 25922, *Salmonella enterica* ATCC 13076, *Bacillus cereus* ATCC 21778, *Vibrio cholerae* ATCC 14033, *Staphylococcus aureus* ATCC 25923, and *Listeria monocytogenes* ATCC 1911. Inhibition zones ranging from 16 to 27.5 mm. At pH 2 (3 h), survival rates were 86.3% (K24) and 83.3% (K36). In 0.3% bile salts, survival rates were 94.14% (K24) and 97.92% (K36) after 3 h. Furthermore, strains K24 and K36 demonstrated strong auto-aggregation, with percentages of 55.61% and 69.66%, respectively. Both strains also showed resistance to several commonly used antibiotics. Based on 16S rRNA gene sequence analysis, strain K24 was identified *as Lactobacillus plantarum,* while strain K36 was identified as *Lactobacillus acidophilus*. These strains warrant further investigation to evaluate their safety and efficacy as probiotic candidates for human use.

***Keywords:*** *LAB, probiotics, meconium, Lactobacillus.*

## 1. INTRODUCTION

Probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits on the host [1, 2]. Probiotics have been shown to prevent and treat oral infections and dental caries [3], as well as enhance the immune system [4]. In addition, the human gut microbiome consists of both beneficial and pathogenic microorganisms. Pathogenic microorganisms are associated with various diseases, including diarrhea, colorectal cancer, and gastric cancer [4]. Probiotics can help mitigate these effects through their ability to prevent and manage conditions such as diarrhea [5] and inflammatory bowel disease [6].

Lactic acid bacteria (LAB) are generally recognized as safe (GRAS) and are among the most widely used microorganisms in probiotic applications in both the medical and food sectors [7]. They can survive under harsh gastrointestinal conditions and produce beneficial compounds such as antimicrobial substances and enzymes [8]. LAB can be isolated from various sources, including fermentation products, the gastrointestinal tract of livestock and aquatic organisms, etc [9]. Currently, numerous studies worldwide, including in Vietnam, have focused on isolating LAB strains-with probiotic potential from many diverse sources, such as cherry fruit [8], food products [4], and the gastrointestinal tract of honeybees [7]. However, Gheziel *et al.* (2019) reported that LAB originating from the human gastrointestinal tract exhibit superior adaptability to the intestinal environment and may persist longer than strains from other sources [10]. Meconium represents a potential source of diverse LAB strains, including *Lactobacillus*, *Streptococcus,* and *Enterococcus*. These strains have been shown to exhibit probiotic activity [11]. Therefore, this study aimed to isolate and identify LAB strains

*Corresponding author: Pham Van Kien (E-mail: kienphamvan2801@gmail.com )

https://doi.org/10.47866/2615-9252/vjfc.4675

from meconium with probiotic properties, including antimicrobial activity against intestinal pathogens, acid and bile salt tolerance, auto-aggregation ability, and antibiotic susceptibility. Selected strains were identified based on the 16S rRNA gene sequence. These findings may provide a basis for the development of probiotic products for human use.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Thirty strains of LAB isolated from meconium were stored at the Department of Bioactive Compounds from Microorganisms, Institute of Biology, Vietnam Academy of Science and Technology. These strains were designated K2, K5, K8, K10, K11, K14, K17, K24, K28, K31, K33, K34, K36, K39, K41, K43, K44, K47, K48, K55, K59, K61, K73, K83, K119, K120, K121, K131, K132, K134.

Indicator microorganisms included: *Escherichia coli* ATCC 25922, *Salmonella enterica* ATCC 13076, *Bacillus cereus* ATCC 21778, *Vibrio cholerae* ATCC 14033, *Staphylococcus aureus* ATCC 25923, and *Listeria monocytogenes* ATCC 1911. All indicator strains were obtained from the same collection.

Indicator microorganism culture environment (Luria - Bertani): yeast extract 5.0 g/L; Peptone 10 g/L; NaCl 10 g/L; pH 7.0.

De Man, Rogosa, Sharpe (MRS) for culturing and activating LAB: Peptone 10 g/L, meat extract 10 g/L, yeast extract 5 g/L, glucose 20 g/L, sodium acetate 5 g/L, dipotassium phosphate 2 g/L, ammonium citrate 2 g/L, $MgSO_4.7H_2O$ 0.2 g/L, $MnSO_4$ 0.04 g/L, Tween 80 1 mL, pH 6.8.

### 2.2. Methods

#### *2.2.1. Antimicrobial activity*

Preliminary antibacterial activity was determined using the spot-on method as previously described by Ngo Thi Phuong Dung *et al.* (2011) [12] with modifications according to Hoa Thi Minh Tu *et al.* (2021) [13]. Thirty LAB strains were cultured in MRS broth at 30°C for 24 h**.** Subsequently, 5 µL of each bacterial suspension was spotted onto a plate containing MRS agar, with 5 strains inoculated per plate. The plates were allowed to dry and then incubated at 30°C for 24 h. These plates were overlaid with a layer of semi-solid LB medium (0.7% agar) inoculated with 0.2% indicator culture (*E. coli, S. enterica, B. cereus, V. cholerae, S. aureus,* and *L. monocytogenes*) and kept at 4°C for approximately 4 h to allow the active compounds to diffuse into the agar medium. A strain was considered inhibitory if the inhibition zone was greater than 2 mm [12]. The positive control was *L. plantarum* JCM 1149 obtained from the Japan Collection of Microorganisms (JCM). The negative control was MRS medium without bacterial inoculation.

#### *2.2.2. Acid tolerance*

Acid tolerance of LAB strains was assessed by measuring the survival rate in a pH 2.0 medium, following the protocol of Keuho Ji *et al.* (2015) [14]. Subsequently, LAB strains showing the strongest antagonism activity were cultured in MRS at 30°C for 18 h, harvested, washed, and resuspended in phosphate-buffered saline (PBS, pH 2.0). Samples were collected at 0 and 3 h and serially diluted, and plated on MRS agar followed by incubation at 30°C for 24 h for viable cell counting. Survival rate was calculated as follows: Survival rate (%) was calculated as $N_1/N_0 \times 100$, where $N_0$ is the initial viable cell count (CFU/mL) at 0 h, and $N_1$ is the viable cell count (CFU/mL) after 3 h.

#### *2.2.3. Bile salts tolerance*

Before reaching the intestinal tract, probiotic bacteria must first survive in the acidic environment of the stomach and bile in the small intestine, typically for approximately 3 h [9]. The average physiological concentration of bile salts in the human small intestine is about 0.3% [15]. Therefore, LAB strains previously selected based on antimicrobial activity were evaluated and surveyed for tolerance to bile salts at a concentration of 0.3% for 3 h. Bile salt tolerance was assessed using the plate count method according to the procedure described by Keunho Ji *et al.* (2015) [14]. The biomass of LAB strains after 18 h of growth in MRS medium at 30°C was cultured in MRS medium containing 0.3% (w/v) bile salt. Samples were collected at 0 and 3 h, serially diluted, and plated onto MRS agar. The plates were incubated at 30°C for 24 h, after which viable colonies were counted.

*2.2.4. Auto-aggregation ability*

Auto-aggregation ability was determined using the method of Kos *et al.* (2003) [16], with the modification of using plate counting instead of OD measurement. Selected LAB strains were cultured in MRS broth at 37°C for 24 h and then harvested by centrifugation at 5000 rpm for 5 min. The harvested biomass was washed twice with PBS buffer (8g NaCl, 0.2g KCl, 1.44g $Na_2HPO_4$, 0.24g $KH_2PO_4$, pH 7.2) and resuspended in PBS buffer. Cell suspensions were adjusted to approximately $10^8$ CFU/mL prior to the assay. Incubation was performed without agitation. Samples at 0 and 5 h were carefully collected from the upper suspension without disturbing the sediment. These samples were plated on MRS agar to determine the CFU/mL. The auto-aggregation rate was calculated according to the following formula: Auto-aggregation rate (%) = (1 - $N_1/N_0$) x 100, where $N_1$ is the number of bacteria after 5 h (CFU/mL), and $N_0$ is the number of bacteria after 0 h (CFU/mL).

*2.2.5. Method validation*

The antibiotic resistance of selected LAB strains was evaluated using the Kirby-Bauer disk diffusion method [17] based on the CLSI standard, 2021 [18]. Antibiotics include Penicillin G (10 µg/mL), Cephalexin (30 µg/mL), Tetracycline (30 µg/mL), Amoxicillin - clavulanate (20/10 µg/mL), Erythromycin (15 µg/mL). Accordingly, the selected bacterial strains were grown in MRS broth at 30°C for 24 h. Prepare a semi-liquid MRS medium (0.7% agar) containing the cultured LAB strain (1%), then pour it into a plate. The well was made with a stainless steel borer with a diameter of 8 mm. Each well was filled with antibiotics, which were then added. Then they were incubated at 30°C for 24 h. The sensitivity of antibiotics is assessed by measuring the diameter of the zone of inhibition around the antibiotic disk. A ruler with millimeter divisions is used to measure. Antibiotic susceptibility was determined as follows: resistant (R) (inhibition zone diameter ≤ 14 mm), intermediate susceptibility (I) (inhibition zone diameter ranging from 14 to 19 mm), and susceptible (S) (inhibition zone diameter ≥ 20 mm).

*2.2.6. Classification of bacterial strains based on 16S rRNA gene sequencing*

Strains of LAB with high probiotic potential were identified based on 16S rRNA gene sequence analysis following the method described by Hoa Thi Minh Tu *et al.* (2021) [13]. The strains were cultured in 5 mL MRS broth at 30°C for 16 h, followed by centrifugation to collect the biomass. The DNA was separated using a commercial kit (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. PCR reaction was performed with ThermoFisher master mix, template DNA, and universal primers 27F: 5'-AGAGTTTGATCCTGGCTCAG-3', 1492R: 5'-GGTTACCTTGTTACGACTT-3'. The PCR products were sequenced using an automated PRISM@3700 Genetic Analyzer (Thermo Fisher Scientific, USA). The obtained sequences were edited and aligned using BioEdit software, and compared with data on NCBI by BLAST program. Phylogenetic trees are constructed using MEGA X software.

## 3. RESULTS AND DISCUSSION

### 3.1. Antimicrobial activity

The capacity to inhibit the growth of pathogenic bacteria is an essential criterion for selecting probiotic candidates, as it contributes to the host defense against microbial infections [19]. The antimicrobial activity of the 30 LAB strains is presented in **Table 1**.

The results showed that all 30 strains tested were able to inhibit the growth of 4 to 6 indicator strains, with inhibition zones measuring between 5 and 30 mm. However, 10 strains, K14, K131, K10, K17, K24, K5, K31, K36, K39, and K48, showed broad-spectrum antibacterial activity and inhibitory effects against all 6 test strains, higher than the reference strain *L. plantarum* JCM 1149, with inhibition zones ranging from 8.5 to 30 mm. Yalian *et al.* (2014) found that LAB isolated from infant feces could inhibit the growth of *E. coli*, with inhibition zones ranging from 9 to 17.9 mm [20]. Similarly, Zavišić *et al.* (2019) reported that *L. fermentum* G-4 originating from the meconium of newborns showed inhibitory activity against *E. coli* (12 mm), *S. aureus* (18 mm), and *S. enterica* (12 mm) [11]. In a study by Hoa Thi Minh Tu *et al.* (2021), 120 LAB strains isolated from fermented products demonstrated antibacterial activity, producing zone of inhibition diameters of 16 - 26 mm [13]. Consistent with these findings, Wenqing Zhang *et al.* (2022) reported that 22 LAB strains screened from local Holstein raw milk exhibited inhibitory activity against *E. coli, S. aureus, and S. enterica*, with inhibition zones ranging from 13 - 17 mm, 12 - 28 mm, and 11 - 15 mm, respectively [21]. Comparing the

results of this study with previous research revealed that the ability of different bacterial strains to inhibit the growth of pathogens varied depending on the strain and its origin. Based on these results, 10 strains exhibiting broad-spectrum antimicrobial activity were selected for further characterization (**Figure 1**).

***Table 1:*** *Antimicrobial activity of 30 selected lactic strains (n=3)*

| No. | Strain | Antimicrobial activity (Inhibition zone mm) | | | | | |
|---|---|---|---|---|---|---|---|
| | | ***E. coli*** **ATCC 25922** | ***S. enterica*** **ATCC 13076** | ***B. cereus*** **ATCC 21778** | ***S. aureus*** **ATCC 25923** | ***L. monocytogenes*** **ATCC 1911** | ***V. cholerae*** **ATCC 14033** |
| Control | JCM 1149 | 9.5±0.3 | 11.5±0.3 | 12±0.5 | 7.5±0.5 | 17±0.2 | 12±0.2 |
| 1 | K2 | 14±0.1 | 8±0.3 | 6±0.4 | - | 8±0.6 | 8.5±0.1 |
| 2 | K5 | 15±0.3 | 17±0.7 | 20.8±0.5 | 10.5±0.1 | 14±0.7 | 14.3±0.1 |
| 3 | K8 | - | 10.3±0.1 | - | 18±0.6 | 14±0.4 | 11±0.6 |
| 4 | K10 | 30±0.5 | 23±0.1 | 10.5±0.5 | 10±0.2 | 18±0.3 | 18±0.1 |
| 5 | K11 | 14.5±0.3 | 6.5±0.3 | 8±0.4 | 7±0.1 | 13±0.4 | 10.5±0.6 |
| 6 | K14 | 17±0.6 | 22±0.3 | 16±0.4 | 12.3±0.3 | 22.3±0.5 | 24±0.7 |
| 7 | K17 | 11.5±0.3 | 11.5±0.5 | 19.3±0.7 | 10±0.1 | 14.5±0.3 | 10.8±0.7 |
| 8 | K24 | 16±0.4 | 20±0.3 | 20.5±0.4 | 16±0.1 | 18±0.1 | 17±0.1 |
| 9 | K28 | 10±0.6 | 9.5±0.5 | 26±0.1 | - | 11±0.3 | 18.5±0.6 |
| 10 | K31 | 13±0.4 | 12.5±0.1 | 16±0.3 | 11.5±0.4 | 17±0.5 | 14.5±0.6 |
| 11 | K33 | 8±0.3 | 6.5±0.1 | - | 9.5±0.3 | 22±0.5 | 8±0.5 |
| 12 | K34 | 9±0.6 | 13±0.3 | 5.8±0.4 | 8.5±0.6 | 10.3±0.7 | 19±0.3 |
| 13 | K36 | 21.5±0.1 | 27.5±0.3 | 24.5±0.4 | 21.5±0.4 | 17.3±0.3 | 17±0.3 |
| 14 | K39 | 14.5±0.3 | 8.5±0.3 | 8.5±0.4 | 8.5±0.1 | 21±0.4 | 15.5±0.6 |
| 15 | K41 | 9.5±0.4 | 28±0.2 | 8±0.3 | - | 9±0.5 | 10.5±0.3 |
| 16 | K43 | 13±0.5 | - | - | 12±0.4 | 22±0.5 | 10.5±0.3 |
| 17 | K44 | 16±0.5 | - | - | 8±0.4 | 20.5±0.4 | 20±0.7 |
| 18 | K47 | - | 16±0.6 | - | 17.3±0.5 | 19±0.5 | 24±0.3 |
| 19 | K48 | 10±0.5 | 8.5±0.1 | 13±0.1 | 20±0.5 | 10±0.6 | 18±0.7 |
| 20 | K55 | 11.5±0.6 | - | - | 20±0.5 | 18.5±0.4 | 14±0.4 |
| 21 | K59 | 12±0.5 | 17±0.4 | 6±0.3 | 12±0.6 | 12±0.5 | 11.5±0.6 |
| 22 | K61 | 11±0.5 | 15±0.5 | - | 9.3±0.5 | 15.5±0.5 | 11±0.5 |
| 23 | K73 | 10.5±0.6 | 11±0.5 | 9.8±0.3 | 7.8±0.4 | 11.5±0.3 | 11±0.4 |
| 24 | K83 | 14±0.5 | 23±0.4 | - | 8±0.6 | 11±0.4 | 12±0.7 |
| 25 | K119 | 5.5±0.3 | - | 5.8±0.3 | 6.8±0.5 | 5.5±0.7 | 5±0.5 |
| 26 | K120 | 6.3±0.4 | - | 7±0.1 | 13.5±0.3 | 7±0.4 | 6.5±0.1 |
| 27 | K121 | 9.8±0.5 | 7±0.4 | 10±0.1 | 15.8±0.5 | 9.5±0.4 | 12.5±0.7 |
| 28 | K131 | 12.5±0.5 | 8.5±0.3 | 12.5±0.6 | 11±0.3 | 16±0.3 | 16.3±0.7 |
| 29 | K132 | 6.8±0.5 | 22±0.7 | 6±0.5 | 10±0.7 | 8.5±0.3 | 9.3±0.3 |
| 30 | K134 | 18.5±0.1 | 12±0.6 | 10±0.3 | - | 20±0.1 | 8.5±0.4 |

*Note:* ***( - ):*** *No resistance*

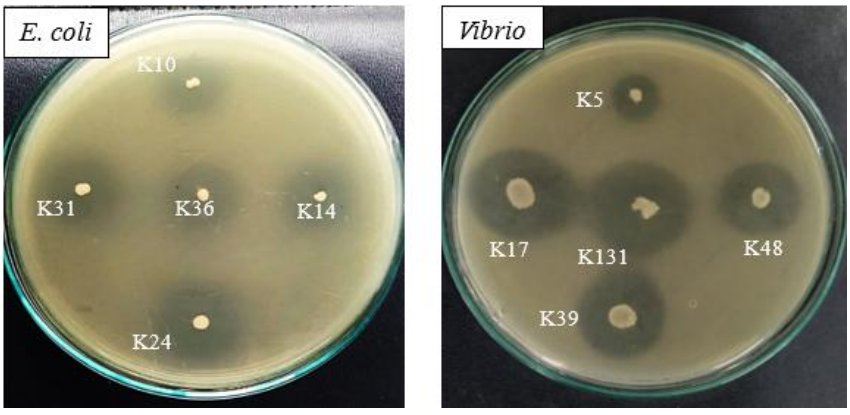


***Figure 1.*** *Antimicrobial activity of selected LAB*

### 3.2. Acid tolerance

LAB are acidophilic in nature, that is, they are tolerant to low pH. However, their growth is affected due to the presence of high concentrations of free acids, as they have growth-inhibitory effects [4]. Potential probiotic strains must be able to survive harsh gastric conditions, typically at pH 2 for at least 3 h, to be considered for further evaluation [22]. The result of the study revealed that all 10 selected strains could withstand a pH 2 for 3 h. However, their survival rates varied significantly, ranging from 26.25% to 86.3% (**Figure 2**). Among these, strains K24, K36, K14, K10, K5, and K131 demonstrated the highest survival rate (86.3%, 83.3%, 73.33%, 70.95%, 64.57% and 62.14%, respectively). Strain K48 had the lowest survival rate at 26.25%. These findings suggest that 6/10 strains had a survival rate at pH 2 above 60%.

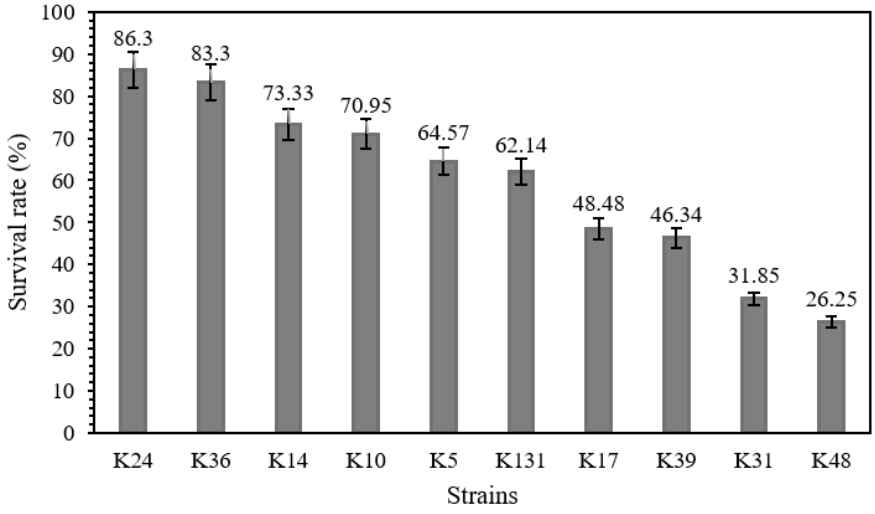


***Figure 2.*** *Acid tolerance of LAB at pH 2 for 3 h*

The survival rates observed in this study were lower than those reported by Nabi Jomehzadeh *et al.* (2020), who examined the survival rate after 2 h in a pH 2.5 environment of 30 LAB strains isolated from infant feces in Southwestern Iran, with 27/30 strains having a survival rate above 60% [23]. This difference is due to the shorter incubation time and at a higher pH of 30 strains of lactic bacteria in the study of Nabi Jomehzadeh *et al.* (2020). In contrast, Duong Nhat Linh *et al.* (2011), with only 4 out of 5 strains surviving at pH 2-3 for 3 h, and none exceeding 60% survival [24]. According to Huynh Ngoc Thanh Tam *et al.* (2023), there are 9/9 strains of lactic bacteria isolated from cherry that are capable of surviving at pH = 2.5 after 3 h, with survival rates of 49 - 59.13% [8]. Thus, this indicates that the survival rate of human-isolated strains of LAB was more resistant to low acidity conditions than those isolated from traditional foods. Additionally, Yasemin Kaya *et al.* (2022) found that LAB isolated from infant feces had a greater ability to withstand acidic conditions than those from sourdough [25]. The ability to survive in an acidic environment depends on the bacterial strain. Strains isolated from feces show better acid tolerance because they have adapted to the low-pH gut environment [9, 25].

### 3.3. Bile salt tolerance

Another challenge to the survival of microorganisms in the human intestinal tract is the presence of bile in the intestine. Before reaching the intestinal tract, probiotic bacteria must first survive in the bile in the small intestine for about 3 h [9]. For the human body, a bile salt concentration of 0.3% is considered the average concentration present in the small intestine [15]. The results showed that all 10 strains exhibited a remarkable survival rate exceeding 70%. Furthermore, 5/10 strains, namely K48, K36, K131, K24, and K14, demonstrated an exceptional survival rate of over 90% (**Figure 3**).

The results of this study are similar to the study of Duong Nhat Linh *et al.* (2011) on the tolerance to bile salts of lactic bacteria isolates from breast milk, with the survival rate of most strains in bile salts of 0.3%, 3 h, over 90% [24]. Another study by Nabi Jomehzadeh *et al.* (2020) on the tolerance to 0.3% bile salt for 8 h of 30 strains of lactic bacteria isolated from infant feces in Southwest Iran showed lower results, with 8 strains surviving over 90%, 13 strains having a survival rate of over 70%, and 9 strains surviving over 50% [23]. This

can be explained by the difference in incubation time in the bile salt medium of 0.3%. Jia-Xin Lin *et al.* (2022), when studying the tolerance to 0.3% concentration of bile salts of 16 strains of lactic bacteria isolated from mulberry fermentation fluid, the results showed that 8 strains had a survival rate of over 90%, 3 strains had a survival rate of over 70%, and the remaining strains all had a survival rate of over 60% [26]. This result indicates that the studied strains exhibited higher survival rates than those reported by Jia-Xin Lin *et al.* (2022). This suggests that strains of LAB isolated from meconium were more resistant to survive the bile salt conditions of the human body than strains isolated from fermentation products. Based on the aforementioned research results, five strains with high survival rates were selected: K24, K10, K14, K36, and K131. These five strains will be used for further studies.

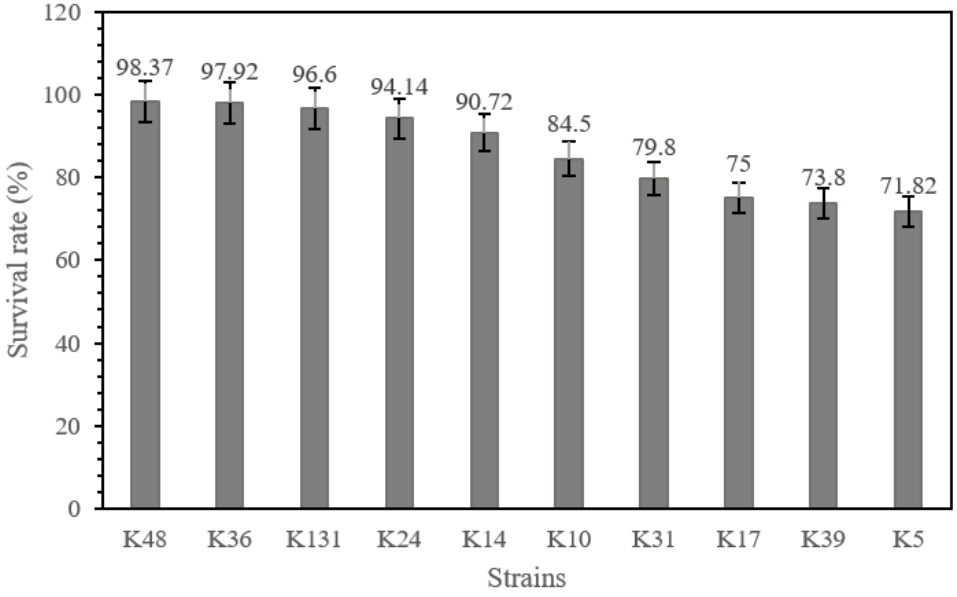


***Figure 3:*** *Ability of LAB to tolerate 0.3% (w/v) bile for 3 h*

### 3.4. Auto-aggregation ability

The ability of LAB to self-aggregate is a key characteristic that facilitates the formation of larger bacterial communities, enhancing their survival and growth within the gastrointestinal tract [27]. This symbiotic relationship contributes to the strain's ability to persist in the host. As a result, auto-aggregation is a significant criterion for screening potential probiotic strains. The auto-aggregation capacity of the 5 selected strains is illustrated in **Figure 4**.

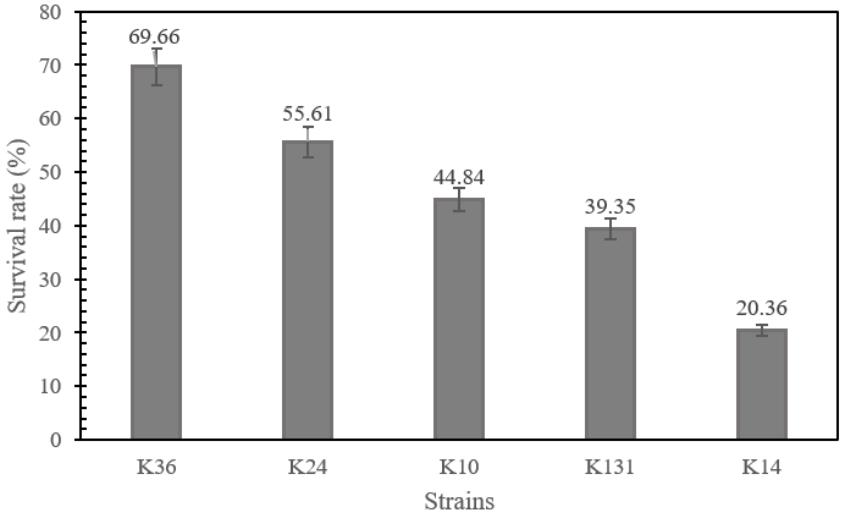


***Figure 4****. Auto-aggregation rate of 5 research strains*

Experimental results showed that all 5 selected bacterial strains exhibited auto-aggregation ability. The K36 strain showed the highest auto-aggregation rate of 69.66%. The K14 strain had the lowest aggregation rate of 20.36%. These findings are consistent with the study by Xing Wang *et al.* (2020) on the auto-aggregation ability of 12 LAB strains isolated from infant feces, with rates ranging from 5% to 60% [28]. Another study by Huynh Ngoc Thanh Tam *et al.* (2023) showed that the auto-aggregation ability of potentially probiotic LAB from acerola was lower, ranging from 10.28% to 49.35% [8]. Shubham Gupta *et al.* (2021), when studying the auto-aggregation ability of LAB strains isolated from traditional fermented fish, also reported lower results, ranging from 30.7% to 45.92% [29]. Thereby, the auto-aggregation ability of the 5 strains isolated from meconium was found to be significantly higher than that of LAB strains obtained from other sources.

### 3.5. Antibiotic resistance assessment

Currently, the overuse of antibiotics is becoming increasingly common. These drugs can inadvertently kill beneficial bacteria in the gut, leading to an imbalance in the gut ecosystem [8]. The level of antibiotic resistance of probiotic bacteria is considered one of the important criteria in the selection of probiotic bacteria [30]. The antibiotic susceptibility of the selected strains is presented in **Table 2**.

***Table 2:*** *Antibiotic susceptibility of 5 study strains*

| No. | Strain | Penicillin G | Cephalexin | Tetracycline | Amoxicillin-clavulanate | Erythromycin |
|---|---|---|---|---|---|---|
| **1** | K10 | R | R | R | R | R |
| **2** | K14 | R | R | R | S | R |
| **3** | K24 | R | R | R | S | I |
| **4** | K36 | R | R | S | S | R |
| **5** | K131 | R | R | R | S | R |

*Notes: R: Resistance, S: Sensitivity, I: Intermediate*

As shown in **Table 2**, all five strains (5/5) exhibited resistance to penicillin G and cephalexin. Resistance to tetracycline and erythromycin was observed in 4/5 strains, whereas only 1/5 strains showed resistance to amoxicillin–clavulanate. In a study by Hadiza Umar Meleh *et al.* (2020), most LAB strains isolated from the vaginas of healthy women were found to be susceptible to amoxicillin-clavulanate [31]. This is likely due to amoxicillin's ability to inhibit cell wall synthesis in Gram-positive bacteria, leading to cell death. The addition of clavulanic acid further enhances the effectiveness of amoxicillin by inhibiting bacterial β-lactamase enzymes that can break down amoxicillin [32]. According to the study of Vikas Jha *et al.* (2022), when studying the tetracycline susceptibility of 9 strains of lactic bacteria isolated from cherry fruit, 6/9 strains of tetracycline resistance were found [4]. Keunho Ji *et al.* (2015) investigated the tetracycline resistance of 12 LAB strains isolated from kimchi and infant feces and found that all 12 strains were susceptible to tetracycline [14]. According to Do Thi Bich Thuy (2022), antibiotic resistance in LAB strains is associated with certain genes located on transposons or plasmids [9, 33]. If antibiotic resistance is high, there is a high risk of transferring antibiotic-resistance genes to pathogens or intestinal bacteria [34]. Nevertheless, the potential transfer of resistance genes from probiotics to pathogenic bacteria remains a concern and warrants further research.

Despite these concerns, moderate antibiotic resistance may provide a functional advantage when probiotics are co-administered with antibiotics. Highly susceptible strains may be eliminated during antibiotic therapy, reducing their efficacy [35]. Therefore, a balanced resistance profile is desirable, ensuring both safety and functional viability.

### 3.6. Identification of selected probiotic LAB

Among 30 LAB strains, strains K24 and K36 exhibited all probiotic properties and were taxonomically classified for subsequent research. The results showed that the K24 strain belonged to the species *L. plantarum*. The K36 strain belongs to the species *L. acidophilus* (**Figure 5**).

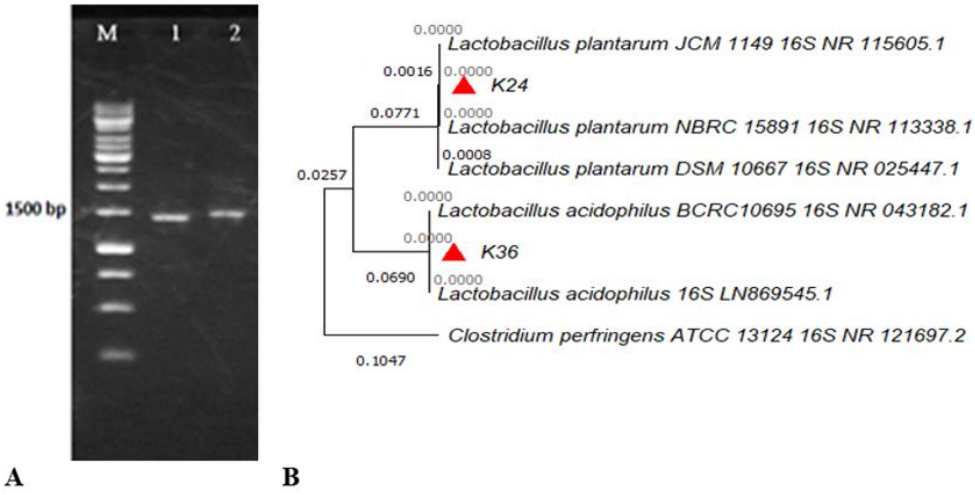


***Figure 5.*** *A. PCR products of 2 strains: M: Maker, 1: K24; 2: K36; B. Phylogenetic tree*

## 4. CONCLUSION

Among 30 screened LAB strains, strains K24 and K36 demonstrated strong resistance to all six pathogenic bacteria, acid and bile salt tolerance, high auto-aggregation, and resistance to certain antibiotics. Molecular identification based on 16S rRNA gene sequencing revealed that strain K24 belongs to *L. plantarum* and strain K36 was identified as *L. acidophilus*. These findings suggest that both strains may be promising candidates for probiotic applications in human health. However, further studies are required to evaluate their safety, including the assessment of transferable antibiotic resistance genes, as well as to confirm their efficacy through *in vivo* models and clinical studies.

## REFERENCES

[1]. C. Hill, F. Guarner, G. Reid, G. R. Gibson, D. J. Merenstein, and B. Pot, "The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic," *Nature Reviews Gastroenterology & Hepatology*, vol. 11, no. 6, pp. 506-514, 2014.

[2]. K. Angmo, A. Kumari, T. C. Bhalla, "Probiotic characterization of lactic acid bacteria isolated from fermented foods and beverages of Ladakh," *LWT*, vol. 66, pp. 428-435, 2016.

[3]. K. Ishikawa, M. Mayer, T. Miyazima, V. Matsubara, E.G. Silva, C. Paula, T. Campos, A. Nakamae, "A multispecies probiotic reduces oral *Candida* colonization in denture wearers," *Journal of Prosthodontics*, vol. 24, no. 3, pp. 194-199, 2015.

[4]. V. Jha, C. Sarang, D. Sawant, K. Nalawade, V. Dhamapurkar, N. Kaur, K. Thakur, S. Amin, P. Mane, and A. Marath, "Exploration of probiotic potential of lactic acid bacteria isolated from different food sources," *American Journal of BioScience*, vol. 3, no. 118-130, pp. 10, 2022.

[5]. B. Johnston, A. Supina and S. Vohra, "Probiotics for pediatric antibiotic-associated diarrhea: A meta-analysis of randomized placebo-controlled trials," *Canadian Medical Association Journal*, vol. 175, no. 4, pp. 377-383, 2006.

[6]. D. Bunt, A. Minnaard, and S. E. Aidy, "Potential modulatory microbiome therapies for prevention or treatment of inflammatory bowel diseases," *Pharmaceuticals*, vol. 14, iss. 6, pp. 506, 2021.

[7]. G.S. Mohamed, S.H.D. Masry, M.A. Nourhan, L. R. Fouad, B. M. Shaher, A.E. Sobhy, "Probiotic potential of lactic acid bacteria isolated from honeybees stomach: Functional and technological insights," *Annals of Agricultural Sciences*, vol. 69, pp. 11-18, 2024.

[8]. N. T. T. Huynh, Y. N. Huynh, C. T. Lam, T. B. Nguyen, "Identification of lactic acid bacteria in acerola fruit (*Malpighia glabra* L.) and potential probiotic properties of selected Lactobacillus strains," *CTU Journal of Science*, vol. 59, no. 6B, pp. 189-197, 2023.

[9]. T. B. T. Do, *Probiotic and Prebiotic*, Hue: Hue University Publishing House, 2023.

[10]. C. Gheziel, P. Russo, M.P. Arena, G. Spano, H. Ouzari, O. Kheroua, D. Saidi, D. Fiocco, H. Kaddouri, and H. Capozzi, "Evaluating the probiotic potential of *Lactobacillus plantarum* strains from Algerian infant feces: Towards the design of probiotic starter cultures tailored for developing countries," *Probiotics and Antimicrobial Proteins*, vol. 11, iss. 1, pp. 113-123, 2019.

[11]. G. Zavisic, S. Petricevic, S. Ristic, M. Rikalovic, N. Jovanovic-Ljeskovic, J. Begovic, and I. Strahinic, "Probiotic potential of *Lactobacillus fermentum* G-4 originated from newborns' meconium," *Journal of the Serbian Chemical Society*, vol. 84, no. 4, pp. 365-376, 2019.

[12]. T. P. D. Ngo, T. Y. L. Huynh, X. P. Huynh, "Isolation and selection of lactic acid bacteria producing anti-bacterial substances," *CTU Journal of Science*, vol. 19a, pp. 176-184, 2011.

[13]. H. T. M. Tu, C. T. Binh, N. X. Phuong, N. P. Nhue, N. K. Thoa, T. T. Thuy, N. T. Trang, and L. T. Xuan, "Antimicrobial activity of several lactic acid bacteria against pathogenic and foodborne microorganisms," *Journal of Military and Pharmaceutical Medicine*, vol. 353, pp. 15-21, 2021 (in Vietnamese).

[14]. [14] K. Ji, N.A. Jang and Y.T. Kim, "Isolation of lactic acid bacteria showing antioxidative and probiotic activities from kimchi and infant feces," *Journal of Microbiology and Biotechnology*, vol. 25, no. 9, pp. 1568-1577, 2015.

[15]. S.E. Gilliland, C.R. Nelson and C. Maxwell, "Assimilation of cholesterol by *Lactobacillus acidophilus*," *Applied and Environmental Microbiology*, vol. 49, no. 2, pp. 377-381, 1985.

[16]. B. Kos, M.J. Suskovic, S. Vukovic, M. Simpraga and J. Frece, "Adhesion and aggregation ability of probiotic strain *Lactobacillus acidophilus* M92," *Journal of Applied Microbiology*, vol. 94, pp. 981-987, 2003.

[17]. A. W. Bauer, M. D. Kirby, J. C. Sherris, and M. Turck, "Antibiotic susceptibility testing by a standardized single disk method." *American Journal of Clinical Pathology*, vol. 45, pp. 493-496, 1966.

[18]. Clinical and Laboratory Standards Institute, "M100 Performance Standards for Antimicrobial Susceptibility Testing," CLSI, 2021.

[19]. P. D. Khoi, T. Q. Hoa, and N. T. N. Nhi, "An overview of in vitro tests used for probiotic screening Lactobacillus spp. potential," *Thu Dau Mot University Journal of Science*, vol. 68, no. 1, pp. 50-61, 2024 (in Vietnamese).

[20]. S. Yalian, L. Xiuyu, Z. Xuan, J. Han, and G. Qing, "Isolation and characterization of lactic acid bacteria producing bacteriocin from newborn infants feces," *Journal of Bacteriology and Mycology*, vol. 1, iss. 2, 2014.

[21]. W. Zhang, S. Lai, Z. Zhou, J. Yang, H. Liu, Z. Zhong, H. Fu, Z. Ren, L. Shen, S. Cao, L. Deng, and G. Peng, "Screening and evaluation of lactic acid bacteria with probiotic potential from local Holstein raw milk," *Frontiers in Microbiology*, vol.13, 2022.

[22]. N. T. D. Huong and D. T. B. Thuy, "Identification and investigation of some beneficial properties of *Lactobacillus fermentum* DC1 strain isolated from Hue pickled mustard greens," *Hue University Journal of Science*, vol. 2, no. 71, 2012 (in Vietnamese).

[23]. N. Jomehzadeh, H. Javaherizadeh, M. Amin, M. Saki, M. AI-Ouqaili, H. Hamidi, M. Seyedmahmoudi, and Z. Gorjian, "Isolation and identification of potential probiotic *Lactobacillus* species from feces of infants in southwest Iran," *International Journal of Infectious Diseases*, vol. 96, pp. 524-530, 2020.

[24]. D. N. Linh, N. V. Minh, T. T. K. Sa, and T. C. Dong, "Probiotic potential of lactic acid bacteria isolated from breast milk," *Ho Chi Minh City Open University Journal of Science*, vol. 6, iss. 1, pp. 67-73, 2011 (in Vietnamese).

[25]. Y. Kaya, T. Erten, M. Vurmaz, H. Ispirli, and O. Simek, "Comparison of the probiotic characteristics of lactic acid bacteria (LAB) isolated from sourdough and infant feces," *Food Bioscience*, vol. 47, 2022.

[26]. J.X. Lin, T. Xiong, Z. Peng, M. Xie, and F. Peng, "Novel lactic acid bacteria with anti-hyperuricemia ability: Screening and in vitro probiotic characteristics," *Food Bioscience*, vol. 49, 2022.

[27]. D. T. B. Thuy, N. T. D. Huong, and D. T. T. Thanh, "Determination of some beneficial properties of lactic acid bacterial strains isolated from mam ruoc Hue," *Hue Journal of Agricultural Science and Technology*, vol. 3, no. 3, pp. 1458–1467, 2019 (in Vietnamese).

[28]. X. Wang, W. Wang, L.V. Haoxin, H. Zhang, Y. Liu, M. Zhang, Y. Wang, and Z. Tan, "Probiotic potential and wide-spectrum antimicrobial activity of lactic acid bacteria isolated from infant feces," *Probiotics and Antimicrobial Proteins*, vol. 13, iss. 1, pp. 90-101, 2020.

[29]. S. Gupta, U. Mohanty, and R. K. Majumdar, "Isolation and characterization of lactic acid bacteria from traditional fermented fish product Shidal of India with reference to their probiotic potential," *LWT*, vol. 146, 2021.

[30]. A. S. Hummel, C. Hertel, W. H. Holzapeel and C. M. Franz, "Antibiotic resistances of starter and probiotic strains of lactic acid bacteria," *Applied and Environmental Microbiology*, vol. 73, iss. 3, pp. 730-739, 2007.

[31]. H. U. Meleh, S. Choo, M.N.M. Desa, S.Y. Chew, P. Rangasamy, H. Hassan, and L. T. L Than, "Isolation and safety characterisation of Lactobacilli strains with antimicrobial properties as potential probiotics for human use," *LWT*, vol. 131, 2020.

[32]. A. Hunttner, J. Bielicki, M. N. Clements, N. Frimodt-Moller, A. E. Muller, J. -P. Paccaud and J. W. Mouton, "Oral amoxicillin and amoxicillin - clauvulanic acid: properties, indications and usage," *Clinical Microbiology and Infection*, vol. 26, iss.7, pp. 871-879, 2020.

[33]. Do Thi Bich Thuy, *Some beneficial properties of lactic acid bacteria*, Hue: Hue University Publishing House, 2022.

[34]. C. Liu, Z. Y. Zhang, K. Dong, J. P. Yuan, and X. K. Guo, "Antibiotic resistance of probiotic strains of lactic acid bacteria isolated from marketed foods and drugs," *Biomedical and Environmental Sciences*, vol. 22, iss. 5, pp. 401 - 412, 2009.

[35]. G. H. Choi, J. I. I. Fugaban, and C. M. Dioso, "Selection of bacteriocinogenic *Bacillus* spp. from traditional fermented Korean food products with additional beneficial properties," *Fermentation*, vol. 7, iss. 4, 2021.

.