



## Investigation of the Probiotic Properties of Some Cattle-Origin Bacterial Isolates (*Lactobacillus* spp., *Enterococcus faecium*, and *Escherichia coli*)

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**Abstract:** In this study, *Lactobacillus* spp. (56 isolates), *Enterococcus faecium* (25 isolates), and *Escherichia coli* (33 isolates) were isolated from bovine ceca, and their probiotic properties were investigated in vitro. The isolates were evaluated for bile salt tolerance at different concentrations (0.6% and 1.5%), survival rates at pH=2 and pH=7, cell surface hydrophobicity, antibacterial activity against the *E. coli* K99 strain, and susceptibility to six antibiotics from five different classes (ampicillin, trimethoprim–sulfamethoxazole, neomycin, enrofloxacin, amoxicillin-clavulanic acid, and tetracycline). All *E. faecium* and *E. coli* isolates, as well as all but two *Lactobacillus* spp. isolates, were able to grow at 0.6% bile concentration. At 1.5% bile concentration, all *E. coli* isolates and all but two *Lactobacillus* spp. and one *E. faecium* isolate exhibited growth. The viability rates at pH=2 were determined as 34–52% for *Lactobacillus* spp. (14 isolates), 35% for *E. faecium* (1 isolate), and 34–55% for *E. coli* (4 isolates). Hydrophobicity was observed in 19 *Lactobacillus* spp., 14 *E. faecium*, and 14 *E. coli* isolates, respectively, while none of the isolates exhibited antibacterial activity against the *E. coli* K99 strain. Ampicillin was identified as the antibiotic to which the isolates showed the highest resistance, with rates of 92.85% for *Lactobacillus* spp., 96% for *E. faecium*, and 100% for *E. coli*. In conclusion, based on their tolerance to bile salts and low pH, hydrophobic properties, and antibiotic resistance profiles, it was revealed that especially the *Lactobacillus* spp. M22 isolate possesses a high potential for use as a probiotic candidate.

**Keywords:** *E. coli*, *E. faecium*, *Lactobacillus* spp., probiotic, cow

## INTRODUCTION

The term *probiotic*, derived from the Greek words pro (for) and bios (life), was first used by Vergin (1954) in the sense of ‘for life’. The concept was introduced into the scientific literature by Lilly and Stillwell (1965), who defined it as a microbial substance produced by one microorganism that stimulates the growth of another. Later, Parker (1974) described probiotics as organisms and substances that contribute to the intestinal microbial balance a definition quite close to the current understanding. In 2001, the FAO/WHO expert committee defined probiotics as “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host,” a definition that

has since gained wide acceptance (FAO/WHO, 2002). Today, probiotics include bacterial species such as *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Streptococcus thermophilus*, and *Escherichia coli* Nissle 1917, as well as yeast species such as *Saccharomyces boulardii* (McFarland and Meta, 2006; Hossain et al., 2012). Probiotics exert their effects in the gastrointestinal tract through mechanisms such as competitive inhibition against pathogens, production of antimicrobial substances, enhancement of intestinal barrier integrity, and modulation of the immune system (Bermudez-Brito et al., 2012). In animal production, the use of probiotics has gained importance, particularly in intensive livestock systems, due to the increasing demand for productivity and the restrictions on antibiotic use. Studies have demonstrated that probiotic supplementation in cattle can positively influence milk yield (Nocek and Kautz, 2006; Boyd et al., 2011), body weight gain (Frizzo et al., 2011), and feed conversion efficiency (Jatkauskas and Vrotniakiene, 2010). Additional benefits such as improved rumen fermentation, enhanced immune response, and reduced disease incidence have also been reported (Desnoyers et al., 2009; Timmerman et al., 2005).

This study was conducted to investigate the in vitro probiotic potential of cattle-derived *Lactobacillus* spp., *E. faecium*, and *E. coli* isolates. The isolates were evaluated in terms of bile and pH tolerance, cell surface hydrophobicity, bacteriocin production, antimicrobial activity, and antibiotic resistance profiles. Based on the obtained results, their suitability for use as probiotics was assessed. Determining isolates with strong probiotic potential is particularly important for promoting healthy livestock production systems and reducing antibiotic usage.

## **MATERIAL AND METHOD**

### ***Sample Collection and Bacterial Isolation***

In this study, cecal samples were collected from 25 clinically healthy cattle in slaughtered located in Suluova (Amasya) and Bafra (Samsun), Türkiye. Samples were aseptically transferred into sterile containers and transported under cold-chain conditions to the Microbiology Laboratory, Faculty of Veterinary Medicine, Ondokuz Mayıs University. Mucosal scrapings were homogenized in 50 mL of physiological saline (PS), vortexed, and serially processed. A 0.1 mL aliquot from each sample was spread, in triplicate, onto three selective media. For the isolation of *Lactobacillus* spp., samples were

plated onto MRS agar and incubated anaerobically at 35°C for 48 h. Colonies exhibiting Gram-positive rod morphology and catalase-negative reactions were identified as presumptive *Lactobacillus* isolates, purified, and stored at -20°C in glycerol-supplemented MRS broth (Victor et al., 2011). For the isolation of *Enterococcus faecium*, samples were streaked onto Bile Esculin Agar (BEA) and incubated aerobically at 37°C for 24 h. Black colonies showing Gram-positive coccoid morphology and catalase negativity were considered presumptive *E. faecium* and stored in glycerol-enriched Tryptic Soy Broth (TSB) (Strompfová et al., 2004). For the isolation of *Escherichia coli*, Eosin Methylene Blue (EMB) agar was used. Colonies with a metallic green sheen, Gram-negative rod morphology, catalase positivity, and oxidase negativity were identified as presumptive *E. coli* isolates. These were purified and preserved at -20°C in glycerol-supplemented TSB (Baker et al., 2013).

#### ***DNA Extraction***

Genomic DNA of presumptive *Lactobacillus* spp., *E. faecium*, and *E. coli* isolates was extracted using the boiling method. A loopful of fresh colonies cultured on MRS or TSA was suspended in 500 µL distilled water, boiled at 100°C for 10 min, and centrifuged at 11000 × g for 3 min. Supernatants were stored at -20°C until PCR analysis (Pasayo et al., 2019).

#### ***Genotypic Identification***

Species-level confirmation was performed using PCR with species-specific primers. Extracted DNA samples were amplified under appropriate cycling conditions to target the diagnostic gene regions for *Lactobacillus* spp., *E. faecium*, and *E. coli*. Primer sequences and expected amplicon sizes are shown in Table 1 (Markiewicz & Biedrzycka, 2005; Jackson et al., 2004; Abd El-Razik et al., 2010).

**Table 1.** Oligonucleotide primer sequences used in the study

Primer	Primer sequence (5'-3')	Amplicon size (bp)	References
Lacto F	TGGAAACAGGTGCTAATACCG	230	Markiewicz and Biedrzycka (2005)
Lacto R	CCATTGTGGAAGATTCCC		
FM1	GAAAAACAATAGAAGAATTAT	215	Jackson et al. (2004)
FM2	TGCTTTTTGAATTCTTCTTTA		
Eco 2083	GCTTGACACTGAACATTGAG	662	Abd El-Razik et al. (2010)
Eco 2745	GCACTTATCTCTCCGCATT		

### ***Bile Tolerance Test***

Bile tolerance was assessed by supplementing MRS broth (for *Lactobacillus* spp.) and TSB (for *E. faecium* and *E. coli*) with natural bovine bile at final concentrations of 0.6% and 1.5%. Isolates were inoculated and incubated at 35 °C for 48 h (*Lactobacillus*) or 24 h (*E. faecium* and *E. coli*). Following incubation, cultures were serially diluted to  $10^{-9}$ . From the  $10^{-5}$ ,  $10^{-6}$ , and  $10^{-7}$  dilutions, 100  $\mu$ L samples were plated onto MRS agar for *Lactobacillus* spp. isolates and onto TSA for *E. faecium* and *E. coli* isolates. Plates were incubated under appropriate conditions. Bacterial growth was evaluated after incubation, and bile tolerance was determined (Strompfova et al., 2004).

### ***pH Tolerance Test***

To determine the pH tolerance of the isolates, MRS broth was used for *Lactobacillus* spp., and TSB was used for *E. faecium* and *E. coli*. The pH was adjusted to 2 and 7 using HCl and NaOH solutions. Bacterial suspensions standardized to 0.5 McFarland were inoculated and incubated anaerobically (for *Lactobacillus*) or aerobically (for *E. faecium* and *E. coli*). After incubation, serial dilutions ( $10^{-1}$ – $10^{-3}$  for pH=2;  $10^{-5}$ – $10^{-7}$  for pH=7) were plated and viable counts determined to assess acid resistance (Strompfova et al., 2004).

### ***Hydrophobicity of the Isolates***

To determine the hydrophobicity of the isolates, TSA and MRS agars were prepared with the addition of 0.03% Congo Red. *Lactobacillus* spp. were plated onto MRS agar, whereas *E. faecium* and *E. coli* were plated onto TSA and incubated under appropriate conditions. After incubation, isolates forming pink–red colonies were classified as hydrophobic, and colorless colonies as non-hydrophobic (Sharma et al., 2006).

### ***Bacteriocin Extraction***

*Lactobacillus* spp. isolates were inoculated into MRS broth, *E. faecium* and *E. coli* isolates were inoculated into TSB and incubated under appropriate conditions. The cultures were centrifuged at 9000 rpm for 30 minutes to obtain the supernatants. For bacteriocin precipitation, ammonium sulfate was added to the supernatants at concentrations ranging from 40% to 80% and stirred at +4°C for 24 hours. The precipitates obtained at the most effective concentration were centrifuged at 14.000 rpm for 1 hour, dissolved in physiological saline, and prepared for subsequent analyses (Foulquie Moreno et al., 2003).

### ***Determination of Antibacterial Activity***

Antibacterial activity of the extracted bacteriocins was tested against *E. coli* K99 using the agar diffusion method. Mueller–Hinton Agar (MHA) plates were prepared, wells were punched into the agar, and bacteriocin suspensions were loaded into the wells. A 100 µg/mL ofloxacin disk served as the positive control. Plates were incubated, and inhibition zones were measured using a caliper (Tag and McGiven, 1971; Reinhamier et al., 1990).

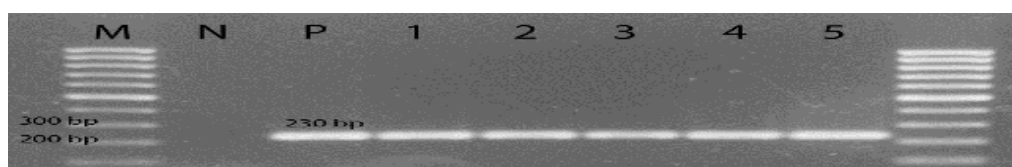
### ***Antibiotic Susceptibility Testing***

Antibiotic susceptibility was determined using the disk diffusion method. MRS agar was used for *Lactobacillus* spp. and MHA for *E. faecium* and *E. coli*. Antibiotic disks included ampicillin (10 µg), trimethoprim–sulfamethoxazole (1.25/23.75 µg), neomycin (30 µg), enrofloxacin (5 µg), amoxicillin–clavulanic acid (20/10 µg), and oxytetracycline (30 µg). Plates were incubated following incubation, the diameters of the inhibition zones were measured and evaluated according to CLSI guidelines (CLSI 2020). Isolates were classified as susceptible (S), intermediate (I), or resistant (R).

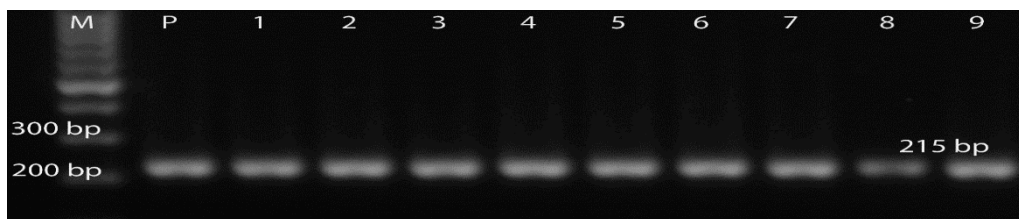
## **RESULTS**

### ***Bacterial Isolation and Genotypic Identification***

A total of 67 *Lactobacillus* spp., 47 *Enterococcus faecium*, and 33 presumptive *Escherichia coli* colonies were initially isolated. Colonies were initially identified based on their morphological and biochemical characteristics. PCR-based genotypic identification produced clear, species-specific amplicons of 230 bp for *Lactobacillus* spp., 215 bp for *E. faecium*, and 662 bp for *E. coli*. Based on these results, 56 *Lactobacillus* spp., 25 *E. faecium*, and 33 *E. coli* isolates were confirmed and stored at –20 °C for further analysis. PCR images are shown in Figure 1, Figure 2, and Figure 3.



**Figure 1.** PCR image of *Lactobacillus* spp. (M: Marker, P: Positive Control, N: Negative Control)



**Figure 2.** PCR image of *E. faecium* (M: Marker, P: Positive Control)



**Figure 3.** PCR image of *E. coli* (M: Marker, P: Positive Control, N: Negative Control, 1-12: Positive *E. coli* isolates)

### ***Bile Tolerance Test Results***

Bile tolerance analysis showed that two *Lactobacillus* isolates (M12, M14) and one *E. faecium* isolate (B22) exhibited no growth at either bile concentration. One *E. faecium* isolate (B14) grew at 0.6% bile but failed to grow at 1.5%. All *E. coli* isolates were able to grow at both bile concentrations.

At 0.6% bile, the lowest viability among *Lactobacillus* spp. was observed in isolate M6 (77%), whereas the highest was recorded for M54 (100%). For *E. faecium*, viability ranged from 87% (B14) to 99% (B13). Among *E. coli* isolates, viability values varied between 93% (E10) and 99% (E3).

At 1.5% bile, *Lactobacillus* spp. viability ranged from 76% (M6) to 98% (M25). *E. faecium* isolates showed viability values between 82% (B12) and 97% (B2). For *E. coli*, the lowest viability was recorded for E1 (85%) and the highest for E19 (99%).

Overall, all isolates tested in this study maintained high survival levels at both 0.6% and 1.5% bile salt concentrations. The isolates M2, M8, M9, M10, M13, M16, M23, M25, M29, M30, M32, M33, M35, M36, M38, M41, M43, M50, M54, M55, B2, B3, B5, B8, B13, B17, B18, B19, B24, E4, E9, E14, E15, E17, E19, E22, E23, E25, E27, E28, E29, E30, E31, E32, and E33 exhibited viability levels above 95% at both bile concentrations.

### *Acid Tolerance Test*

**Table 2.** Acid tolerance test results of isolates for pH=7 (log cfu/ml)

<b>İzolat No</b>	<b>log kob/ml</b>	<b>İzolat No</b>	<b>log kob/ml</b>	<b>İzolat No</b>	<b>log kob/ml</b>	<b>İzolat No</b>	<b>log kob/ml</b>
<b>M1</b>	8.00	<b>M29</b>	9.11	<b>M56</b>	8.54	<b>E3</b>	8.70
<b>M2</b>	8.46	<b>M30</b>	8.33	<b>B1</b>	9.50	<b>E4</b>	8.84
<b>M3</b>	8.85	<b>M31</b>	8.15	<b>B2</b>	8.47	<b>E5</b>	8.97
<b>M4</b>	8.77	<b>M32</b>	8.22	<b>B3</b>	8.96	<b>E6</b>	8.86
<b>M5</b>	8.85	<b>M33</b>	8.68	<b>B4</b>	8.79	<b>E7</b>	8.74
<b>M6</b>	8.77	<b>M34</b>	8.82	<b>B5</b>	8.89	<b>E8</b>	8.92
<b>M7</b>	8.08	<b>M35</b>	8.85	<b>B6</b>	9.45	<b>E9</b>	8.76
<b>M8</b>	8.14	<b>M36</b>	9.00	<b>B7</b>	9.50	<b>E10</b>	8.86
<b>M9</b>	8.98	<b>M36</b>	9.00	<b>B8</b>	8.79	<b>E11</b>	9.00
<b>M10</b>	8.85	<b>M37</b>	8.38	<b>B9</b>	8.96	<b>E12</b>	8.82
<b>M11</b>	8.85	<b>M38</b>	8.96	<b>B10</b>	8.77	<b>E13</b>	8.92
<b>M12</b>	8.11	<b>M39</b>	7.81	<b>B11</b>	8.98	<b>E14</b>	8.95
<b>M13</b>	8.83	<b>M40</b>	8.71	<b>B12</b>	8.33	<b>E15</b>	8.94
<b>M14</b>	7.46	<b>M41</b>	8.79	<b>B13</b>	7.93	<b>E16</b>	8.82
<b>M15</b>	8.62	<b>M42</b>	9.17	<b>B14</b>	8.09	<b>E17</b>	8.68
<b>M16</b>	7.30	<b>M43</b>	8.85	<b>B15</b>	8.96	<b>E18</b>	8.84
<b>M17</b>	9.19	<b>M44</b>	8.94	<b>B16</b>	9.12	<b>E19</b>	9.00
<b>M18</b>	9.23	<b>M45</b>	9.18	<b>B17</b>	8.97	<b>E20</b>	8.90
<b>M19</b>	8.94	<b>M46</b>	8.40	<b>B18</b>	8.90	<b>E21</b>	8.96
<b>M20</b>	9.31	<b>M47</b>	9.49	<b>B19</b>	8.74	<b>E22</b>	8.85
<b>M21</b>	8.75	<b>M48</b>	9.21	<b>B20</b>	9.27	<b>E23</b>	8.70
<b>M22</b>	8.52	<b>M49</b>	8.78	<b>B21</b>	8.24	<b>E24</b>	8.94
<b>M23</b>	9.00	<b>M50</b>	8.85	<b>B22</b>	7.49	<b>E25</b>	8.75
<b>M24</b>	8.84	<b>M51</b>	8.96	<b>B23</b>	9.12	<b>E26</b>	8.70
<b>M25</b>	8.47	<b>M52</b>	8.94	<b>B24</b>	8.77	<b>E27</b>	8.89
<b>M26</b>	8.93	<b>M53</b>	8.78	<b>B25</b>	8.68	<b>E28</b>	8.88
<b>M27</b>	8.84	<b>M54</b>	8.81	<b>E1</b>	8.93	<b>E29</b>	8.83
<b>M28</b>	8.52	<b>M55</b>	8.88	<b>E2</b>	8.88	<b>E30</b>	8.99
<b>E33</b>	8.83	<b>E32</b>	8.98	<b>E31</b>	9.00		

M: *Lactobacillus* spp., B: *E. faecium*, E: *E. coli*

Acid tolerance was assessed by determining isolate viability following incubation at pH=2 and pH=7. At pH=2, a total of 14 *Lactobacillus* spp., one *E. faecium*, and four *E. coli* isolates demonstrated survival. Survival rates were as follows: *Lactobacillus* spp., 34 (M40) -52(M1)%; *E. faecium*, 35%(B11); and *E. coli*, 34 (E1)-55 (E20)%. Among all isolates, the *E. coli* strain E20 exhibited the highest survival rate under acidic conditions. The isolates that remained viable at pH=2 were M1, M2, M3, M7, M18, M22, M26, M29, M32, M40, M51, M52, M54, M55, B11, E1, E19, E20, and E27. The survival rates of all isolates were calculated using their log CFU/mL values at pH=7 as the reference. These reference values are presented in Table 2.

### ***Hydrophobicity Test***

Hydrophobicity was assessed using Congo Red agar, and isolates producing pink–red colonies were classified as hydrophobic. The overall hydrophobicity rates were as follows: *Lactobacillus* spp., 33.92%; *E. faecium*, 56%; and *E. coli*, 42.42%. The isolates identified as hydrophobic were M1, M4, M8, M19, M20, M21, M22, M23, M24, M29, M36, M39, M42, M46, M48, M49, M50, M54, M56, B1, B2, B3, B6, B7, B8, B9, B11, B13, B15, B17, B18, B20, B25, E6, E7, E8, E9, E10, E11, E12, E13, E14, E15, E16, E17, E18, and E19.

### ***Antibacterial Activity of Bacteriocins***

The antagonistic effect of bacteriocins obtained from the isolates against *E. coli* K99 was evaluated using the Disk Diffusion Agar method. However, since no inhibition zones were observed, it was determined that none of the bacteriocins exhibited antibacterial activity.

### ***Antibiotic Susceptibility Profile***

The antibiotic susceptibility profiles of the isolates were determined using the Kirby–Bauer disk diffusion method with ampicillin (10 µg), amoxicillin–clavulanic acid (20/10 µg), oxytetracycline (30 µg), trimethoprim–sulfamethoxazole (1.25/23.75 µg), enrofloxacin (5 µg), and neomycin (30 µg) antibiotic disks. Among the *Lactobacillus* spp. isolates, the highest resistance rates were observed against ampicillin (93%) and trimethoprim–sulfamethoxazole (89%), with most isolates exhibiting resistance to these two antibiotics. In contrast, 75% of the isolates were found to be sensitive to oxytetracycline. Evaluation of multidrug resistance revealed that 91.07% of the 56 *Lactobacillus* spp. isolates were resistant to three or more antibiotics. Among the 51 isolates exhibiting multidrug resistance, 3 (5.35%) were resistant to six antibiotics, 7 (12.5%) to five antibiotics, 20 (35.71%) to four antibiotics, and 18 (32.14%) to three antibiotics.

For *Enterococcus faecium*, the highest resistance was detected against ampicillin (100%) and trimethoprim–sulfamethoxazole (60%), whereas all isolates were sensitive to amoxicillin–clavulanic acid. Assessment of multidrug resistance showed that 24% of the 25 *E. faecium* isolates were resistant to three or more antibiotics. Of the six isolates exhibiting multidrug resistance, three (12%) were resistant to four antibiotics and three (12%) to three antibiotics.

In *Escherichia coli* isolates, the highest resistance rate was recorded for ampicillin (100%), while the lowest resistance was observed for enrofloxacin (9.1%) and amoxicillin–clavulanic acid (3%). Evaluation of multidrug resistance revealed that 12.12% of the 33 *E. coli* isolates were resistant to three or more antibiotics. Among the four multidrug-resistant isolates, two (6.06%) were resistant to five antibiotics and two (6.06%) to four antibiotics.

Based on multiple in vitro assays, isolate M22 (*Lactobacillus* spp.) demonstrated a combination of favorable traits. A summary is shown in Table 3.

**Table 3.** Key probiotic properties of *Lactobacillus* spp. isolate M22

Parameters	Result for M22
Acid tolerance (pH=2)	42.28% viability
Bile tolerance 0.6%	91.93% viability
Bile tolerance 1.5%	89.23% viability
Cell surface hydrophobicity	Positive
Antibiotic profile	AMP (R), AMC (R), ENR (R), OTC (R), NEO (R), SXT (R)

## DISCUSSION

In this study, two *Lactobacillus* isolates (M12, M14) and one *Enterococcus faecium* isolate (B22) exhibited no growth at either bile concentration tested, while isolate B14 demonstrated sensitivity only at 1.5% bile. In contrast, all *Escherichia coli* isolates tolerated both bile concentrations. These findings are consistent with Liu et al. (2019), who reported that all *Lactobacillus* isolates and 75% of *Enterococcus* spp. obtained from panda feces were resistant to 0.3% bile salt. Previous research has also shown that cattle-derived *Lactobacillus* spp. can survive in 0.3% bile (Jobby et al., 2020), and that most LAB strains grow in bile concentrations ranging from 0.05% to 0.3% (Brashears et al., 2003). Similarly, *Bacillus* spp. isolated from cattle exhibited variable resistance to 0.3% bile salt (Naeem et al., 2018). Kassa et al. (2024) further demonstrated that LAB isolates from local chickens exhibited strong survival at 0.3% and 0.6% bile after 12-24 hours of incubation. Collectively, these results highlight that bile tolerance is species- and strain-dependent and confirm that resistance to bile salts is a key criterion for selecting potential probiotic candidates. Regarding acid tolerance, 14 *Lactobacillus* isolates survived exposure to pH=2, with viability ranging from 34-52%. Only one *E. faecium* isolate remained viable, whereas four *E. coli* isolates demonstrated acid tolerance with viability between 34-55%. These results indicate that a large proportion of isolates were sensitive

to highly acidic conditions. Comparable findings were reported by Rodriguez-Palacios et al. (2009), who observed that none of 106 bovine LAB isolates grew at pH 2. Likewise, Adetoye et al. (2018) found that only four *Lactobacillus* strains out of 88 bovine LAB isolates survived at pH 3. In contrast, Kassa et al. (2024) noted exceptionally high survival rates (83.6-97.1%) for LAB isolates exposed to pH 2 for 3–6 hours. Gupta et al. (2023) reported that only 27 of 90 LAB isolates tolerated pH 3, whereas Wu et al. (2025) found that eight of thirteen LAB isolates demonstrated low-pH tolerance. Additionally, Zhang et al. (2025) demonstrated significant interspecies differences in acid-adaptation capacity among *Lactobacillus* spp., with *L. crispatus* showing the highest resilience in simulated gastric fluid over 24 hours. Given that survival in acidic environments is essential for passage through the stomach, acid tolerance remains a critical parameter in probiotic strain selection. An important properties of probiotics is their ability to adhesion to host epithelial cells (Yu and Tsen, 1993; Lin et al., 2007). Wadstroum et al. (1987) noted that hydrophobic interactions may be necessary for colonizing the mucus layer. Zareba et al. (1997) suggested that high cell surface hydrophobicity plays a role in bacterial adhesion to epithelial cells. In the present study, hydrophobicity rates were 33.9% for *Lactobacillus* spp., 56% for *E. faecium*, and 42% for *E. coli*. These results are comparable to those reported by Grajek et al. (2016) for calf-derived *Lactobacillus* (26.9-41.1%) and *Enterococcus* (28.4-34.9%) isolates. Additionally, Marchwińska et al. (2022) found that among 41 LAB isolates from pig feces, seven exhibited high and eleven exhibited moderate hydrophobicity (22-63%). Qin et al. (2022) reported nearly 100% hydrophobicity in *Lactobacillus* isolates obtained from horse feces. Such variability across studies indicates that hydrophobicity is strongly strain-dependent and influenced by the host species of origin. No antibacterial activity was observed against *E. coli* K99 for any of the bacteriocin preparations tested. Liu et al. (2019) previously reported antimicrobial effects among panda-derived isolates against ETEC, *S. aureus*, and *Salmonella*, although these effects were attributed to non-proteinaceous factors. Adetoye et al. (2018) likewise reported inhibitory effects from bovine-derived lactobacilli that were not linked to bacteriocin production. Puphan et al. (2015) showed that 15 bovine LAB isolates inhibited *E. coli* ATCC 25923 and *Salmonella* Typhimurium. Bacteriocin production is known to be highly sensitive to environmental and cultural conditions including growth medium, pH, incubation duration, temperature, and inoculum density.

Co-culture strategies and quorum-sensing regulation can significantly enhance bacteriocin yields in LAB (He et al., 2025). Classical studies have shown that even minor alterations in growth conditions may reduce bacteriocin production by up to two orders of magnitude (Reeves, 1965). Therefore, the absence of observable antibacterial activity in this study may reflect insufficient bacteriocin concentration or unfavorable production conditions. Antibiotic susceptibility patterns varied among isolates. For *Lactobacillus* spp., the highest resistance was observed against ampicillin (92.9%), whereas resistance to other antibiotics remained comparatively low. Conversely, Jobby et al. (2020) reported broad susceptibility in cattle-derived *Lactobacillus* strains. Qin et al. (2022) reported that horse-derived isolates were susceptible to ampicillin and tetracycline but resistant to gentamicin and enrofloxacin. For *E. faecium*, resistance was highest against ampicillin (96%), trimethoprim–sulfamethoxazole (56%), and oxytetracycline (20%), consistent with literature describing substantial variability in antimicrobial resistance depending on host and region (Manu et al., 2003; Thal et al., 1995; De Jong et al., 2018). Among *E. coli* isolates, ampicillin resistance was universal (100%), consistent with previous reports from multiple countries and animal hosts (Marinho et al., 2014; Sipahi, 2023; Makita et al., 2016). Since antibiotic resistance represents a major safety consideration in probiotic selection, each candidate strain must be evaluated carefully for resistance traits and the potential for horizontal gene transfer.

## CONCLUSION

In conclusion, the M22 strain, which performed successfully in all tests, was also resistant to six antibiotics. Therefore, it represents both a potential probiotic candidate and a strain that requires further investigation regarding the transferability of its antibiotic resistance genes. When compared with the literature, some findings showed similarity while others differed. The limited data on probiotic isolation from cattle in our country underscores the significance of this study. It is recommended that the M22 strain be further evaluated through comprehensive *in vivo* and *in vitro* assays.

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## Conflict of interest

There is no conflict of interest stated by the authors.

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