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# EFFECTIVENESS OF *Lactobacillus fermentum* CMUL-54 AND *Lactobacillus fermentum* B978 AS PROBIOTIC CANDIDATES PRODUCING MANNANASE, CELLULASE AND PROTEASE ACTIVITIES FOR POULTRY

Annisa Rahma IRYOS[1], Mirnawati MIRNAWATI[1]✉, Harnentis HARNENTIS[1], Anifah SRIFANI[1], and Gusri YANTI[2]

[1]*Nutrition and Feed Technology, Faculty of Animal Science, University of Andalas, 25175, Padang, Indonesia*
[2]*Agricultural Extension, Faculty of Social, Science, and Education, Prima Nusantara, 26111, Bukittinggi, Indonesia*

✉Email: mirnawati@ansci.unand.ac.id

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**ABSTRACT**: The present research investigated the potential of *Lactobacillus fermentum* strains CMUL-54 and B978 as a probiotic candidates with mannanase, cellulase, and protease activities. The materials used in this research included *L. fermentum* CMUL-54, *L. fermentum* B978, MRS Broth containing oxgall, and various equipment and chemicals for analyzing probiotic candidates, mannanase, cellulase, and protease activities. This study utilized quantitative analysis conducted using a paired two-sample t-test with ten replications. The results revealed that *L. fermentum* CMUL-54 could be significantly ($P<0.01$) used as a probiotic candidate, showing resistance to temperatures of 42°C ($9.9x10^9$±0.71 CFU/ml), gastric pH (72.35±0.80%), bile salt resistance (87.69±3.66%), and hydrophobicity test to the intestine (92.40±0.30%). *Lactobacillus fermentum* CMUL-54 also exhibited significant inhibitory zones against lactic acid bacteria (LAB) and pathogenic bacteria such as *Escherichia coli* (13.27±0.13mm), *Salmonella enteritidis* (13.91±0.13mm), *Staphylococcus aureus* (17.75±0.24mm), high activity mannanase (12.36±0.61U/ml), cellulase (12.42±0.24U/ml) and protease (11.30±0.08U/ml). It is concluded that *L. fermentum* CMUL-54 exhibited superior probiotic properties compared to *L. fermentum* B978, thus positioning it as a more promising candidate for improving broiler performance through enhanced digestion and overall health.

**Keywords:** Enzyme activity, *Lactobacillus fermentum CMUL-54, L. fermentum B978*, Probiotics

## INTRODUCTION

Broiler chickens are a type of poultry that have a rapid growth period, and can be marketed from three to six weeks of age. Therefore, broilers require very high-quality feed intake. Notably, good quality feed comes at a fairly high price, which increases the cost of rations for poultry, especially broilers. Hence, nutritional optimization is required to maximize nutrient provision, optimize feed, and manage production costs. One way to optimize nutrition is by adding feed additives in the form of microbes (probiotics).

Probiotics are living microorganisms that enhance the health of their host by improving the balance of intestinal microflora when ingested adequately (Hill et al., 2014; Harumdewi et al., 2018; Srifani et al., 2024a). The addition of probiotics as feed additives in broiler diets improves the health of broiler and the digestibility of feed. This resulted in improved body weight gain and feed conversion ratio (Melia et al., 2022) and increased the intake of vitamins and other feed substances (Sugiharto et al., 2018; Sabo et al., 2020). Probiotics can also increase the number of beneficial microbes in the digestive tract and stimulate the growth of broiler digestive organs (Mirsalami and Mirsalami, 2024). Furthermore, the use of probiotics in poultry rations can replace antibiotics which have negative impacts including the occurrence of antibiotic resistance residues that can be passed on to humans and endanger health. In addition to producing residues, antibiotics can also cause normal imbalances in the intestinal flora of poultry (Zhou et al., 2020; Xing et al., 2021).

Bacteria can be considered probiotic if they meets several criteria: they must be non-pathogenic, part of the normal intestinal microbiota of a particular host, and remain functional in environments with high gastritis acid and bile salts within the small intestine. They can also grow and metabolize quickly, be available in large quantities in the digestive tract, and be able colonize the intestinal tract at a certain period. Additionally, they can efficiently produce organic acids and antimicrobial properties against pathogenic bacteria in the digestive tract. According to He et al. (2023), the selection of probiotic strains must meet several criteria, including being non-pathogenic, capable to producing antimicrobial substances, resistant to acidic conditions in the gastritis and bile salts in the small intestine. They also be able to



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modulate immune responses and influence metabolic processes in the intestine. Notably, one type of probiotic bacteria is lactic acid bacteria (LAB).

The potential of LAB, such as *Lactobacillus*, can vary depending on the source of microbial isolation. Research by Rahmiati and Mugi (2017) discovered that bacterial isolates from various sources have different characteristics and abilities, both microscopic and macroscopic. Kim et al. (2019) also isolated four types of microbes from various sources and tested them as probiotics, yielding diverse results in terms of gastric pH survivability and bile salts. However, the four microbes were equally effective reducing the odor of pig manure waste. One of the LAB that can be used as probiotics is *Lactobacillus fermentum*.

*Lactobacillus fermentum* is a LAB, gram-positive, facultative anaerobic, and non-pathogenic and also helps maintain microbes in the digestive tract (Karlyshev et al., 2015). Malik and Javed (2024) added that LAB can be cellulolytic as it has the ability to produce cellulase to degrade cellulose. In addition to having LAB properties, *L. fermentum* can be employed as a probiotic (Barone et al., 2016) in rations to improve broiler performance.

Seftiadi et al. (2020) isolated LAB from decomposed palm kernel cake (PKC), where the identified bacteria were *Lactobacillus sp.* It exhibited cellulase activity of 18.4U/ml, mannanase 24.86U/ml, and protease 10.45 U/ml. Furthermore, Mirnawati et al. (2022) conducted sequencing tests using 16S rRNA where the identified bacteria are *L. fermentum* CMUL-54 and assessed the nutritional content with PKC fermentation (fermentation time is four days). The results revealed crude protein at 26.31%, crude fiber at 15.71%, crude fat at 1.45%, nitrogen retention at 63.92%, and metabolic energy at 2752.69 kcal/kg (Mirnawati et al., 2023). The same study also reported enzyme activities such as cellulase activity (18.01% U/ml), mannanase (24.95 U/ml), and protease (10.55 U/ml) (Mirnawati et al., 2023).

This study was conducted using *Lactobacillus fermentum* strains (*CMUL-54 and B978*), which are cellulolytic and manannolytic as a probiotic candidate for broiler.

## MATERIALS AND METHODS

### Study periods and location

This research was executed from 08 January to 30 April 2024 in Animal Biotechnology Laboratory, the Non-Ruminant Nutrition Laboratory, the Feed Industry Technology Laboratory, Andalas University and the Bacteriology Laboratory of Bukittinggi Veterinary Center, West Sumatera, Indonesia.

### Research design

This research was undertaken in the laboratory in several stages. The first step was to isolate *L. fermentum* CMUL-54 and *L. fermentum* B978 on De Man Rogosa and Sharpe (MRS) broth (oxoid CM359B). After that, the bacteria were tested for their ability to produce cellulase enzymes on carboxymethyl cellulase (CMC), mannanase enzyme on mannan, and protease enzyme on casein.

### Method

The method used in this study was quantitative analysis through a two-sample paired t-test with ten replications. The research began with assessing the ability of *L. fermentum* CMUL-54 derived from degraded palm kernel cake (PKC) and *L. fermentum* B978 derived from LIPI (Indonesia Institute of Science) as a probiotic. Probiotic candidate tests that will be performed include the resistance of 42°C, gastric pH survivability, bile salts resistance, hydrophobicity test on the intestine, antagonistic activity, and enzyme activity (mannanase, cellulase, and protease).

### Probiotic testing

#### Resistance to 42°C

Resistance test at 42°C by growing bacteria on MRS Broth media (oxoid CM359B) and placing it at 42°C, then bacterial growth is observed through colonization and colony formation based on the standard plate count method (Zawistowska-Rojek et al., 2022).

#### Gastric pH survivability

The experiment utilized MRS Broth media mixed with HCl 37% (Merck KGaA) to obtain pH 2.5 and for the control, MRS Broth is not given addition of HCL 37% with pH of 6.8. The media was sterilized with an autoclave at 121°C for duration of 15 minutes. Bacteria were isolated from up to 0.5 ml of MRS Broth-HCl and incubated at 37°C for 3 and 6 hours. Then, the absorbance was measured at a wavelength of 600 nm.

#### Bile salt resistance

The experiment involved with adding bile salt concentrations of 0%, 0.3%, and 0.5% to MRS Broth media. The media was sterilized with an autoclave at 121°C for duration 15 minutes. Then, 5 ml of MRS Broth containing 0%, 3%, and 5%

oxgall (Sigma-Aldrich, St. Louis, MO, USA) was added with 0.5 ml of bacterial isolates. Next, the mixture was incubated for 5 hours at 37°C. The treatments were compared with the control, which consisted of MRS Broth with no additional bile salt (0% concentrations). Growth was measured by analysing the absorbance at a wavelength of 600 nm.

**Hydrophobicity test on intestine**

The hydrophobicity test uses stainless steel plates. The stainless steel can be thoroughly cleaned by immersing it in a hot detergent solution (temperature 40-45°C) for 24 hours. Then, the plate was rinsed with hot water until it was not longer foamy and slippery, dried, and marked. To prepare the growth media, 5.22 g MRS Broth was dissolved into 100 ml of distilled water. The growth media and stainless steel were sterillised in the autoclave (temperature 121°C) for 15 minutes. Then, the stainless steel plate was placed into 25 ml of MRS Broth inoculated with 1 ml of bacterial isolate in an erlenmeyer and incubated (temperature 37°C) for 24 hours. After incubation, the stainless steel was swabbed evenly. The swab was homogenized after being placed into a tube containing 10 ml of phosphate buffer solution (A) and then measured at a wavelength of 600 nm. 1 ml of the media's liquid was removed and diluted in 9 ml of phosphate buffer solution for the measurement of liquid phase growth (Ao). Then, the absorbance at a wavelength of 600 nm is measured.

**Antagonistic activity**

The antagonistic effects of *L. fermentum* strains (CMUL-54 *and* B978) against several pathogens were determined by the agar well diffusion method (Hossain, 2024). *Lactobacillus fermentum* isolates were cultured in MRS Broth at 37°C for 24 hours, and the targeted pathogens were also pre-cultured under the circumstances of brain heart infusion (BHI) (Liofilchem, Italy). Mueller Hinton Agar plates were subsequently covered with 200 µL of the test pathogen ($10^7$ CFU/ml). Cell-free supernatant previously centrifuged at 6,000 rpm for 10 minutes was streaked as much as 100 µL on Petri dish. Then, petri dish were incubated (37°C for 24 hours). The antagonistic activity of *L. fermentum* was assessed in terms of inhibition zone formation (mm) around the wells. Each *L. fermentum* isolate was subjected to this procedure four times, with the average outcome being recorded. The target pathogens assessed were *Escherichia coli, Staphylococcus aureus,* and *Salmonella enteritidis*.

**Enzyme activity testing**

**Mannanase activity**

Bacterial isolates were taken 1 ml reacted with 1 ml of manan substrate (0.5 manan plus 10 ml phosphate buffer); all solutions were reacted in a test tube and then placed in a water bath (60°C) for duration at 30 minutes. Take 1 ml of the previous solution, add 1 ml of Nelson AB. After that, the solution is heated over a stove (temperature 100°C) for 30 minutes. After 30 minutes, remove test tube and allow it to cool briefly. After cooling, add 1 ml of phosphomolybdate and 1 ml of distilled water. Absorbance was measured using a spectrophotometer UV-VIS 1800 (Shimadzu USA MFG inc.) with a wavelength of 575 nm.

**Cellulase activity**

Bacterial isolates were taken in 1 ml and reacted with 1% CMC (carboxymethyl cellulose) (Himedia). All solutions were reacted in a test tube and then placed in a water bath at 60°C for 30 minutes. Take 1 ml of the previous solution, add 1 ml of Nelson AB. After that, the solution is heated over a stove (temperature 100°C) for 30 minutes. After 30 minutes, remove test tube and allow it to cool briefly. After cooling, add 1 ml of phosphomolybdate and 1 ml of distilled water. Absorbance was measured using spectrophotometer UV-VIS 1800 (Shimadzu USA MFG inc.) with a wavelength of 575 nm.

**Protease activity**

Pipette 2.5 ml of 1% casein solution and add 1.5 ml of 0.1 M phosphate buffer at pH 7 in a test tube, homogenized with a vortex mixer (Raypa) with vibration of 3. The sample was incubated in a water bath at 37°C for 10 minutes, adding 1 ml of bacterial isolate. Then, the reaction was incubated in a water bath at 50°C for 10 minutes. For control, enzyme activity was stopped by adding 5 ml of 20% Trichloroacetic acid (TCA) (Himedia) solution, homogenized with a vortex, and then cooled in the refrigerator for 30 minutes to coagulate the protein. The reaction for enzyme activity was carried out, and the solution that has been incubated, was then centrifuged (Sigma) at 5,000 rpm at 4°C for duration at 15 minutes, thereafter filtered, and supernatant was observed. Then, the supernatant was pipetted 2 ml and then put into a test tube and add 5 ml of 0.5N NaOH (Himedia) and 0.5 ml of folin ciocalteu (Merck KGaA) reagent to test tube, and cool for 10 minutes. Absorbance was measured using spectrophotometer uv-vis 1800 (Shimadzu USA MFG inc.) a wavelength of 650 nm.

**Statistical analysis**

This study used a paired two-sample t-test with ten replications. Tukey test at a confidence level of 0.01 ($P<0.01$) was used to see the difference in each sample.

## RESULTS

### Probiotic testing

Probiotic testing of *Lactobacillus fermentum* strains (CMUL-54 and B978) can be observed in Table 1.

### Resistance to 42°C

Figure 1 displays the incubation results of *L. fermentum* CMUL-54 and *L. fermentum* B978 after incubation at 42°C. The growth of *L. fermentum* CMUL-54 was better than that of *L. fermentum* B978. Total colonies from *Lactobacillus fermentum* CMUL-54 had 9.9x10$^9$±0.71 CFU/ml. Meanwhile, *L. fermentum* B978 had 8.7x10±1.75 CFU/ml (Table 1).

**Table 1 -** Probiotic test of *Lactobacillus fermentum* CMUL-54 and *Lactobacillus fermentum* B978

| Probiotic test | *Lactobacillus fermentum* CMUL-54 | *Lactobacillus fermentum* B978 |
|---|---|---|
| Resistance to 42°C (CFU/ml) | 9.9x10$^{9\,a}$ ± 0.71 | 8.7x10$^{9\,b}$ ± 1.75 |
| Gastric pH survivability (%) | 72.35$^{a}$ ± 0.80 | 68.87$^{b}$ ± 0.57 |
| Bile salts resistance (%) | 87.69$^{a}$ ± 3.66 | 78.20$^{b}$ ± 3.57 |
| Hydrophobicity test to Intestine (%) | 92.40$^{a}$ ± 0.39 | 85.57$^{b}$ ± 1.10 |
| Antagonistic activity (mm) | | |
| *Escherichia coli* | 13.27$^{a}$ ± 0.13 | 12.24$^{b}$ ± 0.5974 |
| *Salmonella enteritidis* | 13.91$^{a}$ ± 0.13 | 12.81$^{b}$ ± 0.23 |
| *Staphylococcus aureus* | 17.75$^{a}$ ± 0.24 | 16.94$^{b}$ ± 0.15 |

a,b; Means within a row with different superscripts different significantly (P<0.01).

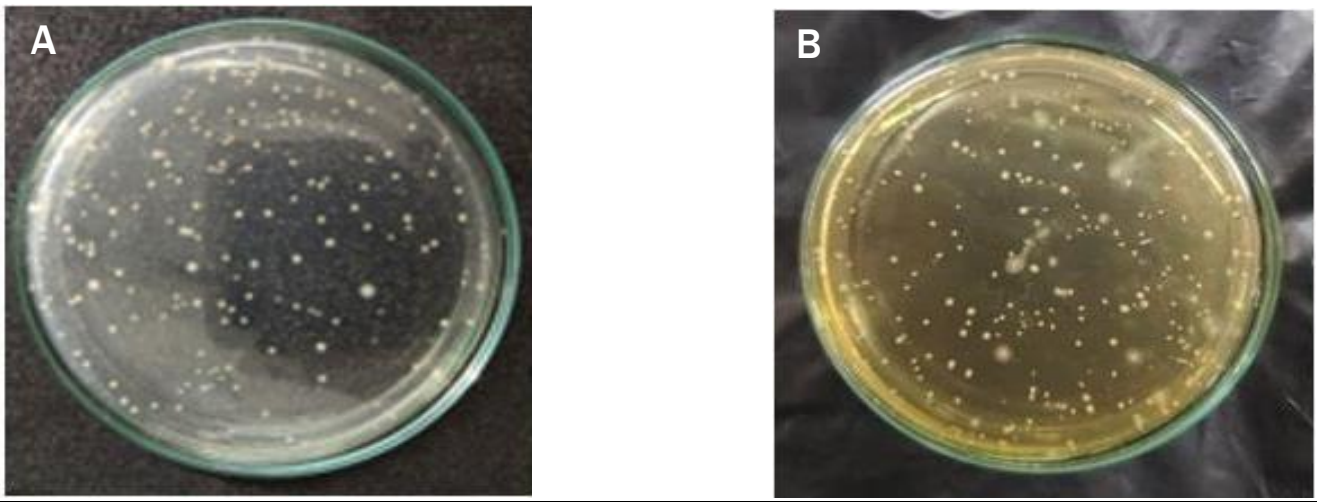


**Figure 1 -** Resistance of *L. fermentum* CMUL-54 (A) and *L. fermentum* B978 (B) at 42°C.

### Gastric pH survivability

The results of the bacterial resistance test are provided in Table 1, where the resistance of *L. fermentum* CMUL-54 (72.35±0.80%) is higher than that of *L. fermentum* B978 (68.87±0.57%). At the 3-hour time interval, *Lactobacillus fermentum* CMUL-54 showed higher resistance than *Lactobacillus fermentum* B978. This higher resistance value indicates that *L. fermentum* CMUL-54 is better able to survive at pH 2.5 conditions in a short time. At the 6-hour time interval, *L. fermentum* CMUL-54 also showed higher resistance compared to *L. fermentum* B978. Although both strains experienced a decrease in resistance, *L. fermentum* CMUL-54 remained superior in terms of resistance to acidic conditions. There is a significant negative relationship between *Lactobacillus fermentum* CMUL-54 and *L. fermentum* B978 (Table 1).

### Bile salt resistance

The bile salt resistance test results can be observed in Table 1, where *L. fermentum* CMUL-54 (87.69±3.66%) is higher than *L. fermentum* B978 (78.20±3.56%). At 0.3% bile salt concentration, *L. fermentum* CMUL-54 showed higher resistance compared to *L. fermenetum* B978. This higher resistance value indicates that *L. fermentum* CMUL-54 is better able to survive in lower bile salt conditions. At 0.5% bile salt concentration, *L. fermentum* CMUL-54 also showed higher

resistance compared to *L. fermentum* B978. Although both strains experienced a decrease in resistance as the bile salt concentration increased, *L. fermentum* CMUL-54 remained superior in terms of resistance at higher bile salt conditions.

**Hydrophobicity Test on Intestine**

Cell wall components such as phospholipids and lipopolysaccharides play a vital role in the hydrophobic interaction of bacterial cells. Table 1 indicated that the hydrophobicity value of *L. fermentum* CMUL-54 (92.40±0.39%) is higher than *L. fermentum* B978 (85.57±1.10%). *Lactobacillus fermentum* CMUL-54 showed a higher resistance value compared to *L. fermentum* B978. This higher resistance value indicates that *L. fermentum* CMUL-54 has a better ability to attach to hydrophobic surfaces in the gut. The difference in resistance values between the two strains was statistically significant indicating that *L. fermentum* CMUL-54 is superior in hydrophobicity compared to *L. fermentum* B978.

**Antagonistic activity**

The results in Table 1 showed that the inhibition of *Lactobacillus fermentum* CMUL-54 is higher than *Lactobacillus fermentum* B978. The average inhibition power produced by each bacterium ranged from 12.24 to 17.75 mm. The difference in the diameter of the inhibition zone between the two strains was statistically significant, indicating that *Lactobacillus fermentum* CMUL-54 was more effective in inhibiting the growth of the pathogenic bacteria.

**Enzyme activity testing**

The enzyme activity testing of *Lactobacillus fermentum* strains (CMUL-54 and B978) can be observed in Table 2.

**Table 2.** Enzyme activity of *Lactobacillus fermentum* CMUL-54 and *Lactobacillus fermentum* B978

| Enzyme activity (U/ml) | *Lactobacillus fermentum* CMUL-54 | *Lactobacillus fermentum* B978 |
|---|---|---|
| Mannanase Activity | 12.36 $^{a}$ ±0.61 | 9.78 $^{b}$ ±0.22 |
| Cellulase Activity | 12.42 $^{a}$ ±0.24 | 8.94 $^{b}$ ±0.54 |
| Protease Activity | 11.30 $^{a}$ ±0.08 | 8.87 $^{b}$ ±0.13 |

a,b; Means within a row with different superscripts different significantly (P<0.01).

**Mannanase activity**

The research results on mannanase activity are summarized in Table 2, where the enzyme activity in *Lactobacillus fermentum* CMUL-54 is higher than in *L. fermentum* B978. Mannanase activity from *L. fermentum* CMUL-54 had 12.36±0.61.U/ml; however, *L. fermentum* B978 had 9.78±0.22 U/ml. There was a significant difference between the mannanase activities of the two bacterial strains. This indicates that *L. fermentum* CMUL-54 is more effective in producing mannanase enzyme than *L. fermentum* B978.

**Cellulase activity**

The results of cellulase activity research can be observed in Table 2, where the highest activity value is reported in *L. fermentum* CMUL-54. Cellulase activity from *L. fermentum* CMUL-54 was 12.42 ±0.24 U/ml. Nevertheless, *L. fermentum* B978 had 8.94 ±0.54 U/ml. There was a significant difference between the cellulase activities of the two bacterial strains. This indicates that *L. fermentum* CMUL-54 is more effective in producing cellulase enzyme than *L. fermentum* B978.

**Protease activity**

The results of protease activity research are provided in Table 2, where the activity value of *L. fermentum* CMUL-54 is higher than that of *L. fermentum* B978. Protease activity from *L. fermentum* CMUL-54 had 11.30 ±0.08 U/ml, but *L. fermentum* B978 had 8.87±0.13 U/ml. These results indicate that *L. fermentum* CMUL-54 is more effective in producing protease enzymes than *L. fermentum* B978 in probiotic applications.

## DISCUSSION

**Resistance to 42°C**

Microbes that are resistant at a temperature of 42°C is a normal body temperature in poultry and their digestive system since, at this temperature, microbes can live and multiply (Yang et al., 2014; Mhone et al., 2022; Srifani et al., 2024b). The growth of *Lactobacillus fermentum* CMUL-54 is better than that of *L. fermentum* B978. Note that bacterial growth is influenced by several factors, one of which is temperature. According to Pellissery et al. (2020), based on the temperature of microbial growth can be divided into mesophiles (20-45°C) and thermophiles (45-65°C). *Lactobacillus fermentum* can grow well at 42°C. Therefore, it can be categorized into mesophile bacteria. These bacteria can be used as probiotics since they can live in poultry's body and digestive tract.

**Gastric pH survivability**

Resistance to acidic environments is a crucial requirement for LAB as probiotics. In accordance with the statement of Mulaw et al. (2019), probiotic microbes must be able to pass through an acidic gastritis. Note that the gastritis has very high acidity; thus, the microbes that live in the gastritis must be able to survive at pH 3 (Sanhueza et al., 2015) or pH 4, which is the pH of the gastric mucus layer (Garcia et al., 2017). As such, microbes that cannot with stand gastric pH due to high acidity can damage cell membranes and intracellular components, ultimately causing death (Guan and Liu, 2020). pH below 2 can directly activate pepsinogen which in turn produces pepsin, a protease with an optimal acidic pH. Pepsin contributes importantly to first-line feed digestion during feed retention in poultry (Svihus, 2014). Proventriculus and gizzard are estimated to have the longest feed retention time, ranging from 30 minutes to 2 hours, before the partially digested chyme is discharged into the small intestine (Han et al., 2019). So during this interval, probiotic isolates must endure the low pH of proventriculus and gizzard.

Based on the result in Tabel 1, *Lactobacillus fermentum* CMUL-54 (72.35%) is higher than *Lactobacillus fermentum* B978 (68.87%). Mulaw et al. (2019) stated that the resistance LAB isolates at pH 2.5 for 3 hours exceeded 50%. These results indicated that these two bacteria can be used as probiotics in terms of resistance to acidic pH. This supports Skenderidis et al. (2020) results, who found that high quality probiotics are resistant to acidic pH and less impacted by it.

**Bile salt resistance**

Resistance to bile salts is a critical criterion for probiotic candidates, as bile salts serve as potent emulsifiers and exposure to bile in gastrointestinal tract offers significant toxicity for bacterial species, hindering their survival and functionally in gut (Shimizu et al., 2023; Foley et al., 2023). Bile is one of the complex conditions in the digestive tract that probiotics must be able to tolerate. Bile contains antimicrobial properties and is an important component of the body's physiocochemical defense system (Long et al., 2017). Bile can damage to bacterial membranes. Probiotics must exhibit resistance to bile salts to endure in gastrointestinal tract and fulfill their functional role as probiotics (Zhang et al., 2020). Elevated resistance to bile salt in bacterial isolate enhances their ability to colonize the host gastrointestinal tract. So, evaluating the potential capacity of probiotics to thrive in presence of bile salt is essential.

Resistance to bile salts is related to the ability of isolates to produce the enzyme bile salt hydrolase (BSH). Some types of Lactobacillus have BSH enzymes that can hydrolyze bile salts, thus changing the physico-chemical properties of bile salts to be non-toxic to LAB (Morinaga et al., 2022). Additionally, BSH enzyme activity can improve bacterial survival in the gut and provide favorable characteristics for probiotic bacteria.

**Hydrophobicity test on intestine**

A high level of hydrophobicity indicates the presence of hydrophobic molecules on the surface of the bacterial cells being tested. Yang et al. (2022) stated that bacteria with a high level of hydrophobicity have the ability to settle on the intestinal surface, multiply, and enter the tissue. One thing that affects the ability to hydrophobicize is the origin of the bacteria. Meanwhile, Panjaitan et al. (2018) stated that the value of microbial hydrophobicity is influenced by bacterial strains, growth media, bacterial age, and bacterial surface structure. *Lactobacillus fermentum* CMUL-54 comes from bacterial isolation from decomposed palm kernel meal (Mirnawati et al., 2023), while *L. fermentum* B978 is obtained from LIPI isolation. The diversity of these factors causes each species and strain to be used to demonstrate various levels of hydrophobicity.

**Antagonistic activity**

These results are lower than the results of research by Srifani et al. (2024b) on the ability of LAB isolates isolated from soymilk waste to inhibit *Escherichia coli* by 22.25 mm, but inhibit *Staphylococcus aureus* and *Salmonella enteritidis* from this study is higher than Srifani et al. (2024b) (*Staphylococcus aureus* by 15.15 mm, and *S. enteritidis* by 12.5mm). According to Riyanto et al. (2020), the strength of an antibacterial power can be measured based on the size of the inhibition formed like considered very strong if it is 20 mm or more, the servant area between 10-20 mm suggests strong, while between 5-10 mm indicates moderate. If it is 5 mm or below, then the antibacterial is considered weak. One that can inhibit pathogenic bacteria is the content of organic acids present in LAB. Organic acids such as acetic acid and lactic acid significantly inhibit gram-negative bacteria since these compounds act as the main antimicrobial for the inhibitory activity of probiotics against pathogens (Chizhayeva et al., 2022). Moreover, the main targets of organic acids are the bacterial cell wall, cytoplasm, and specific metabolism of bacteria, which can cause damage and the death of pathogens (Nair et al., 2017).

**Mannanase activity**

Mannanase activity produced by microbes varies depending on the source. This enzyme can be produced from various sources, including animals, plants, and microorganisms such as bacteria, molds, and yeasts (Kuo et al., 2022). The microbial source of this research is *L. fermentum* CMUL-54, obtained from decomposed PKC isolation (Mirnawati et al.,

2023), while *L. fermentum* B978 was obtained from LIPI. The ability of microbes to produce mannanase has a role in degrading mannose and manooligosaccharides. In accordance with the opinion of Chen et al. (2023) mannanase is an enzyme capable of hydrolyzing manan substrates into manooligosaccharides and small amounts of mannose, glucose, and galactose. So, adding mannanolytic microbes to the ration can produce improvements and increase the nutritional value to ensure that it can be optimally utilized by livestock, especially poultry.

**Cellulase activity**

Cellulolytic bacteria such as *Lactobacillus fermentum* are able to degrade cellulose. In accordance with the opinion of Gurovic et al. (2023), microbes can degrade cellulose since they produce degrading enzymes. Note that cellulase enzymes are generally produced by microbes and can also be produced by animals and plants. However, microbes are the most widely used since microbial growth is faster, they can grow on cheap substrates, and their enzyme production can be more easily increased, such as by using cellulolytic bacteria. Opinion of Murtiyaningsih and Hazmi (2017), cellulolytic bacteria can hydrolyze cellulose by synthesizing cellulase complex enzymes. The isolation of cellulolytic bacteria can improve and increase nutrition in the ration so that poultry can optimally utilize it.

**Protease activity**

Protease is an enzyme that can degrade proteins. According to Rio et al. (2021), protease plays a role in hydrolyzing proteins into amino acids. Microbes are the most widely used source of enzymes. Similarly, Adrio and Demain (2014) mentioned that the selection of microbes as enzyme producers is based on their ability since microbes can be used to meet the high demand for enzymes and support sustainable production. Furthermore, using proteolytic bacteria such as *L. fermentum* can improve the nutritional value of the ration to ensure that it can be optimized optimally by poultry.

## CONCLUSION AND RECOMMENDATION

Based on this study, it can be deduced that both *Lactobacillus fermentum* strains (CMUL-54 and B978) have the potential to be employed as probiotics. However, *L. fermentum* CMUL-54 has the highest results, such as resistance to 42°C ($9.9x10^9$±0.71 CFU/ml), gastritis pH survivability (72.35±0.80%), bile salt resistance (87.69%±3.66%), and hydrophobicity to the intestine (92.40±0.39 %). In addition, it can also inhibit pathogenic bacteria (*Escherichia coli* 13.27±0.13 mm, *Salmonella enteritidis* 13.91±0.12 mm and *Staphylococcus aureus* 17.75±0.15 mm) and have enzyme activities (mannanase 12.36±0.61 U/ml, cellulase 12.42 ±0.24 U/ml, and protease 11.30±0.08 U/ml). the conclusions from this study suggest that *L. fermentum* CMUL-54 exhibits superior probiotic properties compared to *L. fermentum* B978, making it a more promising option for enhancing broiler performance through improved digestion and overall health.

## DECLARATION

**Corresponding author**

Correspondence and request for material should be addressed to Prof. Dr. Ir. Mirnawati, MS; E-mail: mirnawati@ansci.unand.ac.id

**Data availability**

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

**Author's contribution**

Mirnawati and Harnentis contributed to research concepts, technical and logistic support, and supervised the research. G. Yanti contributed to experimental design, data collection and execution. A.R. Iryos contributed to data collection, analyses and write up of the manuscript. A. Srifani contributed to writing the final drafted manuscript.

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**Ethical approval**

This research does not necessitate ethical approval due to its utilization of neither human or animal as research.

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**Competing interests**

The authors have not declared any competing interests.

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