Viability of *Lactobacillus plantarum* and *Lactobacillus pentosus* isolated from solid waste of soy milk as candidate probiotic for poultry

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**Abstract.** This study was aimed to determine the viability of *Lactobacillus plantarum* and *Lactobacillus pentosus* isolated from soy milk waste as a candidate for poultry probiotics in vitro. This research was designed in the form of a laboratory experiment descriptively. This study was conducted in several stages, viability test at 37°C and 42°C, viability test at pH 2 and 7, viability test for 0.3% and 0.5% bile salts, viability test of adhesion [hydrophobicity] and antimicrobial activity against pathogenic bacteria [*Escherichia coli* 0157, *Staphylococcus aureus*ATCC-25293 and *Salmonella* sp]. The result showed that *Lactobacillus plantarum* and *Lactobacillus pentosus* survived and grew at 37°C and 42°C (observation time at 30 and 60 minutes), pH 2, and resistant for 0.3% and 0.5% bile salts. Both bacteria also could inhibit the growth of pathogenic bacteria [active category], which produced clear zones with diameters <30mm. Hydrophobicity test used stainless still found that both bacteria had high hydrophobicity [<40%], 82.82% and 82.71%. This study concluded that both of *Lactobacillus plantarum* and *Lactobacillus pentosus* isolated from soy milk waste have potential as a probiotic for poultry.

1. **Introduction**

Probiotics are currently widely used as an alternative for antibiotics in poultry. The potential of probiotics could be a solution to increase livestock productivity. Probiotics are bacterial cultures that can stimulate the intestinal microflora and can positively modify the digestive tract environment, benefit-able and improve the growth performance and feed efficiency of broiler [1].

A well-known source of probiotics is the group of *lactic acid bacteria* [LAB]. Since it is known as Generally Recognized as Safe [GRAS] they are classified as a safe and non-pathogenic microbe. One of the most common groups of bacteria in the digestive system of poultry is *Lactobacillus*. This strain has been proven to be effective in preventing disease, increasing body weight gain, feed conversion and reducing mortality [2]. *Lactobacillus* can be isolated from a variety of natural ingredients, one of which is from solid waste of soy milk production. The solid waste of soy milk production contains many nutrients, so it can be a good medium for the growth and development of microorganisms such as Lactic Acid Bacteria [LAB].

Aritonang *et al.* [3] have succeeded in isolating LAB from solid waste of soy milk production and found two probiotic candidates namely *Lactobacillus plantarum* and *Lactobacillus pentosus*. Supplementation of *Lactobacillus plantarum* and *Lactobacillus pentosus* probes isolated from solid waste of soy milk production has never been done before in poultry. Allen *et al.* [4] explained that the
microbial requirements that can be used as probiotic agents are resistant to acids and bile salts. Therefore it is necessary to do an in vitro test on *Lactobacillus plantarum* and *Lactobacillus pentosus* as a probiotic candidate.

2. Materials and Methods

2.1. Tested Microorganisms
In this study *Lactobacillus plantarum* and *Lactobacillus pentosus* were isolated from solid soybean milk waste. Both bacteria were obtained from the collection of the Animal Husbandry Technology Laboratory of the Andalas University Faculty of Animal Husbandry, Padang. This research was designed in the form of a laboratory experiment descriptively.

2.2. Test the viability of LAB at 37°C and 42°C
LAB isolates were inoculated as much as 1% into the MRS Broth media which had been sterilized in an autoclave at 121°C for 15 minutes, incubated at 42°C for 30 and 60 minutes. Then 1 % isolate was taken and diluted to 10^{-6} and then planted with spread method on to MRSA and incubated at 37°C for 48 hours [5].

2.3. Test the ability of LAB to live at pH 2
The effect of acidic conditions [pH 2] was tested using MRS broth [Merck] regulated with 1N HCl and MRS broth [Merck, pH 5.7] as a control. Both media were inoculated with 1% isolate, at 37 °C for 90 minutes, then diluted to 10^{-6} and then planted using spread method to MRSA and incubated at 37°C for 48 hours [5].

2.4. Test the survival ability of LAB against bile salts
The culture was taken from 1% isolate and inoculated into MRS broth containing 0.3% and 0.5% oxgall salts [Merck-Germany] for 5 hours at 37°C. Furthermore, the 0.5% oxgall and without the oxgall [control] setting were diluted to 10^{-6} and then planted using the spread method to MRSA and incubated at 37°C for 48 hours [5].

2.5. Hydrophobicity [adhesion] ability test
Hydrophobicity tests were carried out based on the method of Fadda *et al.* [6] using stainless steel plates.

2.6. Antibacterial activity test against pathogens
The antimicrobial activity of lactic acid bacteria against pathogenic bacteria [*Escherichia coli*, *Salmonella enteritidis*, *Staphylococcus aureus*] was carried out using the disk diffusion method [7].

3. Results and discussion

3.1. LAB live ability at 37°C and 42°C
The ability to live in the LAB can be seen from the temperature. The temperature in poultry digestion ranges from 40-42°C [8] so that bacteria used as probiotics must be able to grow and survive at these temperatures. Test of ability to live of BAL at an incubation temperature of 37°C and 42°C was carried out for 30 and 60 minutes. The results showed that the two isolates of *Lactobacillus plantarum* and *Lactobacillus pentosus* could grow well at 37°C and 42°C for 30 and 60 minutes. The isolate from soybean milk solid waste could grow at 15°C and 60°C for 48 hours [3]. These results are in line with a study published by Husmaini *et al.* [9] concluding that probiotics bacteria isolated from the processing waste of virgin coconut oil can adapt and grow well at 42°C. Based on the temperature, the LAB is classified into two groups: 1. The mesophilic group has an optimum temperature of optimum 25°C; 2. The thermophilic group has an optimum temperature of 52°C [10]. Therefore, both isolates are classified as thermophilic bacteria.
3.2. The ability to live of LAB at pH 2
In the poultry digestive system, LAB growth is influenced by pH value. One of the most important standards for the selection of LAB as a probiotic is their viability at a low pH [11]. Part of the poultry digestive system precisely at the proventriculus has a pH value of 2.0-2.5 and 4.8. The duration of observation was carried out at 90 minutes on the MRSA agar enriched with HCl [pH 2]. Data are presented in Table 1. Table 1 showed that Lactobacillus plantarum and Lactobacillus pentosus can survive at pH 2 exhibiting viability of 56.94% - 33.92% respectively. This result is lower than that published by Rossi [12] showing 84.79% and 43.44% respectively. However, the two isolates can be classified as tolerant [13].

### Table 1. Ability to live of LAB at pH2.0

| LAB Isolate       | Duration [minute] | Viability [%] |
|-------------------|-------------------|---------------|
| A [Lactobacillus plantarum] | 90                | 56.02 ±0.94   |
| B [Lactobacillus pentosus]    | 90                | 36.92 ± 2.68  |

The value is expressed as the mean±standard deviation; n=3.

3.3. LAB life ability test on bile salt
The LAB survival test on bile salts was carried out for 5 hours on MRS agar media. The number of surviving colonies was measured as viability value and presented in Table 2. Table 2 shows that isolate A decreased its viability up to 82.64 ± 9.75% is grown on 0.3% of salt. Increasing salt concentration up to 0.5% subsequently decreases its viability up to 39.00 ± 8.10. Meanwhile, the viability of isolate B decreased up to 74.76 ± 8.66 if the oxgall of 0.3% was added and subsequently decreased up to 22.42 ± 2.12 if the salt concentration is increased up to 0.5%. These results are higher than the study conducted by Guan et al. [14] stating that the Lactobacillus plantarum HLX37 strain isolated from fermented milk has the viability of 54.68% in bile salts of 0.3% for 4 hours. Resistance to gastric acid and bile salts is at least 20-40% for the criteria for probiotic bacteria [15].

### Table 2. LAB life ability test on bile salt

| Lactic Acid Bacterial Isolates       | 0.3 % | 0.5% |
|-------------------------------------|-------|------|
| A [Lactobacillus plantarum]         | 82.64±9.75 | 39.00±8.10 |
| B [Lactobacillus pentosus]          | 74.76±8.66 | 22.42±2.12 |

The value is expressed as the mean±standard deviation; n=3

3.4. Ability to inhibit pathogenic bacteria
Results of the inhibitory ability to some pathogenic bacteria can be seen in Table 3 and Figure 1. Inhibition zone diameter formed by isolates A of Lactobacillus plantarum in a row Salmonella enteritidis [28.54 ± 3.74 mm], Escherichia coli [20.21 ± 4.82 mm], Staphylococcus aureus [24.15 ± 0.81mm].The isolate B of Lactobacillus pentosus respectively were Salmonella enteritidis [32.57 ± 4.80], Escherichia coli [20.22±1.87 mm], Staphylococcus aureus [20.48 ± 3.66]. The highest inhibition zone was shown by isolate A, except for Salmonella. In Salmonella, the diameter formed is 35.15 ± 4.80 mm.
### Table 3. Diameter zone of inhibitory test for pathogenic bacteria

| Lactic Acid Bacterial Isolates | The diameter of inhibition [mm] |
|-------------------------------|---------------------------------|
|                               | *Salmonella enteritidis* | *Escherichia coli* | *Staphylococcus aureus* |
| A                             | 28.54 ± 3.74 mm            | 20.21 ± 4.82 mm  | 24.15 ± 0.81 mm       |
| B                             | 32.54 ± 4.80 mm            | 20.22 ± 1.87 mm  | 20.48 ± 3.66 mm       |

Figure 1. Inhibition activity of two LAB against [a] *Salmonella enteritidis* [b] *Escherichia coli* and [c] *Staphylococcus aureus*

The largest diameter is also shown by isolate B against *Salmonella* which reached 35.15 ± 4.80 mm. Based on their inhibition activity, antibacterial power is classified into four categories, namely weak [<5 mm], moderate [5-10 mm], strong [10-20 mm], and very strong [> 20 mm] [16]. The inhibition activity against pathogenic bacteria in this study is categorized very strongly because it could reach > 20 mm. The results of this study are also higher than some studies before, as described by Jain *et al.* [17] which have antibacterial activity against *S. aureus* [20.34 ± 0.02 mm] and *Escherichia coli* [18.65 ± 0.11 mm]. A previous study carried out by Aritonang *et al.* [3] described an average inhibition activity of bacteria isolated from waste in soy milk isolates *Lactobacillus plantarum* and *Lactobacillus pentosus* against *Escherichia coli* bacteria that reached 5.68-9.90 mm and *Staphylococcus aureus* that reached 5.06-9.10 mm.

3.5. Test the ability of hydrophobicity [adhesion]

The hydrophobicity is the ability of LAB to colonize the intestinal mucosa. Hydrophobicity was measured by a 600 nm spindle spectrophotometer and the results are shown in Table 4.

| Lactic acid bacteria | Hydrophobicity [%] |
|----------------------|--------------------|
| A [Lactobacillus plantarum] | 82.82 ± 3,003     |
| K [Lactobacillus pentosus] | 82.71 ± 4,727     |

Table 4 shows that both isolates of lactic acid bacteria have a hydrophobicity of > 80%. The highest hydrophobicity [82.82%] is shown by isolate A. Result obtained in this study is higher than previously described by Rossi [2018], which is 57.78% and B at 51.93% respectively. This result is also higher than previously described by Melia, [18] who isolated BAL from buffalo milk showing hydrophobicity up to 55.49%. Based on the classification described by [19], our isolate is classified as high hydrophobicity.

### 4. Conclusion

From the results of this study, it can be concluded that *Lactobacillus plantarum* and *Lactobacillus pentosus* isolated from soy milk waste can be used as probiotics for poultry.
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