Potential of lactic acid bacteria from asam durian as a probiotic candidate for chicken

Audina Putri¹, Komang Gede Wiryawan*, Toto Toharmat¹, Sri Suharti¹

¹Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University

Abstract. The use of antibiotics as a growth promoter for chickens may develop resistance to pathogenic bacteria in the digestive tract of chickens. Other negative impacts on food safety came from antibiotic residues. One alternative that can be used as a substitute for antibiotics is probiotics. Probiotics derived from lactic acid bacteria (LAB) is safe beside that it is potential to improve growth and chicken health. This study aimed to obtain LAB isolates from asam durian which is a local food in West Sumatera. This study consisted of several steps i.e., isolation, identification, antagonistic test to patogenic bacteria and isolates survival in the gastrointestinal tracts. Results of this study found that 8 isolates of LAB were morphologically identified with negative catalase and gram-positive bacteria. All LAB isolates had inhibitory activity to Escherichia coli. The diameter of the inhibitory zone was used to select the potential of LAB and further tested for their survival in the digestive tract at pH 2, 4 and 6 and 0.5% bile salts. Isolate P1 and isolate P3 had inhibitory zone diameters of 17.35 mm and 16.08 mm, respectively, which could survive at the pH of 2, 4 and 6 and 0.5% bile salts. It is concluded that isolate P1 and isolate P3 from asam durian have the potential as probiotics for chickens because they inhibit the growth of pathogenic bacteria and can survive in the gastrointestinal conditions.

1. Introduction

Antibiotic uses in the poultry farms are not only used for treating diseases but also utilized for an Antibiotic Growth Promotor (AGP). The use of AGP has negative impacts on human health such as stimulate resistant to pathogenic bacteria on antibiotics [1] and accumulate antibiotic residue in the livestock meats [2]. Hence, it is needed to strive for an alternative antibiotic in order to optimize the productivity of broilers with safety products to be consumed in forms of probiotics. Probiotic is a living microorganism that if it is administered, in enough quantity, will provide beneficial effects on the health of host [3]. One of the microbes is often applied as probiotic for chickens originating from lactid acid bacteria. Lactid acid bacteria (LAB) is a fluctuated anaerobe bacterium that is able to live in various ranges of habits in nature such as in plants, digestive tracts of animal and human. One of the potential fermented products is asam durian (fermented durian). Asam durian is a traditional fermented food product from West Sumatera aimed as food preservation. Some durian fermenting methods are through spontaneous and additional starter ways such as adding salts [4,5] and turmeric [6]. The species Lactobacillus has been dominant in asam durian produced in Indonesia and Malaysia [7]. The bacteria of lactid acids that are isolated from asam durian originating from Kotabumi (L.
plantarum and L. casei) and from Kalianda (L. coryneformis) are potentially to be probiotics for poultry [5]. The isolate differences of LAB from asam durian are influenced by types of asam durian such as aspects of variety, maturity level, and chemical content [5]. In addition, a method of asam durian production is also affected by total lactid acid and total LAB [6]. Therefore, needs to be conducted a research to select LAB isolates from asam durian originating from West Sumatera that is spontaneously fermented in order to obtain potential isolates for a probiotic candidate by examining the microbe activity and viability in the digestive tracts based on the requirements of FAO/WHO.

2. Materials and methods

The first step was started by making a asam durian using durians originated from West Sumatera. The arils or flesh of durians were separated from its capsule as many as 250 g and then put into a closed container in anaerobe condition. These durian’s arils were fermented for 7 days in room temperature. The second step was a bacterial isolating of lactid acid from asam durian using the spreading method of Total Plate Count (TPC) in a media of MRS agar+0.5% CaCO₃ and then it was incubated at 41°C temperature for 24-48 hours [8]. The isolated bacteria forming a pellucidum zone is the LAB. The third step was an identification of the selected lactic acid bacteria isolate by observing the morphology of colony and biochemical, covering gram color test, catalase test, and Triple Sugar Iron Agar test [9]. The fourth step was the testing on antimicrobial activity on the pathogenic bacteria (Escherichia coli). This test was defined by checking the established inhibiting zone and diameter of the zone using the calipers [10]. The inhibiting zone of bacteria, in general, referred to the common standard of antibiotics namely sensitive ≥ 20 mm, intermediate 15-19 mm, and resistant < 14 mm [11]. The isolate that has the biggest inhibiting zone was therefore tested on the viability in a digestive tract. The last step was viability test of the LAB in three pH condition (2, 4, and 6) and bile salt 0.5%. In the test of LAB viability on the digestive tracts, the LAB was inoculated on the MRS broth that had been set using HCl 1N and NaOH 1N with three concentrations of pH (2, 4 and 6) and then incubated for 3 hours. The tolerance test on the bile salt used the MRS broth containing 0.5% bile salt (HIMEDIA) and incubated for 5 hours. All treatments were incubated in a range of 24-48 hours at 41°C temperature. The total cells of the LAB before and after treatments were counted using a spreading method of TPC on agar MRS media with incubation at 41°C temperature for 24-48 hours [12,13]. All data except identification were analyzed by T-test to evaluate the ability of antibacterial activity and survive in the gastrointestinal conditions. Identification were analyzed by descriptive in table to describe morphological identification of lactid acid bacteria.

3. Results and discussion

The isolation of LAB originating from asam durian obtained 8 isolates containing the pellucida zones around the colonies. Different isolate strains were chosen to be identified morphologically. The isolated morphological identification of LAB from asam durian is presented in Table 1.

| Isolate Code | Morphological Characteristics | Gram Coloring Test | TSIA Test |
|--------------|-------------------------------|--------------------|-----------|
| P1           | Spherical Silken Silken Spherical Yellowish white Yellowish white Convex + + + Rods - - t/y |
| P2           | Spherical Silken Silken Silken Milky White Milky White Convex + + + Rods - - t/y |
| P3           | Spherical Silken Silken Silken Milky White Milky White Convex + + + Rods - - t/y |
| P4           | Spherical Silken Silken Silken Milky White Milky White Convex + + + Rods - - t/y |
| P5           | Spherical Silken Silken Silken Yellowish white Yellowish white Convex + + + Rods - - t/y |
| P6           | Spherical Silken Silken Silken Yellowish white Yellowish white Convex + + + Rods - - t/y |
| P7           | Spherical Silken Silken Silken Milky white Milky white Convex + + + Rods - - t/y |

Table 1. The morphological identification of selected LAB isolates
P8 Spherical Silken Milky white Convex + Rods - r/y
Note: positive = (+); negative = (-); red slant/yellow butt = r/y.

Based on the test results according to the morphological characteristics of isolated bacteria from asam durians, the identified bacteria were positive gram, negative catalase, and produced acid. The positive gram bacteria which do not produce catalase enzymes are presumed as the LAB due to having common characteristics of LAB [14]. On the test of Triple Sugar Iron Agar, the bacteria were able to ferment lactose and sucrose to produce acids. This is caused by the lactose is converted to lactid acids by LAB. The LAB can be categorized as potential probiotic when it can inhibit pathogen bacteria and survive in the intestinal tract. The identified bacteria morphologically then were tested in the microbe’s activity on E. coli. The antimicrobe activity test on E. coli is presented in Table 2.

Table 2. The antimicrobe activity test on E. coli

| Isolate | Free Cell Supernatant Inhibition zone (mm) | Supernatant pH Neutral | Proteinase-k Enzyme |
|---------|--------------------------------|----------------------|---------------------|
| 1       | 17.35                        | -                    | -                   |
| 2       | 14.48                        | -                    | -                   |
| 3       | 16.08                        | -                    | -                   |
| 4       | 11.01                        | -                    | -                   |
| 5       | 13.09                        | -                    | -                   |
| 6       | 13.71                        | -                    | -                   |
| 7       | 14.40                        | -                    | -                   |
| 8       | 14.44                        | -                    | -                   |

Note: Diameter of inhibition zone ≥ 20 mm = Sensitif; diameter of inhibition zone 15-19 mm Intermediate; diameter of inhibition zone < 14 mm = Resistant [11].

Based on the activity test of microbes, all isolate supernatants of LAB had the inhibiting activity on the Escherichia coli. The LAB isolates that possess the highest inhibition zones were the isolate P1 and isolate P3 i.e., 17.35 mm and 16.08 mm, respectively. This might be due to the organic acids produced by the LAB. The organic acids have bactericidal effect for negative gram bacteria such as Escherichia. This is due to the organic acids produced by the LAB such as lactic acid and acetic acid has the potential to inhibit the growth of pathogenic bacteria. Furthermore, the ability of acid in a dissociated form breaks through the cytoplasm membrane that generates a low intracellular pH and disturbs transmembrane proton motive force because it obstructs protein synthesis. This causes the cell becomes dead [15,16]. Beside organic acids, LAB is also able to produce bacteriocins [17], and peroxide hydrogen [10]. However, all neutralized LAB isolate supernatants (pH 7.0) do not possess any inhibiting activities. This condition indicates that the LAB does not produce bacteriocins.

The isolate that has the highest inhibition zone was further tested for their survival at different pH (2, 4 and 6) and bile salt 0.5%. The LAB as a probiotic candidate has to be able to survive in the digestive tract to protect the host from pathogen. In the intestine condition, the population of LAB will decrease due to the acidic condition. The population of LAB at different in the MRS media (3 hours incubation) and MRS+0.5% bile salt (5 hours incubation) at 41°C is presented in Tabel 3.

Table 3. Population decrease of LAB (Log CFU mL⁻¹) at different pH and 0.5% bile salt

| Isolate | Log CFU mL⁻¹       |
|---------|-------------------|
|         | pH 2              | pH 4              | pH 6              | Bile Salt 0.5% |
| P1      | 0.09±0.2ᵃ         | 0.98±0.12ᵃ         | 0.41±0.78ᵃ         | 1.39±1.17ᵃ     |
| P3      | 1.69±0.99ᵇ        | 1.19±0.42ᵃ         | 0.65±0.64ᵇ         | 1.43±0.78ᵃ     |

Note: different superscripts in the same column mean significant difference (p< 0.05).
All population of LAB isolates decreased at different pH of digestive tract. A slight lowering cell number occurred from pH 4 to pH 6 and bile salt 0.5%. The isolate P1 has the lower cell number than the isolate P3 at pH 2 and pH 4. Decreasing cell numbers of LAB at low pH is caused by hydrogen ion breaks membranes and releasing intracellular components such as Mg, K, and fat from the cell which resulted in the growth inhibition of the microorganisms [18]. Decreasing population of LAB on the bile salt treatment is caused by its conjugation with bactericidal nature on bile salt-sensitive microorganisms, the membrane lipid can be dissolved and causing cell death [19]. Comparing with other fermented durian research, the ability of both LAB isolates is better than tempoyak (fermented durian) originated from Lampung that the LAB was totally inhibited at pH 2 [5].

LAB that tolerant to intestine condition will increase its survival in gastrointestinal tracts. The survival percentage of BAL at different pH (3 hours’ incubation) and MRS+0.5% bile salt (5 h incubation) incubated at 41°C in Table 4.

**Table 4.** The survival of LAB (%) at different pH and bile salt 0.5%

| Isolate | pH 2         | pH 4         | pH 6         | 0.5%Bile Salt |
|---------|--------------|--------------|--------------|---------------|
| P1      | 89.98±2.23a  | 88.70±1.34a  | 95.32±0.88a  | 83.95±1.78a   |
| P3      | 80.34±1.19b  | 86.21±0.57a  | 99.23±0.72b  | 83.47±0.98a   |

Note: different superscripts in the same column mean significant difference (p<0.05).

The isolate P1 can survive better than the isolate P3 at the pH 4 and 0.5% bile salt. The survival ability of isolates 1 and 3 was better than *Pediococcus pentosaceus*. The survival rate of *Pediococcus pentosaceus* was 41.15% after 3 hours’ incubation at pH 2 [20]. The LAB can survive in the acid condition due to the ability of LAB to protect membrane damages from low extracellular pH [19]. Survival assay under bile salts condition showed that all isolates survived at 0.5% bile salts after 5 hours incubation. Lactic acid bacteria isolated from gastrointestinal tract of cemani chicken did not survive under 0.3% bile salts. The survival of the isolates under 0.1% bile salts decreased after 5 hours’ incubation at 0.1% bile salts suspension [21]. Furthermore, the LAB could survive in the bile salt condition due to its ability to metabolize the bile salt using bile salt hydrolases (BSH) [22], so the LAB could hydrolize bile salt and reducing their solubility [23].

**4. Conclusion**

There are 8 isolate of LAB isolated from asam durian originating from West Sumatera that is fermented spontaneously. The selected LAB isolates are gram-positive bacteria and do not produce catalase enzymes and they are able to ferment lactose and sucrose. All isolates possess the antimicrobial activity on *E. coli*. The isolate P1 and P3 had the inhibitory zones diameters of 17.35 mm and 16.08 mm, respectively which are interpreted as sensitive, capable to survive in the intestine tract condition (pH 2, 4, and 6) and bile salt 0.5%. This result indicated that these LAB isolates are potential as probiotic candidate for chicken.

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