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Article

Antibacterial Activity of Lactic Acid Bacteria Isolates Mountain Rice Wash Water (Mayas Rice) Against *Cutibacterium acnes*

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Abstract

This study aims to explore the antibacterial activity potential of fermented dragon fruit peel juice (*Hylocereus polyrhizus*) fermented using Lactic Acid Bacteria (LAB) isolated from rice washing water (Beras Mayas). The process begins with the preparation of dragon fruit peel samples, which are washed, peeled, and mashed to obtain the juice. The juice is pasteurized and inoculated with a 5% LAB starter, with the addition of a 10% sugar solution before being incubated for 3, 7, 12, and 17 days at 37°C. pH measurements were taken during fermentation, showing a significant decrease in pH, reaching the lowest value of 3.76 on day 17. The antibacterial activity was measured using the well-diffusion method with the fermented juice at a concentration of 100%, showing a very strong inhibition zone (>20 mm) against Propionibacterium acnes on day 7. The results indicate that the pH changes during fermentation are closely related to the antibacterial activity.

Keywords: Lactic Acid Bacteria (LAB); Rice water; *Propionibacterium acnes*; Fermentation; Antibacterial

1. INTRODUCTION

Fermentation of rice has become a widely studied topic, particularly in the context of health and products with antibacterial properties. This fermentation process not only improves the nutritional profile of food but also creates bioactive compounds that have the potential to be used as antibacterial agents. Related studies show that products derived from fermentation have significant potential in the health field, particularly as bacterial infection preventatives. The fermentation process not only enhances the nutritional profile of food but also produces bioactive compounds that can potentially be used as antibacterial agents. Therefore, fermentation not only produces high-quality food products but also offers significant health benefits.

Lactic acid fermentation (LAB) provides a range of significant health benefits. This process involves the conversion of carbohydrates into lactic acid, which has the ability to lower pH and inhibit the growth of harmful pathogens berbahaya [1,2]. One of the main advantages of lactic acid fermentation is its ability to produce antibacterial compounds. For example, *Lactobacillus* and *Leuconostoc* generate bacteriocins, which are proteins and peptides with antimicrobial activity against various pathogens, including *Escherichia coli, Listeria monocytogenes, Bacillus cereus, Staphylococcus aureus,* and *Salmonella* spp. [3].

Propionibacterium acnes, now classified as *Cutibacterium acnes*, is a facultative anaerobic, gram-positive bacterium that predominantly inhabits human pilosebaceous follicles. Historically, it has been associated with the formation of follicular biofilms, which enhance resistance to antimicrobial therapies through the production of lipases that modulate sebaceous lipids and contribute to the inflammatory process characteristic of acne. C. acnes can stimulate the immune system via Toll-like receptors (TLR2/TLR6) on keratinocytes and immune cells, triggering the production of pro-inflammatory cytokines such as IL-1β, IL-6, IL-8, and TNF-α, as well as the activation of the NLRP3 inflammasome pathway. This process initiates a cascade of inflammation that leads to the development of papulopustular acne and may result in scar formation if left untreated. The high density of *C. acnes* in the sebaceous zone makes it highly relevant for testing antibacterial agents targeting acne [4–10].

During fermentation, LAB require substrates as a nutrient source to support the production of antibacterial compounds. Red dragon fruit peel is one such agricultural byproduct that has yet to be fully utilized. This fruit, which is widely produced and consumed, particularly in East Kalimantan, holds potential as a substrate for lactic acid bacteria fermentation [11,12].

Therefore, research on the fermentation of lactic acid bacteria as antibacterial agents should be conducted by utilizing the waste from red dragon fruit peel (*Hylocereus polyrhizus*) as the substrate for fermenting LAB isolates that have been isolated from Gunung rice wash water (Mayas rice) [13]. This can become a natural alternative in the development of products for pathogenic bacterial infections.

2. MATERIALS AND METHODS

2.1. Materials

The lactic acid bacteria isolate used was obtained from a previous study [13]. Meanwhile, the Red Dragon Fruit peel was sourced from farmers in Samarinda, then sorted, washed, sliced, and weighed. Additionally, the materials used included sugar, NA medium (Nutrient Agar), plastic wrap, 95% alcohol, and spiritus.

2.2. Instrument

The instruments used include an autoclave, Petri dishes, Bunsen burner, micropipettes, screw micrometer, pH meter, and test tubes.

2.3. Method

2.3.1. Sample Preparation

Fresh Red Dragon Fruit (*Hylocereus polyrhizus*) was obtained through a wet sorting process to remove adhering dirt. The fruit was then washed thoroughly with running water until completely clean and drained. The dragon fruit peel was carefully peeled to ensure no fruit flesh remained. The peeled skin was then cut into smaller pieces and weighed. Next, the red dragon fruit peel sample was crushed using a juicer and filtered to obtain the juice from the peel.

2.3.2. Fermentation Process

The obtained juice from the red dragon fruit peel was pasteurized for 15 minutes at 80°C. After heating, it was cooled to 30°C. A 5% inoculum of the lactic acid bacteria (LAB) starter, which was obtained from previous research by Amanda (2025) with optimal activity, was added along with 10% sugar solution into the juice. The juice, now containing the starter and sugar solution, was incubated in an incubator for 3, 7, 12, and 17 days at 37°C. The test solution was made by taking the soluble portion that formed during fermentation.

2.3.3. pH Measurement

The pH of the fermentation product was measured using a pH meter. The fermented solution was mixed thoroughly, then the pH was measured using a pH meter.

2.3.4. Bacterial Suspension Preparation

Pure culture of *C. acnes* that had been inoculated in Nutrient Agar (NA) medium was taken using three sterilized inoculation loops, then transferred into 10 mL of sterile 0.9% NaCl solution, and homogenized. Next, 2.5 mL of this dilution was taken and placed into a separate test tube containing 7.5 mL of sterile 0.9% NaCl solution. This resulted in a bacterial suspension with a ratio of 1:40.

2.3.5. Antibacterial Activity Test of Fermentation Juice

Antibacterial testing was conducted using the agar well diffusion method, following the procedure outlined by Hao (2024) and Fathoni (2021) with minor modifications. A volume of 10 mL of Nutrient Agar (NA) medium was poured into a petri dish to form the base layer, followed by the addition of 0.1 mL of the homogenized and solidified NA medium. Subsequently, 7 mL of NA medium was added to the petri dish as the second layer. After solidification, four wells, each with a diameter of 7 mm, were created using a punch. Each well was filled with 50 µL of the fermentation extract from isolates A, B, and C, with a 100% concentration, and K(-) using a micropipette. The petri dish was then incubated at 37°C for 24 hours. The antibacterial test was performed in triplicate for each isolate. Observations were made by measuring the inhibition zone using a digital caliper [14,15].

3. RESULT AND DISCUSSION

In this study, the red dragon fruit peel was fermented using Lactic Acid Bacteria (LAB) isolated from the Gunung rice wash water (Mayas rice) of East Kalimantan. The dragon fruit peel was first pasteurized at 80°C to inhibit other microbes, and then fermented for various durations (3, 7, 12, and 17 days) to determine the optimal time for pH reduction produced by each isolate culture. The initial pH of each fermented sample varied, as shown in Table 1.

Table 1. Results of pH Test for Antibacterial Activity of Red Dragon Fruit Peel Fermentation Juice

Isolate Code	pH Value of Fermentation Juice on Day					Average pH Value
	0	3	7	12	17	
Isolate A	4.48	4.33	4.24	4.11	3.98	4.22
Isolate B	4.43	4.28	4.23	3.98	3.79	4.11
Isolate C	4.41	4.22	4.21	4.00	3.76	4.12

The well diffusion method was used to test antibacterial activity because it allows for more accurate measurement of the inhibition zone area, covering the surface of the Nutrient Agar (NA). Previous studies have shown that this method is more effective than the disk diffusion method in generating antibacterial activity. In the test, 4 wells were made on the nutrient agar medium that had been inoculated with C. acnes, and each well was filled with test solutions and a negative control (red dragon fruit peel juice). After incubating for 24 hours, the inhibition zones around the wells were observed to assess antibacterial activity.

Based on Table 1, the pH test of fermentation with varying incubation times showed that the red dragon fruit peel juice underwent pH changes during fermentation. During the adaptation phase (lag phase), the initial pH of the fermented juice was 4.48, 4.43, and 4.41 for isolates A, B, and C, respectively. During the logarithmic (exponential) phase, the pH decreased to 4.33, 4.28, and 4.22 on day 3, and then further decreased on day 7 (4.24, 4.23, and 4.21) and day 12 (4.11, 3.98, and 4.00). In the stationary phase, the pH reached its lowest values (3.98, 3.79, and 3.76). This pH decrease indicates the growth of lactic acid bacteria and acid production, which influences antibacterial activity. Based on the fermentation time, the pH showed a gradual decrease, signaling bacterial growth. At the beginning of fermentation, the pH dropped rapidly because the bacteria were in the exponential phase and highly active in producing acids. However, over time, the pH decrease slowed as the bacteria entered the stationary phase. This was due to the reduced availability of substrate for the bacteria and the accumulation of acidic byproducts, which began to inhibit bacterial activity.

The decrease in pH (below 4.6) is crucial for food safety because most pathogenic microorganisms cannot thrive in an acidic environment. Furthermore, the pH reduction can alter the flavor profile and increase the acidity of various foods and beverages, making it an effective natural preservative. During fermentation, various compounds are produced, including organic acids such as lactic acid, acetic acid, hydrogen peroxide, and bacteriocins. These compounds exert antimicrobial effects that can inhibit the growth of microorganisms, contributing to acne control and skin care (16–20).

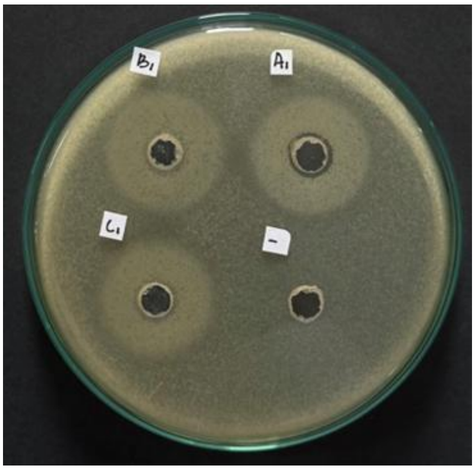

Figure 1. Antibacterial Activity of Fermentation Juice

Based on Table 2, the results of the antibacterial activity test indicate that the red dragon fruit peel fermentation juice at a concentration of 100% exhibited a very strong inhibition zone (>20 mm) on day 7 for isolates A, B, and C, with inhibition zones measuring 27.53 mm, 26.6 mm, and 27.1 mm, respectively. All test results showed inhibition zones of 20 mm or greater, which are categorized as strong (21). Figure 1 presents evidence of the antibacterial activity displayed by isolates A, B, and C.

The decrease in pH through the production of organic acids (primarily lactic acid) is one of the mechanisms contributing to the antibacterial activity of LAB against pathogens by lowering the environmental pH, which disrupts the target bacterial membranes and inhibits vital enzymes, as well as facilitates the entry of other antimicrobial compounds. This claim is consistently supported by studies reporting the identification of organic acids (lactic, citric, succinic acids) as the primary mediators of LAB's antibacterial activity in various contexts (22–25).

Lactic acid production occurs through the sugar-derivative fermentation pathway, activation of enzymatic pathways related to pH reduction, and the regulation of bacteriocin synthesis genetically encoded (pln operon in some Lactobacillus plantarum strains). When pH decreases, several pathogenic bacteria show reduced viability due to disturbances in cell membranes, respiration, and metabolite transport, as reported in various LAB strains and their pathogens (22,24–28).

Table 2. The results of the antibacterial test of isolates A, B, and C against *C. acnes* at various fermentation times

Fermentation Day	Isolate Code	Average Inhibition Zone Diameter (mm) ± SD	Inhibition Zone Category
3	Isolate A	26.37 ± 0.55	Very strong
	Isolate B	25.86 ± 0.16	Very strong
	Isolate C	24.2 ± 0.2	Very strong
	K (-)	-	-
7	Isolate A	27.53 ± 0.40	Very strong
	Isolate B	26.6 ± 0.6	Very strong
	Isolate C	27.1 ± 0.17	Very strong
	K (-)	-	-
12	Isolate A	24.9 ± 0.4	Very strong
	Isolate B	24.63 ± 0.5	Very strong
	Isolate C	24.36 ± 0.64	Very strong
	K (-)	-	-
17	Isolat A	23.73 ± 0.30	Very strong
	Isolat B	23.51 ± 0.41	Very strong
	Isolat C	23.2 ± 0.37	Very strong
	K (-)	-	-

Fermentation of juice or fruit extract containing fruit peels can produce antibacterial products against foodborne pathogens as well as skin microbes through a combined mechanism involving organic acids, bacteriocins, and secondary compounds. This is relevant for the development of natural preservative materials, probiotic supplements, and microbiome-based topical products derived from fruit peels (29–32).

Many studies have shown that fruit peel waste from fruits such as oranges, mangoes, bananas, pineapples, apples, and jackfruit has been used as a substrate for the production of SCP (single-cell protein), lactic acid, ethanol, bioethanol, and antimicrobial compounds through microbial fermentation involving Saccharomyces cerevisiae, Lactobacillus spp., Aspergillus spp., and Monascus spp. (33–40).

Lactobacillus spp. (*L. plantarum*, *L. casei*, *Lactobacillus spp.*) is widely used for fermenting fruits or fruit peel extracts to produce lactic acid, food bioproduct precursors, and antibacterial potential through the production of organic acids and bacteriocins. Fruit peels function as a carbon substrate that supports the growth rate and synthesis of antimicrobial metabolites (33,36,37,40).

Lactic Acid Bacteria (LAB) isolation from fermentation products highlights the ability of various LAB strains, such as *L. plantarum* and *L. rhamnosus*, to suppress the growth of fungi and mycotoxins produced by *A. flavus*. In addition, research on *L. fermentum* shows its antimicrobial activity, which can be used as a natural preservative in food products. Furthermore, the production of reuterin by *L. coryniformis* under aerobic conditions can produce antimicrobial compounds in various conditions. Therefore, several LAB isolates were found not to contain antibiotic resistance genes, making them better candidates for probiotic applications. This highlights the importance of selecting the right LAB isolates for developing probiotic products that are not only effective but also safe for health (16,41–43).

4. CONCLUSION

The highest inhibition on day 7 was shown by isolate A (27.53 ± 0.40 mm), isolate C (27.1 ± 0.17 mm), and isolate B (26.6 ± 0.6 mm), all of which fall into the very strong category with a pH range of 4.21-4.24. Therefore, the pH changes during fermentation are closely related to antibacterial activity.

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