

Fermentation of Kepok Banana Peel Waste by *Lacticaseibacillus casei* to Enhance Antibacterial Activity Against *Escherichia coli* and *Staphylococcus aureus*

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ABSTRACT: Pathogenic bacteria are microorganisms that can cause various diseases, including infections associated with *Escherichia coli* and *Staphylococcus aureus*. Conventional treatment of bacterial infections generally involves antibiotics; however, excessive antibiotic use may contribute to bacterial resistance, highlighting the need to explore alternative antibacterial sources, including organic waste. This study aimed to evaluate the ability of *Lacticaseibacillus casei* to ferment kepok banana peel waste and enhance its antibacterial activity against *E. coli* and *S. aureus*. The experiment included a positive control (ciprofloxacin), an uninoculated fermentation control, and fermentation with *L. casei* starter for 5, 7, and 9 days. Antibacterial activity was evaluated using the disk diffusion method. The uninoculated fermentation showed weak activity, detected only on day 7 against *E. coli* (4.11 ± 1.38 mm) and on days 7 and 9 against *S. aureus*. Fermentation with *L. casei* showed the highest activity against *E. coli* on day 7 (5.05 ± 0.56 mm), categorized as moderate, whereas all treatments against *S. aureus* showed weak activity. Antibacterial activity against *E. coli* decreased after day 7, indicating that prolonged fermentation did not consistently improve inhibitory activity. These findings suggest that fermentation of kepok banana peel waste with *L. casei* may enhance antibacterial activity under specific fermentation conditions, particularly after 7 days against *E. coli*.

KEYWORDS: Antibacterial; Fermentation; Kepok Banana Peel; *Lacticaseibacillus casei*.

1. INTRODUCTION

Pathogenic bacteria are microorganism that have the potential to cause various diseases. The diseases caused by pathogenic bacteria are influenced by various factors, such as environmental conditions and the host's immune response. Infections caused by pathogenic bacteria can have serious health consequences because they can lead to a wide range of diseases [1]. One of the diseases caused by infection with pathogenic bacteria such as *Escherichia coli* is diarrhea. Diarrhea is a disease can be transmitted through food or beverages contaminated with pathogenic agents, which then infect the intestines [2]. According to World Health Organization data from 2024, approximately 1.7 billion cases of diarrhea occur in children each year, with a death toll of 433.832 children under the age of 5 and approximately 50.851 deaths among children aged 5-9 years [3]. Another pathogenic bacterium that can also cause various types of diseases is *Staphylococcus aureus*. *Staphylococcus aureus* is a pathogenic bacterium that can cause various skin diseases [4]. In 2018 the World Health Organization reported that nearly 900 million people worldwide suffered from skin diseases [5].

Convention treatment for diseases caused by bacterial infections generally involves the administration of antibiotics, however excessive use of antibiotics carries the risk of inducing bacterial resistance [6]. The development of alternative therapies is necessary, one of which involves the use of natural materials, specifically organic waste. One type of organic waste with potential for further utilization is banana peel waste. Mulyani et al. reported that kepok banana peels contain several phytochemical constituents, including alkaloids, flavonoids, tannins, and saponins, which may contribute to their antibacterial activity against pathogenic bacteria [7].

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One way to maximize the bioactive potential of kepok banana peel waste is through fermentation using lactic acid bacteria. This fermentation process driven by microbial activity can alter the characteristics of the fermented material due to the microbes' ability to break down the components present in the material [8]. One group of probiotic microorganisms that often plays a role in fermentation is lactic acid bacteria, such as *Lactocaseibacillus*. These bacteria have the ability to convert carbohydrates into lactic acid, thereby lowering the pH of the environment and inhibiting the growth of pathogenic microorganisms as well as those that cause spoilage [9].

Fauziah et al. [10] reported that the ethanol extract of kepok banana peel exhibited antibacterial activity against *Escherichia coli*, with an inhibition zone of 28 mm at 100% extract concentration, which was categorized as very strong antibacterial activity. Similarly, Hesturini et al. [11] reported that fermentation enhanced the antibacterial activity of srikaya (*Annona squamosa*) leaf extract against *Staphylococcus aureus*, as indicated by an increase in the inhibition zone from 19 mm in the non-fermented extract to 26.0 mm after fermentation.

Despite these findings, previous studies have primarily focused on the antibacterial activity of natural extracts, whereas this study focuses on the utilization of organic waste through fermentation as a source of antibacterial agents. Thus, this study is expected to serve as a basis for the development of functional products derived from waste that can be utilized as a source of antibacterial solutions as well as contribute to the sustainable management of organic waste. Therefore, this study aims to evaluate the ability of banana peel waste fermentation by *Lactocaseibacillus casei* to enhance antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*.

2. MATERIALS AND METHODS

2.1. Material

Kepok banana peel waste was obtained as a by-product from a banana-chip producer in Cibereum, Tasikmalaya City, West Java, Indonesia. The material was supplied by the producer as kepok banana peel and was selected in a mature condition, characterized by a yellow peel without visible signs of decay or deterioration. Because the material was collected as food-processing waste, formal botanical authentication and voucher specimen deposition were not performed. The microbiological materials used in this study included *Lactocaseibacillus casei* ATCC-393, *Escherichia coli* ATCC-25922, *Staphylococcus aureus* ATCC-6538P PK/5. Mueller-Hinton agar (MHA), de Man, Rogosa and Sharpe (MRS) agar, and MRS broth were used as microbiological culture media. Other materials included distilled water, 0.9% NaCl, 1% BaCl₂, 1% H₂SO₄, 96% ethanol, 1% FeCl₃, concentrated H₂SO₄, 2 N HCl, dilute ammonia, crystal violet, safranin, Lugol's iodine, immersion oil, Mayer's reagent, Wagner's reagent, and Dragendorff's reagent.

2.2. Procedure

2.1.1. Preparation of Kepok Banana Peel Waste

The kepok banana peels are cut into pieces, washed thoroughly under running water to remove any dirt, and drained.

2.1.2 Preparation of *Lactocaseibacillus casei* Starter

Lactocaseibacillus casei was revived by transferring one loopful of culture onto slanted MRS agar and incubating it at 37°C for 48 h [12]. One loopful of the revived culture was subsequently inoculated into 10 mL of sterile MRS broth and incubated at 37°C for 24 h [13]. The resulting 24-h broth culture was used directly as the fermentation starter. For each fermentation batch, 10 mL of starter culture was added to 100 g of kepok banana peel and 100 mL of sterile distilled water. The viable cell concentration of the starter was not determined by plate counting or optical-density measurement; therefore, the inoculum was standardized based on culture age, incubation conditions, and inoculum volume rather than cell density.

2.1.3 Fermentation Process and Variation in Condition

The fermentation experiment consisted of two conditions: an uninoculated control without starter addition and a treatment inoculated with *Lactocaseibacillus casei*. For each fermentation batch, 100 g of cleaned kepok banana peel was transferred into a sterilized glass jar [14], followed by the addition of 100 mL of sterile

distilled water. For the inoculated treatment, 10 mL of the prepared *L. casei* starter culture was added [15], whereas no starter was added to the uninoculated control. Fermentation was conducted at room temperature for 5, 7 and 9 days in the absence of direct light. These fermentation periods were selected to represent conditions before, at, and after the previously reported 7-day optimum fermentation period for kepok banana peel [16], thereby allowing assessment of the effect of fermentation duration on antibacterial activity. At each designated time point, the fermentation mixture was filtered through a 0.22- μ m pore-size filter to separate the liquid fraction from suspended solids and microbial biomass. The resulting filtrate was subsequently used for antibacterial activity testing [13]. The filtration step was intended to minimize the carryover of bacterial cells and further cellular fermentation prior to antibacterial testing; however, sterility of the filtrate after filtration was not independently verified.

2.1.4 Presumptive Characterization of Lactic Acid Bacteria in Fermentation Filtrates

Presumptive characterization of lactic acid bacteria was performed on fermentation filtrates obtained on day 7 from both the uninoculated control and the *Lacticaseibacillus casei*-inoculated treatment. Serial dilutions of the fermentation filtrates were prepared in 0.9% NaCl solution and cultured on MRS agar. Colonies recovered on MRS agar were subsequently examined by Gram staining and microscopic observation to assess Gram reaction and cellular morphology [17]. This procedure was intended to determine the presence of bacteria with characteristics consistent with lactic acid bacteria in the fermentation system rather than to confirm the identity of *L. casei* at the species level.

2.1.5 Macroscopic Characteristics of the Fermentation Filtrate

The macroscopic characteristics of the fermentation filtrates were evaluated descriptively by observing color, odor, and physical appearance. These observations were included as supportive indicators of changes occurring during fermentation and to identify gross differences between the uninoculated control and the *Lacticaseibacillus casei*-inoculated treatment. The observations were qualitative and were not intended as a formal sensory evaluation..

2.1.6 pH Testing of the fermentation Filtrate

pH testing is performed by immersing the pH meter electrode into the prepared sample solution, the pH value is then read on the pH meter's display [18].

2.1.7 Qualitative phytochemical screening

Qualitative phytochemical screening was performed on the fermentation filtrates obtained from both the uninoculated control and the *Lacticaseibacillus casei*-inoculated treatment at 5, 7, and 9 days. The purpose of this screening was to determine whether major classes of secondary metabolites with potential antibacterial relevance remained detectable during fermentation and to provide supporting information for interpreting the observed antibacterial activity. The screening included alkaloids, flavonoids, tannins, and triterpenoids. Because the analysis was qualitative, the results were interpreted only as the presence or absence of these metabolite classes and not as changes in their concentrations.

2.1.8 Antibacterial Activity Testing

The test bacteria, *Escherichia coli* and *Staphylococcus aureus*, were subcultured aseptically by transferring one loopful of bacterial culture onto slanted Mueller-Hinton agar (MHA) and incubating at 37°C for 24 h [19]. A 0.5 McFarland standard was prepared by mixing 0.05 mL of 1% BaCl₂ with 9.95 mL of 1% H₂SO₄ and homogenizing the mixture [20]. The rejuvenated bacterial cultures were then suspended in 0.9% NaCl solution and homogenized using a vortex mixer. The turbidity of each bacterial suspension was adjusted by visual comparison with the 0.5 McFarland standard before use in the antibacterial assay [19].

Antibacterial activity testing was performed using the disk diffusion method [21]. A volume of 10 mL 10 mL of sterile Mueller-Hinton agar (MHA) was poured into each Petri dish and allowed to solidify [22]. The bacterial suspension was spread evenly across the surface of the Mueller Hinton Agar medium using a sterile cotton swab, then each agar disc was dipped into the test solution and placed on a petri dish containing the medium, then incubated for 24 hours in an incubator at 37°C, after which the resulting inhibition zones were measured using a venier caliper [21].

2.1.9 Data Analysis

Statistical analysis was performed using the IBM SPSS statistic version 25, employing the parametric Pearson Correlation Test (*Pearson Product-Moment Correlation*) if the data is normally distributed, however if the data not normally distributed, the analysis was conducted using the nonparametric *Kruskal-wallis* test.

3. RESULTS AND DISCUSSION

3.1. Organoleptic Test Results

The filtrate from the fermentation of kepok banana peel waste was subjected to organoleptic testing. The results of the organoleptic testing showed that there were differences in color and odor between the filtrate produced by fermentation without the addition of starter culture and the filtrate produced by fermentation with the addition of a bacterial starter culture. As shown in **Table 1**.

Table 1. Organoleptic Test Results

Treatment	Color	Odor	Consistency
Fermentation C (-) D5	Brownish yellow	Sour	Liquid
Fermentation C (-) D7	Dark yellow	Sour	Liquid
Fermentation C (-) D9	Yellow	Sour	Liquid
Fermentation (TC) D5	Pale yellow	Characteristic of fermentation	Liquid
Fermentation (TC) D7	Pale yellow	Characteristic of fermentation	Liquid
Fermentation (TC) D9	Pale yellow	Characteristic of fermentation	Liquid

Description: C (-) Control Negative; (TC) Treatment Control; (D) Day

Based **Table 1**, the color of the filtrate without the addition of starter bacteria tends to range from yellow to brownish yellow. This is due to a browning reaction, which causes the color to darken. This reaction occurs because polyphenolic compounds react with the assistance of the enzyme polyphenol oxidase, resulting in the formation of brown-colored compounds [23]. In the fermentation filtrate with the addition of lactic acid bacteria, the resulting filtrate is pale yellow, this is because the addition of the starter causes an increase in acidity and these acidic conditions play a role in reducing the activity of the polyphenol oxidase enzyme, thereby inhibiting the browning process [24].

The odor produced by the fermentation filtrate without the addition of starter bacteria is sour, this is due to the uncontrolled natural fermentation activity of local microorganisms. The filtrate produced from fermentation with the addition of starter bacteria yields a characteristic fermented odor similar to that of tape (fermented cassava), as fermentation with *Lacticaseibacillus casei* starter produces organic acids during the process, these organic acids react with alcohol to form sweet characteristic aromatic ester [25].

3.2. pH Test Results

pH testing was conducted to determine the acidity level of the filtrate produced from the fermentation process without the addition of starter bacteria and the acidity level of the filtrate produced from fermentation with the addition of starter bacteria capable of providing antibacterial activity. As shown in **Table 2**.

Table 2. pH Test Results

Fermentation condition	Fermentation time (days)	pH (Mean $\pm$ SD)
Fermentation C (-) D5	5	4.80 $\pm$ 0.02
Fermentation C (-) D7	7	5.08 $\pm$ 0.02
Fermentation C (-) D9	9	5.55 $\pm$ 0.01
Fermentation (TC) D5	5	3.88 $\pm$ 0.01
Fermentation (TC) D7	7	3.94 $\pm$ 0.03
Fermentation (TC) D9	9	3.92 $\pm$ 0.01

Based on the test result in **Table 2** there is a difference in pH values between the negative control and treatment control, but the resulting pH values still meet the requirements for fermentation pH. In the negative control, the resulting pH was in the range of 4-5 this occurred because natural fermentation by local microorganisms was less stable, resulting in lower organic acid production and a less acidic pH. In the treated control fermentation, the resulting pH was stable at pH 3, in the treat control the addition of starter bacteria was able to control the fermentation process more stably, thereby producing a higher amount of organic acid and resulting in a more acidic and stable pH.

3.3. Phytochemical Screening Results

Phytochemical screening was conducted to determine the presence of specific classes of secondary metabolites in natural material, the results of the phytochemical screening are presented based on visual color changes [27]. The results of the phytochemical screening showed positive results for flavonoids, tannins, and triterpenoids. The results of the phytochemical screening are shown in **Table 3**.

Table 3. Phytochemical Screening Results

Metabolite Group	Description		Phytochemical Screening Results
	C (-)	TC	
Alkaloids	-	-	No characteristic precipitate was observed, indicating the absence of alkaloids.
Flavonoids	+	+	Orange yellow to reddish brown
Tannins	+	+	Green, greenish, bluish black
Saponins	-	-	No foam
Triterpenoids	+	+	Brownish ring

Description: C (-) Control Negative; (TC) Treatment Control; Negative (-); Positive (+)

The results of the phytochemical screening indicated that there is no difference in the content of secondary metabolites produced from the fermentation filtrate, whether starter bacterial were added, even though fermentation was carried out over varying time periods. This indicates that the fermentation process does not eliminate or alter the presence of secondary metabolites contained in the fermentation filtrate.

3.4. Results of Lactic Acid Bacteria Characterization

Lactic acid bacteria characterization was performed to determine the presence of absence of lactic acid bacteria in the filtrate from the negative control fermentation without the addition of a starter culture, as well as in the filtrate from the treatment control fermentation with the addition of a *Lactocaseibacillus casei* starter

culture. This characterization was performed by staining the bacteria to observe the shape and color characteristics of the colonies. Characterization was performed on the fermentation filtrate at the optimum fermentation time on the (day 7).

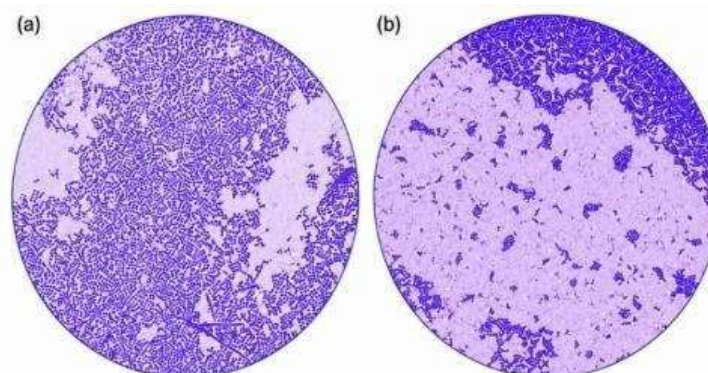


Figure 1. Bacterian staining (a) controls negative D7 fermentation; (b) treatment control D7 fermentation, 40× magnification

Based on **Figure 1**, bacterial characterization was performed on the day-7 fermentation filtrates from both the uninoculated control and the *Lactocaseibacillus casei*-inoculated treatment. Day 7 was selected because this fermentation period produced the highest antibacterial activity against *Escherichia coli* in the inoculated treatment and was therefore considered the most relevant time point for further characterization. Bacteria recovered from both fermentation conditions were able to grow on de Man, Rogosa and Sharpe (MRS) agar. Gram staining and microscopic observation showed predominantly purple-stained, coccoid cells, indicating a Gram-positive reaction. These findings were considered consistent with the presence of presumptive lactic acid bacteria; however, the isolates were not identified at the species level. Therefore, the bacteria observed in the *L. casei*-inoculated treatment cannot be conclusively assigned to *L. casei*, and those recovered from the uninoculated control may represent naturally occurring lactic acid bacteria or other Gram-positive microorganisms associated with the fermentation material.

3.5. Results of Antibacterial Activity Testing

The antibacterial activity test was conducted to assess the inhibitory activity of the fermentation filtrate against *Escherichia coli* and *Staphylococcus aureus*, as indicated by the formation of clear zones. The antibacterial activity test was performed using the disk diffusion method.

The results of the inhibition zone diameters from the antibacterial activity test against *Escherichia coli* are shown in **Table 4**, with visual representation shown in **Figure 2**. The antibacterial test was conducted on fermentation filtrate of the control negative fermentation without the addition of starter bacteria, the treatment control fermentation with the addition of starter bacteria at varying fermentation times of 5, 7, and 9 days and the positive control the antibiotic ciprofloxacin.

Table 4. Category of the inhibitor zone of fermentation filtrate against *Escherichia coli*

Treatment	Mean±SD	Category	P-Value
Control (+) Ciprofloxacin	19.65±0.93	Strong	
Fermentation C (-) D5	-	-	
Fermentation C (-) D7	4.11±1.38	Weak	
Fermentation C (-) D9	-	-	0.008
Fermentation (TC) D5	-	-	
Fermentation (TC) D7	5.05±0.56	Moderate	
Fermentation (TC) D9	1.8±0.21	Weak	

Description: <5 mm (Weak), 5-10 mm (Moderate), 10-20 mm (Strong), >20 mm (very Strong)

Based on the test results in **Table 4** a direct comparison on day 7 shows an increase in the diameter of the inhibition zone between the negative control, which produced an inhibitor zone 4.11±1.38 mm (weak), and the treated control, which produced an inhibition zone of 5.05±0.56 mm (moderate). In the negative control fermentation, antibacterial activity was lost by day 9 this was because the optimum fermentation time for kepok banana peel was on day 7 which produced the highest ethanol content and was thus able to provide an antibacterial effect [28].

Consistent with this pattern, antibacterial activity in the *Lactocaseibacillus casei*-inoculated treatment decreased on day 9, resulting in an inhibition zone of 1.80 ± 0.21 mm, which was classified as weak. The mechanism underlying this decline cannot be determined from the present study because neither residual carbohydrate concentrations nor individual bioactive metabolites were quantitatively measured. Prolonged fermentation may alter the concentration, composition, stability, and biological activity of fermentation-derived compounds through microbial biotransformation or degradation. Lactic acid bacteria are known to enzymatically transform plant phenolic compounds, potentially producing metabolites with biological activities that differ from those of their precursors [29]. Therefore, the reduced antibacterial activity observed on day 9 may reflect changes in the concentration, composition, stability, or bioactivity of antibacterial constituents rather than simple depletion of the fermentation substrate.

The data obtained, excluding the positive control, were subjected to statistical analysis. Based on the results of the Kruskal-Wallis test (p-value <0.05) for *Escherichia coli*, a p-value of 0.008 <0.05 was obtained, indicating a significant difference between the diameter of the inhibition zone and the duration of fermentation. The results of the post hoc analysis using the Mann-Whitney test showed that the comparison between a 5 day and a 7-day fermentation period revealed a significant difference this indicates that a 7-day fermentation period provides the most optimal antibacterial activity against *Escherichia coli*.

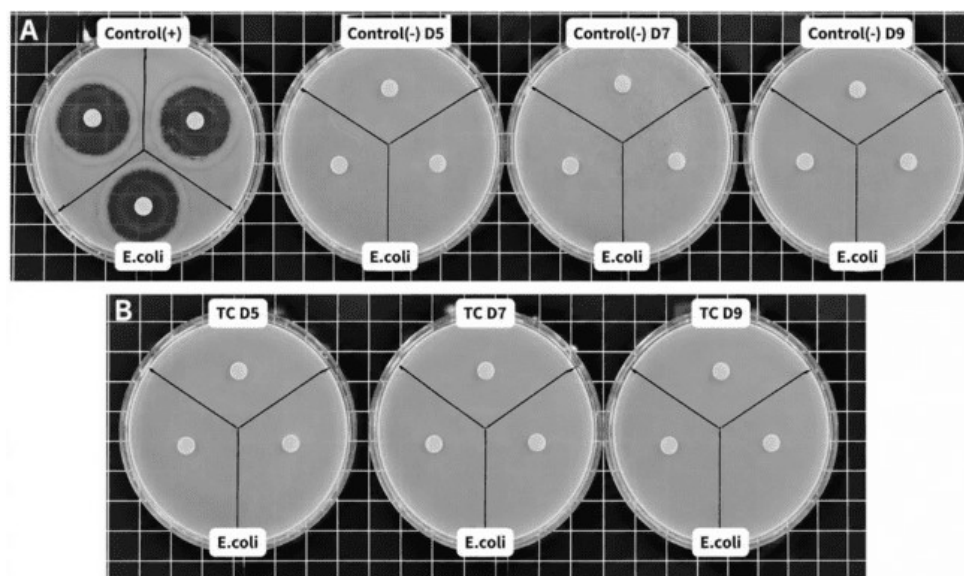


Figure 2. Inhibition zones produced by the fermentation filtrate against *Escherichia coli* bacteria; (A) ciprofloxacin positive control; (B) negative control day 5; (C) negative control day 7; (D) negative control day 9; (E) treatment control day 5; (F) treatment control day 7; (G) treatment control day 9

The results of the inhibition zone diameters from the antibacterial activity test against *Staphylococcus aureus* are shown in **Table 5**, with visual representation shown in **Figure 3**.

Table 5. Category of the inhibitor zone of fermentation filtrate against *Staphylococcus aureus*

Treatment	Mean±SD	Category	P-Value
Control (+) Ciprofloxacin	22.31±0.48	Very Strong	
Fermentation C (-) D5	-	-	
Fermentation C (-) D7	3.94±0.51	Weak	
Fermentation C (-) D9	1.65±0.38	Weak	
Fermentation (TC) D5	3.53±0.58	Weak	0.243
Fermentation (TC) D7	3.26±0.73	Weak	
Fermentation (TC) D9	3.80±0.68	Weak	

Description: <5 mm (Weak), 5-10 mm (Moderate), 10-20 mm (Strong), >20 mm (very Strong)

Based on **Table 5** when compared the addition of starter cultures did not always produce larger inhibition zones than the negative control, on day 7 the negative control produced an inhibition zone of 3.94 ± 0.51 mm, which was larger than that of the treated control, which was 3.26 ± 0.73 mm. the addition of starter culture demonstrated more consistent activity because it was able to produce inhibition zones at all fermentation time points, namely 5, 7 and 9 days, whereas in the negative control fermentation, activity did not appear on day 5 and tended to decrease on day 9, indicating that the addition of bacterial starter does not directly increase antibacterial activity but plays a role in maintaining the stability of antibacterial activity throughout the fermentation process.

The data obtained, excluding the positive control, were subjected to statistical analysis. Based on the results of the Kruskal-Wallis test (p-value <0.05), the test results for *Staphylococcus aureus* yielded a p-value of

0.243 > 0.05, indicating that there was no significant difference between the inhibition zone diameter and the duration of fermentation.

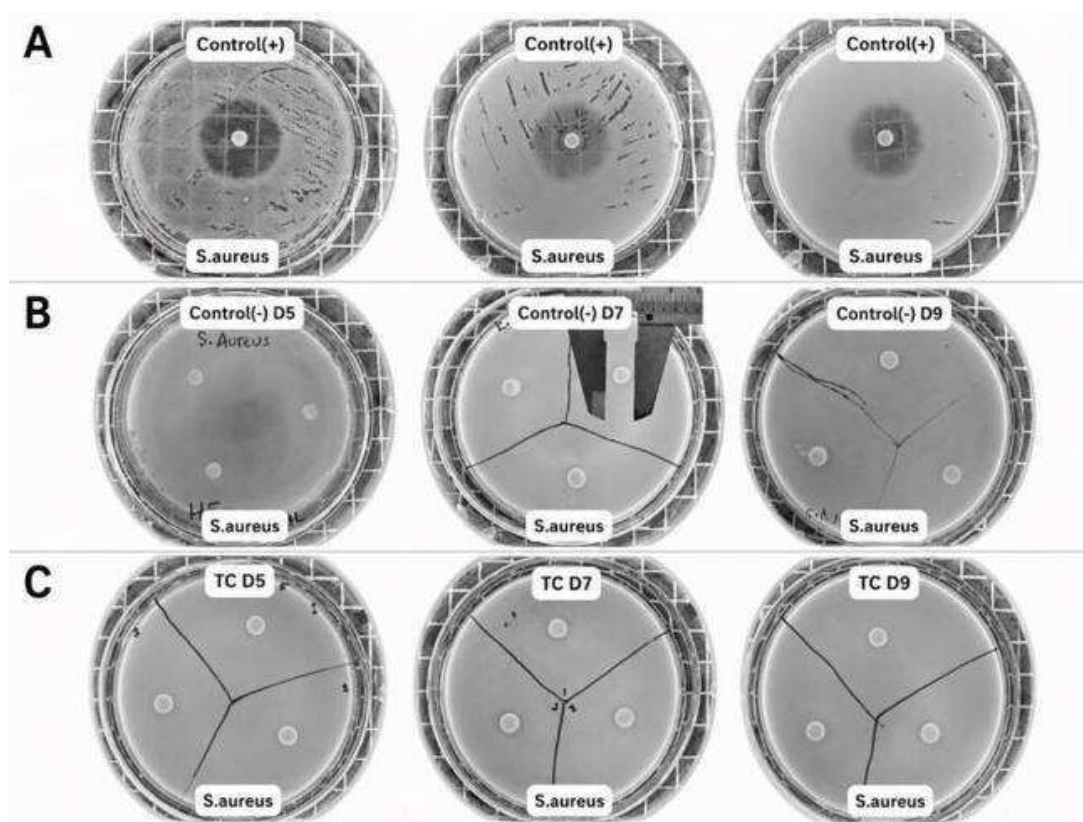


Figure 3. Inhibition zones produced by the fermentation filtrate against *Staphylococcus aureus* bacteria; (A, B, C) ciprofloxacin positive control; (D) negative control day 5; (E) negative control day 7; (F) negative control day 9; (G) treatment control day 5; (H) treatment control day 7; (I) treatment control day 9

Overall, the results of the antibacterial activity tests against the two test bacteria *Escherichia coli* and *Staphylococcus aureus* were also supported by pH test data on the fermentation filtrates from the both treatments, the pH resulting from the control treatment fermentation was more acidic compared to the negative control, proving that the fermentation process by lactic acid bacteria was occurring, but this pH decrease was not yet accompanied by a significant increase in inhibitory activity. Additionally, this could be attributed to the secondary metabolites in the fermentation filtrate, as evidenced by the phytochemical screening test, which showed that their concentration were still not high enough furthermore the concentrations were still not high low 10 mg of starting material per disk and thus was not yet capable of producing a large inhibition zone.

3.6. Data Analysis Results

Statistical analysis showed that not all data groups were normally distributed, or that the significance value was < 0.05 for some groups. This means that the data did not meet the criteria for the normality test. The analysis was continued using the nonparametric *Kruskal-Wallis* test.

The results of the nonparametric *Kruskal-Wallis* test on *Escherichia coli* bacteria yielded a p-value of 0.008 ($p < 0.05$), indicating a significant between the diameter of the inhibition zone and the duration of fermentation. Therefore a post hoc test using *Mann-Whitey* test is required. The results of the *Mann-Whitey* test showed that the comparison between a 5 day and a 7-day fermentation period yielded a significant difference. And a 7-day fermentation period provides the most optimal antibacterial activity against *Escherichia coli*.

4. CONCLUSION

Fermentation of kepok banana peel waste, both with and without *Lacticaseibacillus casei* exhibited weak antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. The addition of *Lacticaseibacillus casei* tended to further enhance the consistency of activity at certain treatment time points but did not yet show a strong and uniform increase. Thus, the fermentation of kepok banana peels using *Lacticaseibacillus casei* has the potential to enhance antibacterial activity particularly under optimal conditions specifically on day 7 with the addition of a starter against *Escherichia coli*.

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