



Antibacterial Potential of Bacterial Isolates from Keluak (*Pangium edule*) Fermentation Against *Salmonella typhimurium*

¹Fazza Safira, ²Guntur Trimulyono

^{1,2}Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya, Surabaya, Indonesia.

*Corresponding Author e-mail: fazzasafira12@gmail.com

Received: July 2026; Revised: September 2026; Accepted: September 2026; Published: September 2026

Abstract: This study aimed to isolate bacterial strains from fermented keluak and evaluate their antibacterial activity against *Salmonella typhimurium*. Bacterial isolation was performed using the pour-plate technique. The isolates were characterized macroscopically based on colony morphology and microscopically using Gram staining and endospore staining. Physiological characterization was further conducted using catalase and motility tests. Antibacterial activity was evaluated using the agar well diffusion method, in which the wells were filled with cell-free supernatants obtained from the bacterial isolates, distilled water as the negative control, and erythromycin as the positive control. Antibacterial activity was determined by measuring the diameter of the inhibition zones using a vernier caliper. A total of 12 bacterial isolates were obtained, six of which were identified as lactic acid bacteria (LAB). Among the LAB isolates, K21A exhibited the largest inhibition zone (8.85 mm), whereas K32B showed the smallest inhibition zone (1.45 mm). In comparison, the positive control produced an average inhibition zone of 16.25 mm. These findings demonstrate that LAB isolated from fermented keluak produce cell-free supernatants with antibacterial activity against *S. typhimurium*. Thus, fermented keluak may serve as a potential source of antibacterial LAB with prospective applications in the prevention or management of bacterial gastroenteritis.

Keywords: Antibacterial activity; gastroenteritis; inhibition zone; lactic acid bacteria (LAB); well diffusion

How to Cite: Safira, F., & Trimulyono, G. (2026). Antibacterial Potential of Bacterial Isolates from Keluak (*Pangium edule*) Fermentation Against *Salmonella typhimurium*. *Bioscientist: Jurnal Ilmiah Biologi*, 14(3), 1595–1609. <https://doi.org/10.33394/bioscientist.v14i3.22026>



<https://doi.org/10.33394/bioscientist.v14i3.22026>

Copyright© 2026, Safira et al

This is an open-access article under the [CC-BY-SA](#) License.



INTRODUCTION

Salmonella typhimurium is a Gram-negative, bacillus-shaped, facultative intracellular pathogenic bacterium (Perez & Hinton, 2025). It can proliferate in diverse environments, including livestock, wild animals, and plant surfaces (Perez & Hinton, 2025). Infection with *Salmonella* spp. commonly occurs through the consumption of food contaminated with animal or human fecal material (Bintsis, 2017). Foods at high risk of contamination include animal-derived products such as eggs, meat, poultry, fish, milk and dairy products, and crustaceans (EFSA, 2015; Bintsis, 2017). *Salmonella* infection is frequently associated with gastrointestinal disorders, particularly gastroenteritis (Rianto et al., 2018). Gastroenteritis may develop when *S. typhimurium* enters the gastrointestinal tract and persists in the body for more than 18 hours (Rianto et al., 2018). Clinical manifestations may include fever, vomiting, headache, diarrhea, and abdominal pain, which generally persist for approximately 2–5 days (Prihdanani, 2015; Rianto et al., 2018). Consequently, salmonellosis-associated gastroenteritis remains an important public health concern worldwide, including in Indonesia (Rianto et al., 2018).

Several therapeutic approaches are used to manage gastroenteritis, including antibiotic treatment. The selection of antibiotics should consider several criteria, including their tolerability, ability to reach adequate concentrations in the intestine, and appropriate spectrum of activity against the target bacteria (Falakh & Asri, 2020). According to the WHO's "Antibiotic Awareness Book" (2022), antibiotic use should be

adjusted according to the appropriate dosage, drug selection, and duration of treatment based on the type of disease and the patient's clinical condition. However, excessive or inappropriate antibiotic use can contribute to the development of antimicrobial resistance (WHO, 2022). Antimicrobial resistance is a major concern because resistant bacteria may no longer respond effectively to conventional treatment, thereby increasing the need for alternative approaches to control pathogenic bacterial growth. One promising strategy is the utilization of lactic acid bacteria (LAB), which are known to produce antimicrobial compounds capable of inhibiting pathogenic microorganisms. Fermented foods, including fermented keluak, may therefore represent potential sources of LAB isolates capable of producing antibacterial metabolites that could be developed as an alternative approach for inhibiting the growth of *S. typhimurium*.

Lactic acid bacteria are generally Gram-positive, coccal or rod-shaped, non-spore-forming bacteria that grow under anaerobic or facultatively aerobic conditions (Quinto et al., 2014; Fauziyah et al., 2023). LAB are commonly associated with fermented foods and beverages because they contribute to fermentation and may suppress spoilage microorganisms through the production of organic acids and other antimicrobial metabolites (Suyono et al., 2024). Their antimicrobial activity is associated with several compounds, including lactic acid, which lowers environmental pH, as well as acetic acid, diacetyl, hydrogen peroxide, carbon dioxide, and bacteriocins (Ravindran et al., 2016). Bacteriocins, in particular, have potential as naturally derived antimicrobial agents capable of inhibiting the growth of pathogenic bacteria (Syukur et al., 2011; Mastuti, 2022). These properties indicate that LAB may provide a useful biological approach for controlling pathogenic microorganisms.

One Indonesian food ingredient that undergoes natural fermentation involving microbial activity is keluak. Keluak (*Pangium edule*) is a plant native to Indonesia belonging to the family *Flacourtiaceae* and is widely distributed across diverse environmental conditions (Samudry et al., 2018). The seed flesh is commonly used as both an ingredient and a flavoring component in various traditional Indonesian dishes. During natural fermentation, keluak seeds support the activity of diverse microorganisms that may produce specific metabolites. This fermentation environment may therefore provide a potential source of beneficial microorganisms, including LAB capable of producing antimicrobial compounds that inhibit pathogenic bacteria.

Previous research by Mamuaja & Lumoindong (2017) demonstrated that bacterial isolates obtained from keluak fermentation could produce antimicrobial compounds capable of inhibiting pathogenic bacteria, including *Bacillus* sp., *Salmonella* sp., and *Escherichia* sp. Similarly, Berlian et al. (2022) reported an increase in LAB populations during the fermentation of pado fish products prepared using keluak seeds. However, previous studies have primarily focused on the general antibacterial activity of bacterial isolates and the occurrence of LAB in keluak-based fermented products. Studies specifically investigating the isolation and selection of presumptive LAB from keluak fermentation, followed by evaluation of the antibacterial activity of the cell-free supernatant from each isolate against *S. typhimurium*, remain limited.

Accordingly, the novelty of this study lies in the isolation of bacteria from keluak fermentation, the selection of isolates presumptively identified as LAB, and the evaluation of the antibacterial activity of cell-free supernatants from individual isolates against *S. typhimurium*. The use of cell-free supernatants enables the antibacterial effects of extracellular compounds or metabolites produced by LAB to be evaluated in the absence of viable bacterial cells. Therefore, this study aimed to evaluate the antibacterial potential of cell-free supernatants obtained from bacterial isolates derived

from fermented keluak (*P. edule*) against the growth of *S. typhimurium*, a bacterial pathogen associated with gastroenteritis.

METHOD

This study employed an exploratory design with a quantitative approach to evaluate the antibacterial potential of cell-free supernatants produced by lactic acid bacteria (LAB) isolated from fermented keluak (*Pangium edule*) against the growth of *Salmonella typhimurium*. Antibacterial activity was assessed using an agar well diffusion assay. Each well received 50 µL of LAB supernatant, 0.5% erythromycin as the positive control, or sterile distilled water as the negative control. Following incubation for 24 h, the resulting inhibition zones were measured in millimeters. Inhibition-zone diameter data were analyzed descriptively to characterize the antibacterial potential of each isolate. Inferential statistical analysis was not performed because the study was exploratory and was intended to provide an initial assessment of bacterial growth inhibition. The study was conducted from January to April 2026 at the Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Surabaya State University.

Materials and Equipment

The equipment used in this study included an autoclave, laminar airflow cabinet, microscope, incubator, vortex mixer, analytical balance, Petri dishes, beakers, test tubes, measuring cylinders, 250-mL Erlenmeyer flasks, Bunsen burner, inoculating loop, micropipettes, blue and yellow pipette tips, cork borers, inoculating needles, magnetic stirrer, hot plate, colony counter, vernier caliper, spectrophotometer, and centrifuge.

The materials consisted of keluak seeds, De Man-Rogosa-Sharpe broth (MRSB), De Man-Rogosa-Sharpe agar (MRSA), Bacto agar, nutrient agar (NA), sulfide-indole-motility (SIM) medium, 1% CaCO₃, sterile distilled water, erythromycin, crystal violet solution, iodine, safranin, 5% malachite green, 70% alcohol, 96% alcohol, 3% hydrogen peroxide (H₂O₂), immersion oil, 0.85% NaCl, 0.9% NaCl, BaCl₂, H₂SO₄, 0.1 N NaOH, 1% phenolphthalein, and a pure culture of *S. typhimurium*.

Isolation and Purification of Bacteria from Fermented Keluak

Naturally fermented keluak seeds were used as the sample source. The samples were purchased from a traditional market and selected based on the following criteria: intact shells, absence of holes, and relatively heavy weight. Seed maturity was determined based on dark-brown coloration, absence of visible mold, a slightly soft texture, absence of excessive dryness or foul odor, and a slightly moist consistency. The samples were stored in a dry, open area at room temperature, approximately 28–30°C.

Bacterial isolation was performed using the pour-plate method. One gram of keluak seed flesh was weighed, homogenized using a mortar and pestle, and transferred into a test tube containing 9 mL of 0.85% NaCl solution. The suspension was homogenized using a vortex mixer. Subsequently, 1 mL of the suspension was transferred into another test tube containing 9 mL of 0.85% NaCl and homogenized to obtain a 10⁻² dilution. Serial dilution was continued until a final dilution of 10⁻⁶ was obtained (Nisa et al., 2020). Aliquots from each dilution were inoculated into Petri dishes containing MRSA supplemented with 1% CaCO₃ using the pour-plate technique and incubated at 37°C for 24 h. Colonies surrounded by clear zones on the MRSA medium were considered presumptive LAB isolates.

Presumptive LAB colonies were purified to obtain single isolates. Selected

colonies were picked using a sterile inoculating loop and streaked onto MRSA plates using the four-quadrant streak method. The plates were incubated in an inverted position at 37°C for 24 h. Well-isolated colonies that appeared in the fourth quadrant were selected as pure isolates. These isolates were then inoculated onto MRSA slants, incubated at 37°C for 24 h, and stored at 4°C until further characterization.

Colony Morphology

Colony morphology was examined macroscopically based on colony form, elevation, margin, and color. These characteristics were used to distinguish among the bacterial isolates obtained from fermented keluak.

Microscopic Characterization

1. Gram staining

A drop of sterile distilled water was placed on a clean microscope slide, and a small amount of bacterial isolate was transferred onto the slide using an inoculating loop and spread to form a smear. The smear was heat-fixed by briefly passing the slide over a flame several times. Crystal violet was applied to the smear and allowed to stand for 1 min, after which the slide was rinsed with water. Iodine solution was then added and left for 1 min. The smear was subsequently decolorized with 96% alcohol for 30 s and counterstained with safranin. After air-drying, the preparation was examined under a microscope at 1000× magnification using immersion oil.

2. Endospore staining

A bacterial isolate was placed on a glass slide containing sterile distilled water and heat-fixed until dry. The smear was covered with filter paper, and malachite green solution was applied to the paper. After 5 min, the preparation was rinsed with running water and allowed to dry. The slide was then examined under a microscope at 1000× magnification using immersion oil.

Physiological Characterization

1. Catalase test

A bacterial isolate was smeared onto a clean microscope slide, after which 2–3 drops of 3% H₂O₂ solution were added. The reaction was observed immediately. The absence of oxygen bubbles was interpreted as a negative catalase reaction.

2. Motility test

A bacterial isolate grown on MRSA medium was picked using a sterile inoculating loop and inoculated into SIM medium in a test tube using the stab-inoculation method. The culture was incubated at 37°C for 24 h. Growth beyond the stab line indicated motility, whereas growth confined to the stab line indicated non-motility (Giyatno & Retnaningrum, 2020).

Preparation of the Test Bacterium

A single colony from a pure culture of *S. typhimurium* was inoculated onto NA slant medium under aseptic conditions and incubated at 37°C for 24 h (Annisa & Mursyid, 2020). The optical density of the bacterial suspension was measured using a UV-Vis spectrophotometer at 600 nm. The suspension was standardized to 0.5 McFarland, corresponding to an absorbance range of 0.08–0.10 and approximately 1.5×10^8 CFU/mL (Altuner, 2025). When the turbidity exceeded the standard, the suspension was diluted using 0.9% NaCl. Conversely, when the turbidity was below the 0.5 McFarland standard, additional bacterial colonies were added until the required turbidity was achieved (Sari et al., 2019; Isnaini & Trimulyono, 2024).

The McFarland standard was prepared by mixing 0.05 mL of 1% BaCl₂ with 9.95 mL of H₂SO₄ solution, followed by homogenization using a vortex mixer (Rosmania &

Fitri, 2020; Rizki et al., 2022).

Preparation of Positive and Negative Controls

Erythromycin at a concentration of 0.5% was used as the positive control. A total of 25 mg of erythromycin powder was dissolved in 5 mL of distilled water and vortexed until homogeneous. Subsequently, 50 µL of the erythromycin solution was dispensed into each well using a micropipette. Sterile distilled water was used as the negative control.

Preparation of Bacterial Supernatants

Each bacterial isolate was cultured in MRSB medium and incubated for 24 h. The cultures were homogenized using a vortex mixer, after which 10 mL of each culture was transferred into a 15-mL centrifuge tube. The samples were centrifuged at 6,000 rpm for 5 min. Following centrifugation, the clear supernatant was separated from the cell pellet and collected using a micropipette. The resulting cell-free supernatant was used for antibacterial activity testing using the agar well diffusion method.

Antibacterial Activity Assay

Antibacterial activity was evaluated using the agar well diffusion method. A 1-mL aliquot of a pure *S. typhimurium* suspension was inoculated into a Petri dish containing 15 mL of NA medium, gently homogenized, and allowed to solidify (Sarifuddin, 2022). Three wells, each approximately 5 mm in diameter, were created in the solidified agar using a sterile cork borer. The wells were filled separately with 50 µL of bacterial supernatant obtained from fermented keluak isolates, 50 µL of 0.5% erythromycin as the positive control, and 50 µL of sterile distilled water as the negative control. The plates were then incubated at 37°C for 24 h (Fitrianarni et al., 2014; Isnaini and Trimulyono, 2024). After incubation, inhibition zones formed around the wells were observed and measured using a vernier caliper.

Determination of Total Acidity

Total acidity was determined by titration and expressed as lactic acid equivalents (Sudarmadji, 1997; Widedianto et al., 2017). A 10-mL aliquot of sample was used for the analysis (Widedianto et al., 2017). Before titration, two drops of 1% phenolphthalein indicator were added to the sample. The sample was then titrated with 0.1 N NaOH until a persistent pink endpoint was observed. Total acidity was calculated using the following equation (Widedianto et al., 2017):

$$\text{Acidity level} = \frac{V_1 \times N \times B}{V_2 \times 1000} \times 100\%$$

Notes:

V1 = Volume of NaOH (ml)

V2 = Volume of sample (ml)

N = Normality of NaOH (0.1 N)

B = Molecular mass of lactic acid (90)

Data Analysis

Inhibition zones were observed after 24 h of incubation. The formation of a clear zone around a well was interpreted as evidence of the sensitivity of *S. typhimurium* to the antibacterial compounds present in the LAB supernatants derived from fermented keluak isolates. The diameters of the inhibition zones were measured in millimeters using a vernier caliper. Vertical and horizontal diameters were measured and calculated according to Toy et al. (2015) using the following equation:

$$D = \frac{(D_v - D_c) + (D_h - D_c)}{2}$$

Notes:

D = Diameter of the barrier zone

D_v = Vertical diameter

D_h = Horizontal diameter

D_c = Well diameter (± 5 mm)

The inhibition-zone data were analyzed descriptively to characterize the antibacterial potential of each isolate. Because this study was exploratory and intended to provide an initial assessment of the antibacterial activity of cell-free supernatants derived from fermented keluak isolates against *S. typhimurium*, no inferential statistical tests were conducted to compare treatments or examine relationships among variables.

RESULTS AND DISCUSSION

Morphological, Microscopic, and Physiological Characterization of Colonies

This study was conducted by isolating bacteria from fermented keluak (*P. edule*) samples using serial dilutions up to a dilution of 10⁻⁶, resulting in a total of 12 bacterial isolates with diverse characteristics. The isolates were then characterized based on macroscopic, microscopic, and physiological observations. The results of this characterization are presented in Table 1 and Table 2. Isolate codes are based on the sample name, dilution level, replicate number, and colony code on the same Petri dish.

Table 1. Morphological characteristics of bacterial isolates from Keluak (*P. edule*) fermentation

Isolate	Macroscopic Characteristics			
	Colony Shape	Elevation	Edge Shape	Color
K21A	Circular	Pulvinate	Entire	White
K21B	Circular	Flat	Entire	White
K61A	Circular	Convex	Entire	Yellowish white
K12A	Rhizoid	Umbonate	Curled	Yellowish white
K32A	Circular	Raised	Entire	Yellowish white
K62A	Circular	Pulvinate	Entire	White
K51A	Irregular	Raised	Curled	Yellowish white
K61B	Circular	Flat	Entire	Yellowish white
K12B	Circular	Raised	Entire	Cream
K22B	Spindle	Umbonate	Curled	White
K32B	Circular	Raised	Entire	Yellow
K62B	Circular	Flat	Entire	White

Table 2. Microscopic and physiological characteristics of bacterial isolates from Keluak (*P. edule*) fermentation

Isolate	Microscopic		Physiological		Cell Shape
	Gram Stain	Endospore Stain	Catalase Test	Motility Test	
K21A	+	-	-	-	Coccus
K21B	+	-	-	-	Bacill
K61A	+	-	-	-	Bacill
K12A	+	-	-	-	Coccus
K32A	+	-	-	-	Bacill

Isolate	Microscopic		Physiological		Cell Shape
	Gram Stain	Endospore Stain	Catalase Test	Motility Test	
K62A	+	-	-	-	Bacill
K51A	+	-	+	+	Coccus
K61B	-	-	+	+	Coccus
K12B	+	-	+	+	Bacill
K22B	-	-	+	+	Bacill
K32B	+	-	+	+	Bacill
K62B	-	-	+	+	Coccus

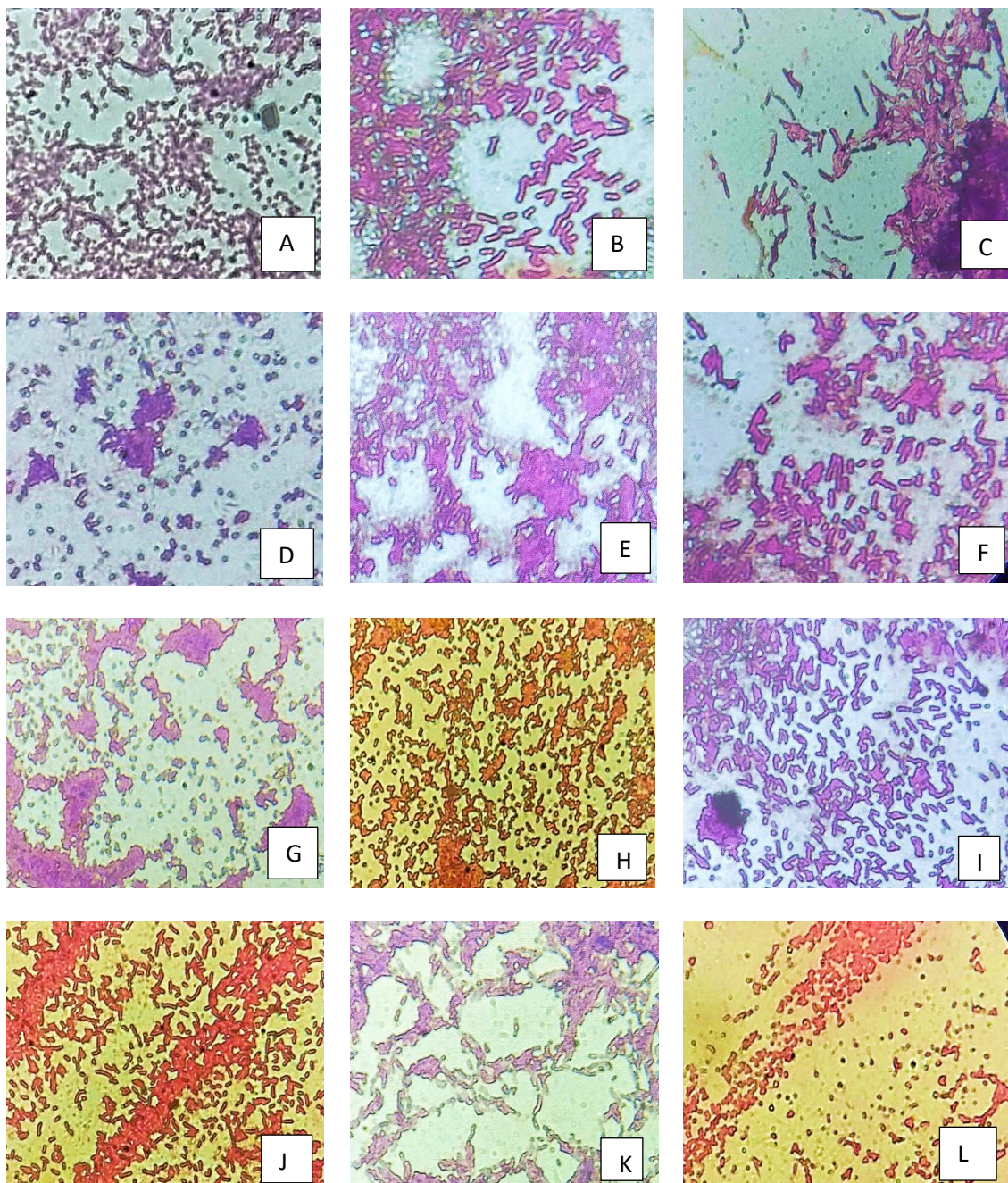


Figure 1. Gram stain results for bacterial isolates: A) K21A, B) K21B, C) K61A, D) K12A, E) K32A, F) K62A, G) K51A, H) K61B, I) K12B, J) K22B, K) K32B, L) K62B (magnification 1000x)

Based on the results of the isolation process, 12 isolates were obtained, 6 of which were suspected to be BAL, as indicated by the presence of a clear zone around the colonies growing on MRSA + 1% CaCO₃ medium. This occurs because the addition of CaCO₃ causes the acid produced by the bacteria to react with the base CaCO₃, resulting in the formation of a clear zone around the colonies (Suryani et al., 2017; Indrayanti & Nur, 2025). Based on observations of bacterial colony morphology, the 12 isolates obtained exhibited diverse characteristics, including round, spindle-shaped, and irregular forms. The colonies displayed convex, protruding, flat, pulvinate, and umbonate elevations. The colony edges were either smooth or curved. Colony colors ranged from white to yellowish-white. Morphologically, some colonies suspected to be BAL shared characteristics with those described in a study by Kurnia et al. (2020), as cited in Canrika et al. (2024), which noted that BAL colonies are white to yellowish-white, round in shape, and have smooth edges.

Microscopic and physiological characterization of isolates suspected to be BAL namely isolates K21A, K21B, K61A, K12A, K32A, and K62A revealed characteristics such as Gram-positive, endospore-negative, catalase-negative, and non-motile. These characteristics are consistent with a study conducted by Jeyagowri et al. (2023), which noted that BAL isolates generally exhibit characteristics such as being Gram-positive, spore-negative, catalase-negative, and non-motile. The presence of isolates that did not exhibit BAL characteristics namely K51A, K61B, K12B, K22B, K32B, and K62B indicates diversity in the isolates obtained from the fermented product. This is consistent with the study by Melizah et al. (2018), which reported that isolates suggesting *Staphylococcus* sp., in addition to *Lactobacillus* sp. isolates, were obtained from fermented bekasam. Based on these results, characterization was performed to determine the group of microorganisms before conducting further testing for their antibacterial activity. However, phenotypic characterization was not performed in this study; the data were used only for preliminary classification into BAL or non-BAL groups and therefore cannot be used to determine identity down to the genus or species level.

Antibacterial Test

Based on antibacterial activity testing, as determined by the formation of a clear zone around the well, which was measured using a vernier caliper in millimeters (mm). The results of the average diameter measurements are shown in Table 3.

Table 3. Results of antibacterial activity tests on bacterial isolates from Keluak (*P. edule*) fermentation against *S. typhimurium*

Supernatant sample	Average Diameter of the Clear Zone (mm)	Category
K21A	8.85	Currently
K21B	8.15	Currently
K61A	6.5	Currently
K12A	6.2	Currently
K32A	6.5	Currently
K62A	7.15	Currently
K51A	3.4	Weak
K61B	3.5	Weak
K12B	4.1	Weak
K22B	2.65	Weak
K32B	1.45	Weak
K62B	5.75	Currently

Supernatant sample	Average Diameter of the Clear Zone (mm)	Category
Positive control (erythromycin 0.5%)	16.25	Strong
Negative control (distilled water)	0	Does not hinder

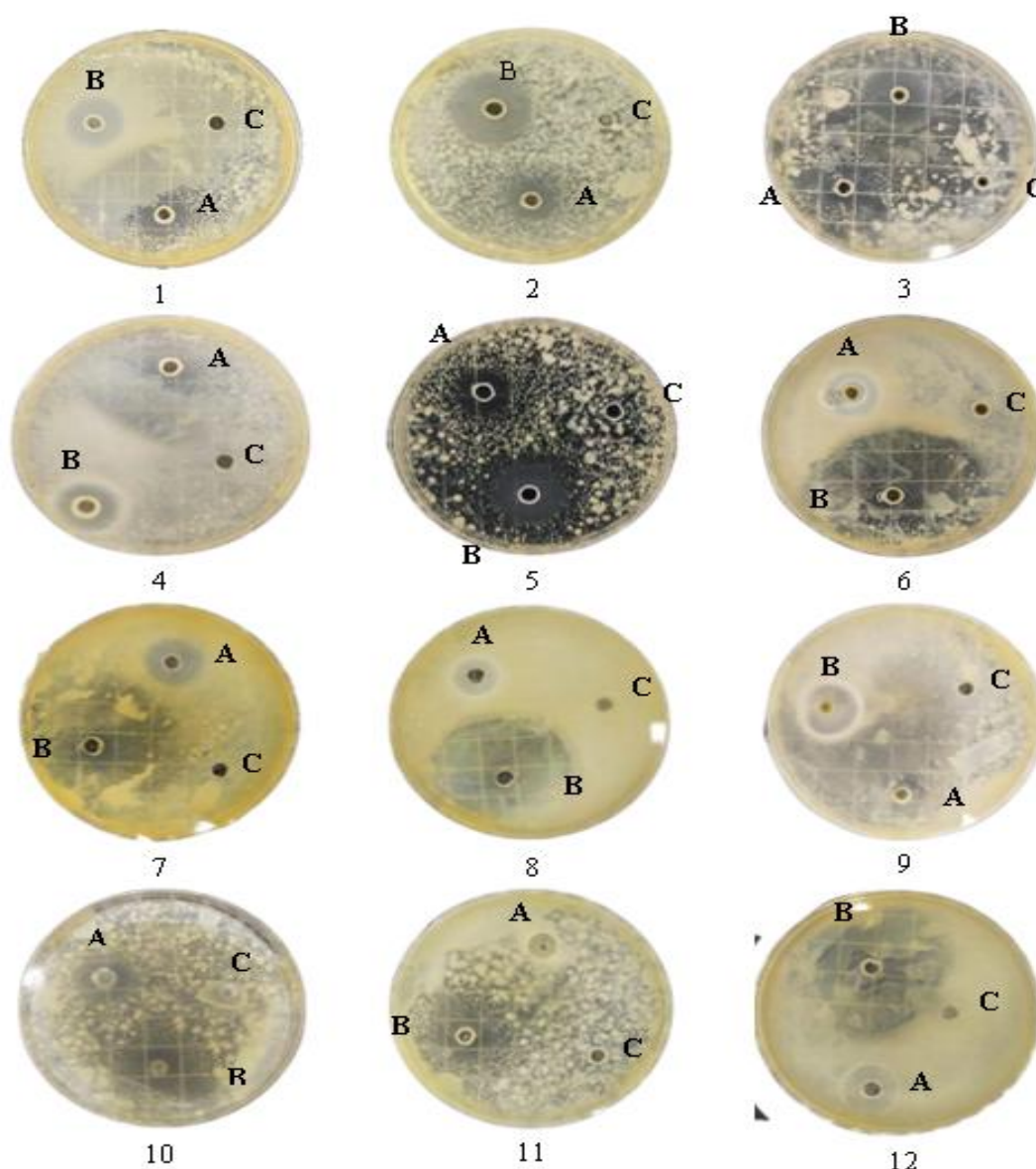


Figure 2. Inhibitory zone diameter in the antibacterial activity test results 1. K21A 2. K21B 3. K61A 4. K12A 5. K32A 6. K62A 7. K51A 8. K61B 9. K12B 10. K22B 11. K32B 12. K62B (Note: A = Bacterial isolate supernatant, B = Positive control (erythromycin 0.5%), C = Negative control (distilled water))

The results of the study on antibacterial activity showed that the supernatant obtained from antibacterial compounds isolated from keluak bacteria produced by fermentation exhibited antibacterial activity against the growth of *S. typhimurium*. This activity was observed through the formation of a clear zone around the well. A clear zone is a transparent area free of bacterial growth and is measured in millimeters (mm) (Afifi et al., 2018). Based on the results obtained, all BAL isolates produced inhibition zones classified as moderate, as their average diameters ranged from 6–10 mm

(Kumowal et al., 2019). The largest inhibition zone diameter for the BAL isolate K21A was 8.55 mm, which was classified as moderate because the result fell within the 6–10 mm range (Kumowal et al., 2019). The inhibition zone diameter results for K21A were still within the range reported in a previous study by Pelyunta et al. (2019), who found that cell-free supernatants from BAL isolates produced inhibition zones against *S. typhimurium* ranging from 3 to 9 mm.

Differences in activity among isolates are related to differences in each isolate's ability to produce antibacterial inhibition zones. This is supported by Pelyuntha et al. (2019), who stated that cell-free supernatant plays a role in the growth of *Salmonella* bacteria, while its inhibitory activity varies among isolates. Therefore, the larger inhibition zone of isolate K21A is related to the isolate's ability to produce more active antimicrobial metabolites compared to other isolates. However, this hypothesis cannot yet be confirmed because inhibitory activity is also influenced by compounds diffusing into the agar medium, as this study used the well diffusion method. The well diffusion method was chosen because it is considered more effective than the disk diffusion method for testing antibacterial activity (Haryati et al., 2017; Aqila & Asri, 2025). Another advantage of this method is the ease of measuring the diameter of the inhibition zone, which forms not only on the surface of the medium but also spreads into the interior of the medium as a result of bacterial growth (Retnaningsih et al., 2019).

An interesting finding in this study is that several isolates, although not classified as BAL, exhibited inhibition zones against *S. typhimurium*. These results are consistent with the study by Voidarou et al. (2020), which found that bacteria other than BAL such as *Bacillus*, *Micrococcaceae*, *Bifidobacterium*, *Brachybacterium*, *Brevibacterium*, and *Propionibacterium* can also be involved in the fermentation process. These results indicate that bacteria other than BAL are capable of producing antibacterial compounds. One such antibacterial compound produced by non-BAL bacteria, BAL, and fungi is phenyl lactic acid (Figueroa et al., 2024). In addition, microorganisms present in fermented materials produce various metabolites such as organic acids (lactic acid, acetic acid), hydrogen peroxide, diacetyl, and bacteriocins (Patrick, 2012; Karyawati et al., 2024). Therefore, the inhibitory activity exhibited by bacteria other than BAL in this study cannot be directly attributed to lactic acid production, as metabolic compounds depend on the species and strains of microorganisms involved. These findings are more appropriately considered preliminary evidence of the antibacterial potential of bacteria other than BAL, which requires further investigation. The identification of the active compounds in these isolates has not yet been conducted in this study.

This study used the antibiotic erythromycin as a positive control. The results from the positive control showed a larger inhibition zone compared to the supernatant of the isolates. This difference is related to the differing characteristics of the test materials, as antibiotics are compounds that can inhibit pathogenic bacteria through various mechanisms of action (Suksma et al., 2024). In contrast, the supernatant is a mixture of metabolites produced by bacterial growth, the active components of which have not yet been specifically identified (Wulansari et al., 2019). The larger diameter of the positive control's inhibition zone indicates greater inhibition in the test; however, it cannot be used as a direct measure to compare the antibacterial potential between erythromycin and the supernatant. This is because the formation of inhibition zones is influenced not only by the diffusion capacity of the inoculated antibacterial agent but also by the growth rate of the tested microorganisms and the bacteria's sensitivity to that antibacterial agent (Wilapangga et al., 2018).

Total Acid

The total acid content of each bacterial isolate was determined and expressed as lactic acid equivalents, as presented in Table 4.

Table 4. Results of acid content determination

Isolate Code	Volume NaOH (ml)	Total Acid (%)
K21A	11.2	1
K21B	9	0.81
K51A	8	0.72
K61A	9.4	0.84
K61B	8.4	0.72
K12A	9	0.81
K12B	9	0.81
K22B	8	0.72
K32A	9	0.81
K32B	8	0.72
K62A	9.8	0.88
K62B	8.3	0.74

The total acid content of the bacterial isolates was determined using a titration method. Titration was performed using 0.1 N NaOH until the endpoint was reached, as indicated by a color change in the solution to pink (Kurnia et al., 2020). The titrant solution consisted of liquid cultures in MRS Broth that had been centrifuged to obtain the supernatant, which contained the organic acids (Kurnia et al., 2020). The measured lactate acid levels varied among isolates. Isolate K21A had the highest acid content at 1%, with an inhibition zone diameter of 8.55 mm. Several isolates, such as K51A (0.72%; 3.4 mm), K61B (0.72%; 3.5 mm), K22B (0.72%; 2.65 mm), and K32B (0.72%; 1.45 mm), produced low lactate acid levels, as indicated by their smaller inhibition zones.

These results indicate a trend whereby higher acid concentrations result in larger inhibition zones. This is consistent with the study by Risna et al. (2022), which found a negative correlation between the pH of the BAL supernatant and the diameter of the inhibition zone, meaning that isolates with lower pH tend to exhibit higher inhibitory activity against pathogenic bacteria. A limitation of this study in determining the relationship between acid concentration and inhibition zone diameter is the absence of statistical correlation analysis. Furthermore, the measurement of total lactic acid concentration cannot be used to identify specific organic acids responsible for antibacterial activity; thus, the resulting inhibition zones may stem not only from acidic compounds but also from other organic compounds. This is because BAL contains several key compounds involved in antibacterial activity, including organic acids (lactic acid, acetic acid), hydrogen peroxide, diacetyl, and bacteriocins (Patrick, 2012; Karyawati et al., 2024).

CONCLUSION

A total of 12 bacterial isolates were obtained from fermented keluak (*Pangium edule*), of which six isolates—K21A, K21B, K61A, K12A, K32A, and K62A—were presumptively identified as lactic acid bacteria (LAB). The isolates exhibited in vitro antibacterial activity against *Salmonella typhimurium*, with isolate K21A producing the largest inhibition zone (8.85 mm). These findings indicate that cell-free supernatants derived from bacterial isolates associated with keluak fermentation possess

antibacterial potential against *S. typhimurium*. Further taxonomic identification and characterization of the isolates are required, together with analyses of the specific metabolites or bioactive compounds responsible for the observed inhibitory activity.

RECOMMENDATION

Keluak samples used for bacterial isolation should be fully mature, as indicated by a brown color, slightly soft texture, absence of mold and excessive dryness, no unpleasant odor, and a slightly moist consistency. Further studies are recommended to evaluate the antibacterial activity of the isolates against other pathogenic bacteria. All procedures, from bacterial isolation to total acid determination, should be conducted under aseptic conditions to minimize contamination. In addition, pH should be measured before antibacterial testing to distinguish inhibition caused by LAB-derived bioactive compounds from inhibition resulting solely from acidic conditions.

ACKNOWLEDGMENT

The author would like to express sincere gratitude to the academic advisor for the assistance and guidance provided throughout the research process and the preparation of this manuscript. The author also extends gratitude to everyone who assisted the author, from the sample preparation process through the completion of this article.

REFERENCES

- Afifi, R., Erlin, E., & Rachmawati, J. (2018). In vitro antibacterial testing of star fruit (*Averrhoa bilimbi* L.) leaf extract against the inhibition zone of the acne-causing bacterium *Propionibacterium acnes*. *Quagga: Journal of Education and Biology*, 10(1), 10–17. <https://doi.org/10.25134/quagga.v10i01.803>
- Altuner, E. M. (2025). Inoculum standardization: Ensuring reliable antimicrobial study outcomes. *Altuner.me*, 5(29), 1–5.
- Annisa, R. N., & Mursyid, M. (2020). Antimicrobial efficacy of olive oil as a toothpaste additive against *Streptococcus mutans*. *BIOMA: Journal of Biology and Its Education*, 2(2), 1–8.
- Aqila, N. A., & Asri, M. T. (2025). Aktivitas antibakteri ekstrak daun nanas terhadap pertumbuhan bakteri *Ralstonia solanacearum*. *LenteraBio: Berkala Ilmiah Biologi*, 14(2), 196–203. <https://doi.org/10.26740/lenterabio.v14n2.p196-203>
- Berlian, S., Syah, D., Nuraida, L., & Nurjanah, S. (2022). Identification of microflora and lactic acid bacteria in pado, a fermented fish product prepared with dried *Pangium edule* seed and grated coconut. *Food Science and Technology*, 42, e19921.
- Bintsis, T. (2017). Foodborne pathogens. *AIMS Microbiology*, 3(3), 529–563. <https://doi.org/10.3934/microbiol.2017.3.529>
- Canrika, R. E., Utami, I. W., & Retno, E. K. (2024). Isolation and characterization of lactic acid bacteria (LAB) from the fermentation starter (*Lactobacillus casei*) in mandai cempedek vinegar (*Artocarpus champedon*). *PHARMACIA*, 2(1), 16–23.
- European Food Safety Authority. (2015). European Food Safety Authority and European Centre for Disease Prevention and Control. *EFSA Journal*, 13, 4329.
- Falakh, M. F., & Asri, M. T. (2022). Testing the antimicrobial potential of lactic acid bacteria isolates from siwalan sap (*Borassus flabellifer* L.) against *Salmonella typhi*. *LenteraBio: Journal of Biology*, 11(3), 514–524.

- Fauziyah, Z., Nia, L., Ndani, J. F., Lingga, R., & Helmi, H. (2023). Identification and probiotic potential of lactic acid bacteria in local fermented foods. *Jurnal Bios Logos*, 13(2), 54–64.
- Figueroa, R. H. H., López-Malo, A., & Mani-López, E. (2024). Antimicrobial activity and applications of fermentates from lactic acid bacteria: A review. *Sustainable Food Technology*, 2(2), 292–306. <https://doi.org/10.1039/D3FB00241A>
- Fitrianarni, D., Ibrahim, M., & Trimulyono, G. (2014). In vitro antibacterial activity of cow's milk yogurt and soy milk yogurt against *Shigella dysenteriae*. *LenteraBio*, 3(1), 97–102.
- Giyatno, D. C., & Retnaningrum, E. (2020). Isolation and characterization of exopolysaccharide-producing lactic acid bacteria from kersen fruit (*Muntingia calabura* L.). *Journal of Basic Sciences*, 9(2), 42–49.
- Haryati, S. D., Darmawati, S., & Wilson, W. (2017). Perbandingan efek ekstrak buah alpukat (*Persea americana* Mill.) terhadap pertumbuhan bakteri *Pseudomonas aeruginosa* dengan metode disk dan sumuran. *Prosiding Seminar Nasional & Internasional*, 1(1).
- Indrayati, S., & Nur, Y. M. (2025). Isolasi dan karakterisasi bakteri asam laktat dari feses luwak (*Paradoxurus hermaphroditus*). *BIOMA: Jurnal Biologi Makassar*, 10(1), 118–125.
- Isnaini, A. A., & Trimulyono, G. (2024). In vitro antibacterial activity of coconut yogurt against *Streptococcus mutans*. *LenteraBio: Journal of Biology*, 13(3), 393–401. <https://doi.org/10.26740/lenterabio.v13n3.p393-401>
- Jeyagowri, N., Ranadheera, C. S., Manap, M. Y., Gamage, A., Merah, O., & Madhujith, T. (2023). Phenotypic characterisation and molecular identification of potentially probiotic *Lactobacillus* sp. isolated from fermented rice. *Fermentation*, 9(9), 807. <https://doi.org/10.3390/fermentation9090807>
- Karyawati, A. T., Darmakusuma, D., Mauboy, R. S., Amalo, D., & Mutis, A. (2024). The potential of lactic acid bacteria as producers of antibacterial compounds and extracellular enzymes. *Journal of Biotropical Sciences*, 21(2), 56–65.
- Kumowal, S., Fatimawali, F., & Jayanto, I. (2019). Antibacterial activity of nanoparticles derived from white galangal (*Alpinia galanga* [L.] Willd.) extract against *Klebsiella pneumoniae*. *Pharmacon*, 8(4), 781–790.
- Kurnia, M., Amir, H., & Handayani, D. (2020). Isolation and identification of lactic acid bacteria from a traditional food of the Rejang tribe in Bengkulu Province: “Lemea.” *Journal of Chemistry Education and Science*, 4(1).
- Mamuaja, C. F., & Lumoindong, F. (2017). Antimicrobial activity of keluak (*Pangium edule*) seed extract as natural preservatives of tuna fish ball. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 20(3), 592–601. <https://doi.org/10.17844/jphpi.v20i3.19815>
- Mastuti, S. (2022). The potential of bacteriocins produced by lactic acid bacteria against *Staphylococcus aureus* and *Escherichia coli*. *Sdani Husada Scientific Journal of Health*, 11(1), 25–30.
- Melizah, A., Husin, S., & Alkaf, S. (2018). Identification of lactic acid bacteria isolate from fermentation food bekasam. *Bioscientia Medicina: Journal of Biomedicine and Translational Research*, 2(1), 16–23.
- Nisa, K., Jannah, S. N., & Rukmi, I. (2020). Isolation and antifungal activity of lactic acid bacteria from plastic-packaged glutinous rice tape against *Fusarium* sp. *Jurnal Akademika Biologi*, 9(2), 1–7.
- Patrick, O. M. (2012). Lactic acid bacteria in health and disease. *Rwanda Journal of Health Sciences*, 1(1), 39–50.

- Pelyuntha, W., Chaiyasut, C., Kantachote, D., & Sirilun, S. (2019). Cell-free supernatants from cultures of lactic acid bacteria isolated from fermented grape as biocontrol against *Salmonella Typhi* and *Salmonella Typhimurium* virulence via autoinducer-2 and biofilm interference. *PeerJ*, 7, e7555. <https://doi.org/10.7717/peerj.7555>
- Perez-Sepulveda, B. M., & Hinton, J. C. (2025). Microbe profile: *Salmonella Typhimurium*: The master of the art of adaptation. *Microbiology*, 171(1), 001521.
- Prihdanani, S. S. (2015). Testing the antibacterial activity of garlic (*Allium sativum* L.) against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium*, and *Pseudomonas aeruginosa* to enhance food safety. *Agricultural Informatics*, 24(1), 53–58.
- Quinto, E. J., Jiménez, P., Caro, I., Tejero, J., Mateo, J., & Gírbés, T. (2014). Probiotic lactic acid bacteria: A review. *Food and Nutrition Sciences*, 5(18), 1765–1775.
- Ravindran, L., Manjunath, N., Darshan, R. P., & Manuel, S. G. (2016). In vitro study analysis of antimicrobial properties of lactic acid bacteria against pathogens. *Journal of Biological Innovation*, 5(2), 262–269.
- Retnaningsih, A., Primadhamanti, A., & Marisa, I. (2019). Uji daya hambat ekstrak etanol biji pepaya terhadap bakteri *Escherichia coli* dan *Shigella dysenteriae* dengan metode difusi sumuran. *Jurnal Analis Farmasi*, 4(2), 122–129. <https://doi.org/10.33024/jaf.v4i2.2242>
- Rianto, A., Isrul, M., Anggarini, S., & Saleh, A. (2018). Isolation and identification of endophytic fungi from cashew leaves (*Anacardium occidentale* L.) as antibacterial agents against *Salmonella typhimurium*. *Jurnal Mandala Pharmakon Indonesia*, 4(2), 109–121.
- Risna, Y. K., Harimurti, S., Wihandoyo, W., Widodo, W., & Sukarno, A. S. (2022). Aktivitas antibakteri bakteri asam laktat (BAL) yang diisolasi dari saluran pencernaan itik lokal asal Aceh terhadap *Salmonella pullorum* dan *Escherichia coli*. *Jurnal Agripet*, 22(2), 169–174. <https://doi.org/10.17969/agripet.v22i2.24297>
- Rosmania, & Fitri, Y. (2020). Calculating bacterial counts in a microbiology laboratory using a modified spectrophotometric method. *Journal of Science Research*, 22(2), 76–86.
- Salsabilla, K. N., & Trimulyono, G. (2022). Isolation and antagonistic testing of lactic acid bacteria from kepok banana tape against *Escherichia coli*. *LenteraBio: Scientific Journal of Biology*, 11(3), 430–440.
- Samudry, E. G., Sukainah, A., & Mustarin, A. (2018). Quality analysis of fermented keluak (*Pangium edule* Reinw.) using soil and rice husk ash as media. *Journal of Agricultural Technology Education*.
- Sari, P., Mentari, I. N. F., & Kurniawan, E. (2019). Antibacterial activity of sea sponge (*Calyspongia* sp.) ethanol extract against *Streptococcus mutans*, the causative agent of dental caries. *Media of Medical Laboratory Science*, 3(2), 52–62.
- Sarifuddin, N. (2022). Formulation and testing of the antibacterial activity of a mouthwash made from beluntas leaf extract (*Pluchea indica* Less.) against *Streptococcus mutans*. *Biolearning Journal*, 9(1), 9–12.
- Sudarmadji, S., Haryono, B., & Suhardi. (1997). *Analytical procedures for foodstuffs and research*. Liberty.
- Suksma, N. A., Utarti, E., & Arimurti, S. (2024). Growth pattern and degradation activity of caffeine-degrading bacteria consortium. *Jurnal Ilmu Dasar*, 25(1), 59–66. <https://doi.org/10.19184/jid.v25i1.32609>

- Suryani, S., Nofiandi, D., Mukhtar, H., Siska, M., Dharma, A., & Nasir, N. (2017). Molecular identification of the lactic acid bacterium *Lactobacillus paracasei* present in the oil layer of VCO. *Jurnal Katalisator*, 2(2), 79–87.
- Suyono, M. N., Sunarti, T. C., & Merydanini, A. (2024). Pemanfaatan bakteri asam laktat (BAL) amilolitik untuk fermentasi tepung terigu dan gandum. *Jurnal Sumberdaya Hayati*, 10(2), 61–66.
- Syukur, S., Purwati, E., & Ibrahim, S. (2011). The potential of lactic acid bacteria to produce bacteriocins as antimicrobials and the measurement of their molecular weight by SDS-PAGE from cocoa fermentation isolates. *Journal of Chemical Research*, 4(2), 94.
- Toy, T. S., Lampus, B. S., & Hutagalung, B. S. (2015). Testing the inhibitory effect of *Gracilaria* sp. seaweed extract on the growth of *Staphylococcus aureus*. *e-GiGi*, 3(1).
- Voidarou, C., Antoniadou, M., Rozos, G., Tzora, A., Skoufos, I., Varzakas, T., Lagiou, A., & Bezirtzoglou, E. (2021). Fermentative foods: Microbiology, biochemistry, potential human health benefits, and public health issues. *Foods*, 10(1), 69. <https://doi.org/10.3390/foods10010069>
- Widedianto, I. N., Antara, N. S., & Wijaya, I. M. M. (2017). Growth of *Lactobacillus casei* subsp. *rhannosus* on media supplemented with kolang-kaling flour. *Journal of Agroindustry Engineering and Management*, 5(2), 1–9.
- Wilapangga, A., & Syaputra, S. (2018). Analisis antibakteri metode agar cakram dan uji toksisitas menggunakan BSLT (*Brine Shrimp Lethality Test*) dari ekstrak metanol daun salam (*Eugenia polyantha*). *Indonesian Journal of Biotechnology and Biodiversity*, 2(2), 50–56. <https://doi.org/10.47007/ijobb.v2i2.20>
- World Health Organization. (2022a). *The WHO AWaRe (Access, Watch, Reserve) antibiotic book*.
- World Health Organization. (2022b). *Understanding the complexities behind antimicrobial drug resistance*.
- Wulansari, A., Aqlinia, M., Wijanarka, W., & Raharja, B. (2019). Isolasi bakteri endofit dari tanaman bangle (*Zingiber cassumunar* Roxb.) dan uji aktivitas antibakterinya terhadap bakteri penyebab penyakit kulit *Staphylococcus epidermidis* dan *Pseudomonas aeruginosa*. *Berkala Bioteknologi*, 2(2).