



**ISOLATION OF ANTIBIOTIC PRODUCING MICROORGANISMS FROM CASSAVA ROOT  
TAPAI BY TRADITIONAL FERMENTED**

**Isolasi Mikroorganisme Penghasil Antibiotik dari Tapai Umbi Ubi Kayu Hasil  
Fermentasi Tradisional**

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**ABSTRACT**

Antibiotic resistance among pathogenic microorganisms has become a growing global health concern, necessitating the search for new natural sources of antimicrobial compounds. Cassava tapai, a traditional Indonesian fermented food, contains molds, yeasts, and bacteria with potential antibiotic-producing abilities. This study aimed to isolate and characterize antibiotic-producing microorganisms from cassava tapai fermented with local Sulawesi 'Ragi'. Isolation, colony morphology characterization, and antibiotic activity tests were conducted using the Kirby–Bauer method against *Escherichia coli* ATCC 35218 and *Staphylococcus aureus* ATCC 25923. Inhibition zones were measured with a digital caliper, and data were analyzed using one-way ANOVA followed by Tukey's HSD test or Kruskal–Wallis followed by the Mann–Whitney U test ( $p < 0.05$ ). Three isolates Bb1, Bb2, and Wk3 exhibited significant antibiotic activity, with Bb1 showing stronger inhibition against *E. Coli* ATCC 35218. These results highlight confirms the potential of cassava tapai as a promising source of antibiotic-producing microorganisms for future antimicrobial discovery.

**Keywords:** *Antibiotic producing microorganisms, Bacteria, Cassava tapai, Molds, Yeasts*

**ABSTRAK**

Resistensi antibiotik pada mikroorganisme patogen merupakan masalah kesehatan global yang terus meningkat, sehingga diperlukan eksplorasi sumber alami baru penghasil senyawa antimikroba. Tapai ubi kayu sebagai makanan fermentasi tradisional Indonesia mengandung mikroorganisme dari kelompok kapang, khamir, dan bakteri yang berpotensi menghasilkan metabolit sekunder antimikroba. Penelitian ini bertujuan untuk mengisolasi dan mengkarakterisasi mikroorganisme penghasil antibiotik dari tapai ubi kayu hasil fermentasi tradisional menggunakan ragi lokal Sulawesi. Penelitian dilakukan melalui isolasi, karakterisasi morfologi koloni, dan uji aktivitas antibiotik dengan metode Kirby–Bauer terhadap *Escherichia coli* ATCC 35218 dan *Staphylococcus aureus* ATCC 25923. Zona hambat diukur menggunakan jangka sorong digital, dan data hasil uji dianalisis menggunakan ANOVA satu arah dilanjutkan uji Tukey HSD untuk data yang normal dan homogen, serta uji Kruskal–Wallis dilanjutkan Mann–Whitney U untuk data yang tidak memenuhi asumsi. Hasil penelitian menunjukkan tiga isolat terpilih, yaitu Bb1, Bb2, dan Wk3, memiliki aktivitas antibakteri yang signifikan terhadap kedua

bakteri uji. Isolat Bb1 menunjukkan aktivitas lebih tinggi terhadap *E. Coli* ATCC 35218, sedangkan isolat Bb2 dan Wk3 menunjukkan aktivitas lebih lemah. Temuan ini menegaskan potensi tapai ubi kayu sebagai sumber baru mikroorganisme penghasil antibiotik alami.

**Kata kunci:** Bakteri, Kapang, Khamir, Mikroorganisme penghasil antibiotik, Tapai ubi umbi kayu

## INTRODUCTION

Indonesia is rich in diverse fermented foods, reflecting significant cultural and culinary heritage. The fermentation process is commonly carried out at the household and small industry level, producing distinctive products with unique flavors, aromas, and textures. One example is cassava tapai, a popular traditional food made from fermented cassava tubers using ragi starter (*ragi tapai*) (Murwani et al. 2024).

Isolation of antibiotic-producing microorganisms from traditionally fermented cassava tapai involves identifying microbial strains with antibiotic properties. The results of Renner et al., (2024) showed that the cassava fermentation process produces a variety of microbial communities with potential industrial applications. Various bacteria and fungi, including *Bacillus subtilis*, *Lactobacillus plantarum*, and *Aspergillus niger*, have been isolated during cassava fermentation. These microorganisms not only contribute to the fermentation process but also exhibit antibiotic properties against pathogens such as *Escherichia coli* and *Staphylococcus aureus* (Adegbehingbe et al. 2019).

The search for new antibiotic compounds remains an urgent priority because the effectiveness of existing antibiotics is decreasing while resistance among infectious microorganisms continues to increase. Cassava tapai, produced through a traditional fermentation process using yeast starter cultures, contains microorganisms from the mold, yeast, and bacterial groups (Muhiddin et al. 2019). The dominant microorganisms found in cassava tapai are *Pichia jadinii*, *Saccharomyces cerevisiae*, *Bacillus subtilis*, *Lactobacillus plantarum*, and *Pseudomonas fragi* (Gozoua et al. 2023). The ability of these microorganisms to produce antibiotic substances is thought to be related to secondary metabolites classified as

saponins, steroids, tannins, flavonoids, and alkaloids (Haye 2020). These findings highlight the importance of exploring traditional fermented foods as potential sources of antimicrobial compounds.

Several studies in Indonesia have attempted to investigate the potential of microorganisms from fermented foods as producers of antibiotic substances, one of which is cassava tapai. In South Sulawesi Province, cassava tapai is locally known as "Poteng" and is generally produced on a household scale using local yeast starter cultures containing molds, yeasts, and bacteria. The results of quantitative analysis of local tapai yeast used in producing mixed sweet potato and cassava tapai showed microbial counts of  $1.0 \times 10^4$  cfu/g mold,  $8.0 \times 10^3$  cfu/g yeast, and  $5.7 \times 10^4$  cfu/g bacteria (Muhiddin, Mamin & Hasanuddin, 2021).

Based on these facts, the main problem of this study can be formulated as follows: Are there antibiotic-producing microorganisms present in cassava tapai fermented using local Sulawesi ragi starters from Bone, Polewali Mandar, and Takalar Regencies?

The ability of microorganisms to produce antibiotic compounds is closely related to their metabolic activity during fermentation. During substrate degradation, microorganisms such as *Bacillus*, *Lactobacillus*, and *Aspergillus* are known to produce secondary metabolites that act as defense mechanisms against competing microorganisms (Yu et al. 2021; Zhu et al. 2023; Nicolas 2025). These bioactive compounds, including saponins, flavonoids, and alkaloids, have been reported to exhibit antibiotic properties. Therefore, exploring microbial communities in traditional fermented foods provides a valuable theoretical basis for discovering new sources of natural antibiotics (Shamsudin et al. 2022; Wang et al. 2023).

Therefore, this study aimed to isolate and characterize antibiotic-producing microorganisms from cassava tapai fermented with local starter yeast from Bone, Polewali Mandar, and Takalar Regencies. The hypothesis proposed in this study is that cassava tapai fermented using local Sulawesi yeast contains microorganisms capable of producing antibiotic compounds that inhibit the growth of pathogenic bacteria.

## MATERIALS AND METHODS

### Place and Time of Research

The research was conducted at the Science Laboratory and Biology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Negeri Makassar, Makassar, South Sulawesi, Indonesia, from April to November 2023.

### Materials

The tools used in this study were a set of microbiology equipment including Laminar Air Flow (LAF), microscope, micropipette, autoclave, incubator, analytical balance, centrifuge, vortex mixer, refrigerator, petri dishes, and test tubes. The materials used in this study were Cassava tapai samples were obtained from Bone Regency, South Sulawesi (-4.547, 120.207), Polewali Mandar Regency, West Sulawesi (-3.876, 118.917), and Takalar Regency, South Sulawesi (-5.423, 119.449), Indonesia. Pure cultures of *E. coli* ATCC 35218 and *S. aureus* ATCC 25923 bacteria, disc paper, 70% alcohol, distilled water, ampicillin, and chloramphenicol were also used. Bacterial growth media were Nutrient Agar (NA) and Nutrient Broth (NB). Fungal growth media were Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB). The general medium for microorganism growth was Plate Count Agar (PCA).

### Methods

#### Samples and Sources of Isolates

Cassava tapai samples were obtained from Bone Regency, Wonomulyo District of Polewali Mandar Regency, and Galesong

District of Takalar Regency. From each location, three independent samples ( $\pm 500$  g per sample) were collected aseptically in sterile containers and transported under cold conditions to the Science Laboratory, Universitas Negeri Makassar, for further analysis.

### Isolation of Microorganisms

Isolation of microorganisms was carried out using the pour plate method on Plate Count Agar (PCA) medium (Cappuccino and Welsh 2018; Hogg 2005; Muhiddin et al. 2018). A total of 10 g of cassava tapai sample was suspended in 90 mL of sterile distilled water to obtain a  $10^{-1}$  dilution, followed by serial dilutions up to  $10^{-6}$ . From each dilution, 0.1 mL was transferred into sterile petri dishes, then poured with molten PCA (40–50 °C) and rotated in a figure-eight motion. Plates were incubated at 37 °C for 24 h. Distinct colonies were sub-cultured on Nutrient Agar (NA) for bacteria and Potato Dextrose Agar (PDA) for fungi.

### Colony Characterization

Bacterial and fungal colonies were characterized based on morphological features including colony color, shape, margin, and elevation. Isolates with different colony morphologies were selected for antibiotic activity testing (Jeong et al. 2023).

### Antibiotic Activity Test (Kirby-Bauer Method)

Selected isolates were cultured. Bacterial colonies were cultured in NB medium for 24 hours and fungal colonies in PDB medium for 48 hours. Test bacteria *E. coli* and *S. aureus* were grown in NB medium until  $OD_{600} = 0.5$ . Each bacterial suspension was spread evenly on NA plates. Sterile disc papers dipped into isolate cultures were placed on the inoculated plates at equal distances. Positive controls were ampicillin and chloramphenicol, while negative controls were sterile NB medium and distilled water. Plates were incubated at 37 °C for 48 hours.

## Measurement and Data Analysis

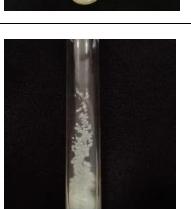
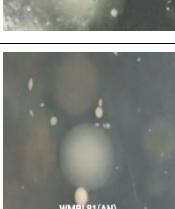
The inhibition zones around the discs were measured in millimeters using a digital caliper. Each test was performed in triplicate ( $n = 3$ ), and the results were expressed as mean  $\pm$  standard deviation (SD). Statistical analyses were carried out using one-way ANOVA followed by Tukey's HSD test for normally distributed and homogeneous data, while the Kruskal-Wallis test followed by the Mann-Whitney U test was applied for data that did not meet the assumptions of normality or homogeneity. Differences were considered significant at  $p < 0.05$ .

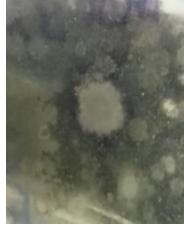
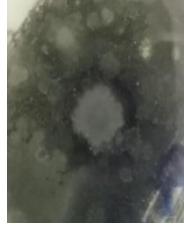
## RESULT AND DISCUSSION

### Isolation of Microorganisms

Isolation from cassava tapai samples collected from Bone, Polewali Mandar, and Takalar yielded several bacterial and fungal colonies with diverse morphologies (Table 1). These isolates were coded according to origin: B (Bone), W (Polewali Mandar), and G (Takalar). After preliminary characterization, isolates with distinct colony morphologies were selected for antibiotic activity screening.

**Table 1.** Morphological Characteristics Colonies of Bacterial and Fungal Isolates Isolated from Cassava Tapai

Code	Colony Morphological Characteristics	Colony Image
Bb1	Color: White Shape: round with raised margins Elevation: flat Edge: undulate Texture: slimy and moist	 
Bb2	Color: White Shape: round Elevation: convex Edge: entire Texture: smooth	 
Bk1	Color: White Shape: irregular Elevation: flat Edge: wavy Texture: slimy and moist	 
Bk3	Color: White Shape: round Elevation: convex Edge: fibrous Texture: dry and slippery	 
Wb1	Color: White Shape: round Elevation: raised Edge: entire Texture: slimy and moist	 

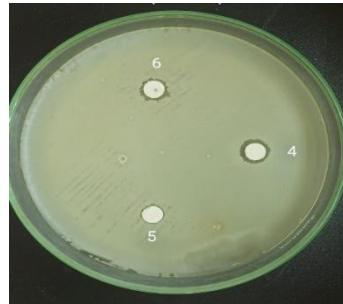
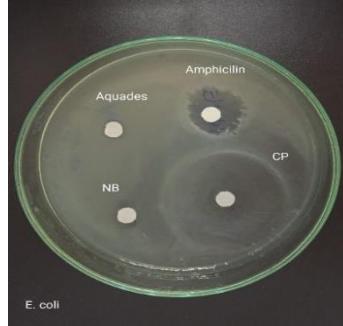
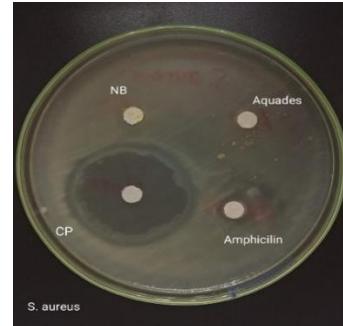
Code	Colony Morphological Characteristics	Colony Image
Wb2	Color: White Shape: round Elevation: flat Edge: entire Texture: slimy and moist	 
Wk1	Color: cream Shape: irregular Elevation: flat Edge: lobate Texture: smooth	 
Wk2	Color: cream Shape: round Elevation: convex Edge: entire Texture: slimy and moist	 
Wk3	Color: white/green Shape: round Elevation: convex Edge: entire Texture: filamentous	 
Wk4	Color: clear white Shape: irregular round Elevation: raised Edge: entire Texture: smooth	 
Gk1	Color: White Shape: round Elevation: raised Edge: entire Texture: smooth	 
Gk2	Color: White Shape: round Edge: entire Elevation: raised Size: medium Transparency: opaque Texture: smooth	 

### Screening of Antibiotic Activity

The screening of antibiotic activity was conducted to evaluate the ability of bacterial and fungal isolates to inhibit the growth of pathogenic bacteria *E. coli* ATCC 35218 and *S. aureus* ATCC 25923. The presence of inhibition zones around the

isolates indicates their antimicrobial potential (Sellam et al. 2025). Representative results showing the formation of inhibition zones for bacterial isolates, fungal isolates, and control treatments are presented in Table 2.

**Table 2.** The Results of Antibiotic Activity Testing

Isolates and Controls	The Existence of Inhibition Zones	
	<i>E. coli</i> ATCC 35218	<i>S. aureus</i> ATCC 25923
Bacteria		
Fungi		
Control		

#### Information:

Bacterial Isolate Code  
 Code 1 : Bb1  
 Code 2: Bb2  
 Code 3 : Wb1  
 Code 4 : Wb2

Fungal Isolate Code  
 Code 4 : Wk2  
 Code 5: Wk3  
 Code 6 : Wk4

Control  
 + : Chloramphenicol  
 + : Ampicillin  
 - : Aquadest  
 - : NB medium

Quantitative analysis of the antibiotic activity was performed by measuring the diameter of inhibition zones formed around the isolates. The test aimed to determine the effectiveness of selected isolates in

suppressing the growth of *E. coli* ATCC 35218 and *S. aureus* ATCC 25923. The size of the inhibition zone indicates the strength of the antibiotic activity, with larger diameters representing stronger inhibition

(Bamigbade et al. 2023). The mean inhibition zone diameters obtained from the

preliminary antibiotic screening of the isolates are summarized in Table 3.

**Table 3.** Inhibition Zone Diameters of Bb1 Isolate

Isolates and Controls	Inhibition Zone Diameter (mm)	
	<i>E. coli</i> ATCC 35218	<i>S. aureus</i> ATCC 25923
Bb1	38.29	13.62
Bb2	15.97	6.39
Wb1	5.44	5.83
Wb2	5.50	5.20
Bk1	5.20	5.20
Bk3	5.20	5.20
Wk1	5.20	5.20
Wk2	7.80	6.36
Wk3	6.01	6.42
Wk4	6.72	6.09
Chloramphenicol	41.59	32.16
Ampicillin	18.54	6.05
Aquadest	6.46	6.10
Medium NB	6.35	6.00

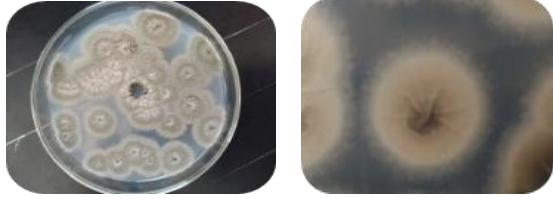
The antibiotic activity assay revealed varying inhibition zone diameters produced by bacterial and fungal isolates against *E. coli* ATCC 35218 and *S. aureus* ATCC 25923. Among all isolates, Bb1 exhibited the strongest inhibitory effect, with inhibition zones of 38.29 mm against *E. coli* ATCC 35218 and 13.62 mm against *S. aureus* ATCC 25923. Bb2 and Wk3 showed moderate inhibition, while the positive controls chloramphenicol and ampicillin produced larger zones, confirming the reliability of the assay. Measurement of inhibition zones is

widely used as a standard indicator of antibacterial potential in microbial screening assays (Adoh & Enabulele, 2025).

The selected isolates showing potential antibiotic activity during the preliminary screening were designated as Bb1, Bb2, and Wk3. The morphological characteristics of these isolates, including colony color, form, elevation, and edge, were observed on agar plates. Representative images of the colonies are presented in Table 4.

**Table 4.** Morphological Characteristics of Selected Isolates

Isolate	Characteristics				Picture	
	Code	Color	Form	Elevation		
Bb1		white	round with raised margin	flat	undulated	 
Bb2		white	round	convex	entire	 

Isolate	Characteristics				Picture
	Code	Color	Form	Elevation	
Wk3	white/green	round	convex	entire	

The morphological observation of isolates Bb1, Bb2, and Wk3 revealed clear differences in colony characteristics on agar plates. Isolate Bb1 exhibited white, round colonies with flat elevation and undulated margins, while Bb2 produced white, convex, round colonies with entire edges. In contrast, Wk3 developed white to greenish colonies, also round and convex with entire margins. These morphological variations indicate phenotypic diversity among the isolates, which may reflect underlying genetic and physiological differences.

Colony morphology encompassing traits such as color, form, elevation, and edge is widely used as a preliminary parameter for microbial identification and classification (Froböse et al. 2021; Kovács 2023). Such diversity often arises as an adaptive response to environmental and nutrient

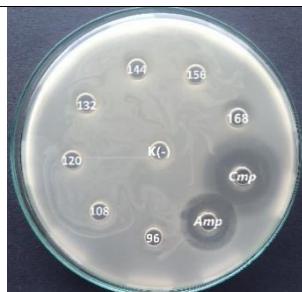
conditions, influencing the visual manifestation of microbial growth (Rodrigues et al. 2023). Furthermore, differences in spatial distribution, medium composition, and incubation time can significantly affect colony size and morphology (Xue et al. 2021).

#### Determination of Inhibitory Zone of Selected Isolates

Isolates Bb1, Bb2, and Wk3, which showed promising antibiotic activity, were selected for further activation. Activation was conducted for 48 hours for Bb1 and Bb2 and 168 hours for Wk3. Their antibiotic activity against *E. coli* ATCC 35218 and *S. aureus* ATCC 25923 was evaluated using the agar diffusion method, with inhibition zones indicating antibiotic production as presented in Table 5.

**Table 5.** Antibiotic Activity of Selected Isolates

Isolates Code	The Existence of Inhibition Zones	
	<i>E. coli</i> ATCC 35218	<i>S. aureus</i> ATCC 25923
Bb1		
Bb2		

Isolates Code	The Existence of Inhibition Zones	
	<i>E. coli</i> ATCC 35218	<i>S. aureus</i> ATCC 25923
Wk3		

The antibiotic activity of the selected isolates Bb1, Bb2, and Wk3 was evaluated against *E. coli* and *S. aureus* using the Kirby–Bauer disc diffusion method. The diameters of the inhibition zones with mean

and standard deviation from three repetitions are presented in Tables 6–8. Ampicillin and chloramphenicol served as positive controls, while TSB and PDB media were used as negative controls.

**Table 6.** Inhibition Zone Diameters of Bb1 Isolate

Test Bacteria	Groups	Repetitions (mm)			Average $\pm$ SD
		1	2	3	
<i>E. coli</i> ATCC 35218	Bb1	19.3	18.6	19.1	18.95 $\pm$ 0.49 <sup>c</sup>
	K(+1)	26.6	25.9	25.7	26.25 $\pm$ 0.49 <sup>b</sup>
	K(+2)	34.2	34.3	33.3	34.25 $\pm$ 0.07 <sup>a</sup>
	K(–)	0	0	0	0 $\pm$ 0 <sup>d</sup>
<i>S. aureus</i> ATCC 25923	Bb1	12.4	11.8	0	12.1 $\pm$ 0.42 <sup>a</sup>
	K(+1)	37.6	34.1	23.1	35.85 $\pm$ 2.47 <sup>a</sup>
	K(+2)	22.4	34.2	22.6	28.3 $\pm$ 8.34 <sup>a</sup>
	K(–)	0	0	0	0 $\pm$ 0 <sup>a</sup>

Information:

K(+1) : Ampicillin 100 ppm

*E. coli* ATCC 35218 : Different letters indicate significant differences ( $p < 0.05$ , Tukey HSD).

K(+2) : Chloramphenicol 100 ppm

*S. aureus* ATCC 25923 : Same letters indicate no significant difference ( $p > 0.05$ , Mann–Whitney U).

K(–) : TSB medium

The Bb1 isolate exhibited antibiotic activity against both *E. coli* ATCC 35218 and *S. aureus* ATCC 25923. Statistical analysis using one-way ANOVA followed by Tukey's HSD test for *E. coli* ATCC 35218 showed a highly significant difference among treatments ( $p < 0.05$ ), with inhibition zones increasing in the order K(–) < Bb1 < K(+1) < K(+2), indicating that Bb1 had moderate antibiotic activity compared to the positive

controls. In contrast, the Kruskal–Wallis test for *S. aureus* ATCC 25923 revealed a significant overall difference ( $p < 0.05$ ), but the Mann–Whitney post hoc test showed no significant pairwise differences ( $p > 0.05$ ). These findings suggest that Bb1 demonstrated stronger antibiotic potency against *E. coli* ATCC 35218 than against *S. aureus* ATCC 25923.

**Table 7.** Inhibition Zone Diameters of Bb2 Isolate

Test Bacteria	Groups	Repetitions (mm)			Average $\pm$ SD
		1	2	3	
<i>E. coli</i> ATCC 35218	Bb2	17.1	16.9	9.9	17 $\pm$ 0.14 <sup>b</sup>
	K(+1)	23.2	22.2	21	22.7 $\pm$ 0.71 <sup>b</sup>
	K(+2)	33.2	32.6	31.3	32.9 $\pm$ 0.42 <sup>b</sup>
	K(−)	0	0	0	0 $\pm$ 0 <sup>c</sup>
<i>S. aureus</i> ATCC 25923	Bb2	10.5	9.6	0	10.05 $\pm$ 0.64 <sup>c</sup>
	K(+1)	36.4	34.1	35.2	35.25 $\pm$ 1.63 <sup>a</sup>
	K(+2)	26.3	23.3	22.3	24.8 $\pm$ 2.12 <sup>b</sup>
	K(−)	0	0	0	0 $\pm$ 0 <sup>d</sup>

Information:

K(+1) : Ampicillin 100 ppm

*E. coli* ATCC 35218 : Same letters indicate no significant difference ( $p > 0.05$ , Mann–Whitney U)

K(+2) : Chloramphenicol 100 ppm

*S. aureus* ATCC 25923 : Same letters indicate no significant difference ( $p > 0.05$ , Mann–Whitney U)

K(−) : TSB medium

The Bb2 isolate exhibited antibiotic activity against both *E. coli* ATCC 35218 and *S. aureus* ATCC 25923. Statistical analysis using the Kruskal–Wallis test revealed significant differences among treatments for both test bacteria ( $p < 0.05$ ). For *E. coli* ATCC 35218, the inhibition zones increased in the order K(−) < Bb2 < K(+1) < K(+2), indicating that Bb2 showed moderate antibiotic activity compared to the positive controls. Similarly, for *S. aureus* ATCC

25923, the inhibition zones followed the order K(−) < Bb2 < K(+2) < K(+1), demonstrating a weaker inhibitory effect of Bb2 against the Gram-positive bacterium. Overall, these findings indicate that Bb2 exhibited stronger antibiotic activity against *E. coli* ATCC 35218 than against *S. aureus* ATCC 25923, consistent with the general trend that Gram-negative bacteria were more susceptible to the tested isolates.

**Table 8.** Inhibition Zone Diameters of Wk3 Isolate

Test Bacteria	Groups	Repetitions (mm)			Average $\pm$ SD
		1	2	3	
<i>E. coli</i> ATCC 35218	Wk3	10.8	10.2	0	10.5 $\pm$ 0.42 <sup>c</sup>
	K(+1)	23.2	22.2	22.8	22.7 $\pm$ 0.71 <sup>b</sup>
	K(+2)	31.6	32.5	32.6	32.05 $\pm$ 0.64 <sup>a</sup>
	K(−)	0	0	0	0 $\pm$ 0 <sup>d</sup>
<i>S. aureus</i> ATCC 25923	Wk3	0	0	0	0 $\pm$ 0 <sup>d</sup>
	K(+1)	38.4	28.9	35.9	33.65 $\pm$ 6.72 <sup>a</sup>
	K(+2)	38.1	26.6	26.3	32.35 $\pm$ 8.13 <sup>a</sup>
	K(−)	0	0	0	0 $\pm$ 0 <sup>d</sup>

Information:

K(+1) : Ampicillin 100 ppm

*E. coli* ATCC 35218 : Same letters indicate no significant difference ( $p > 0.05$ , Mann–Whitney U)

K(+2) : Chloramphenicol 100 ppm

*S. aureus* ATCC 25923 : Same letters indicate no significant difference ( $p > 0.05$ , Mann–Whitney U)

K(−) : PDB medium

The Wk3 isolate exhibited antibiotic activity against *E. coli* ATCC 35218 but showed no inhibition against *S. aureus*

ATCC 25923. Statistical analysis using the Kruskal–Wallis test revealed significant differences among treatments for both test

bacteria ( $p < 0.05$ ). For *E. coli* ATCC 35218, the inhibition zones increased in the order  $K(-) < Wk3 < K(+1) < K(+2)$ , indicating that Wk3 possessed weak antibiotic activity compared to the positive controls. In contrast, no inhibition zone was observed for *S. aureus* ATCC 25923 in the Wk3 treatment, suggesting that the antibiotic compounds produced by Wk3 were active primarily against Gram-negative bacteria rather than Gram-positive ones.

Based on the findings of this study, isolates Bb1, Bb2, and Wk3 exhibited antibiotic activity with varying levels of effectiveness against *Escherichia coli* ATCC 35218 and *Staphylococcus aureus* ATCC 25923. The stronger inhibitory activity observed against *E. coli* compared to *S. aureus* suggests that the antibiotic compounds produced by these isolates may be more effective against Gram-negative bacteria. This observation is consistent with recent reports indicating that several environmental microorganisms are capable of producing secondary metabolites with antibiotic activity against both Gram-negative and Gram-positive pathogens (Adoh and Enabulele 2025). However, further research is necessary to substantiate the presence of antibiotic compounds within these isolates through comprehensive chemical analyses, including phytochemical screening, Gas Chromatography–Mass Spectrometry (GC–MS), and Liquid Chromatography–Mass Spectrometry (LC–MS), to elucidate the nature and composition of the bioactive metabolites responsible for the observed inhibitory effects (Verma et al. 2025; Oliveira et al. 2024; Pardo-Esté et al. 2024).

## CONCLUSION

This study successfully isolated and characterized antibiotic-producing microorganisms from cassava tapai fermented with local Sulawesi yeast. The selected isolates, Bb1, Bb2, and Wk3, demonstrated significant antibiotic activity *E. coli* ATCC 35218 and *S. aureus* ATCC 25923, indicating their potential as promising sources of natural antibiotic compounds. These findings highlight the potential of traditional fermented foods as valuable reservoirs for discovering new

antimicrobial-producing microorganisms. Further studies are recommended to purify and identify the active antibiotic compounds produced by these isolates, as well as to evaluate their stability, spectrum of activity, and potential applications in pharmaceutical or food preservation fields.

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