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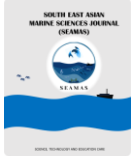

Isolation of Antibiotic-Producing Microorganisms from *Camponotus melanus* in the Mangrove Ecosystem against Pathogenic Bacteria

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Abstract

Microorganisms that live in extreme conditions in mangrove ecosystems have unique defence mechanisms that enable them to produce secondary metabolites that function as antibiotics. One of them is the wood ant (*Camponotus melanus*), which is commonly found in mangrove ecosystems and plays a significant role in these ecosystems. This study aims to identify bacteria associated with *C. melanus* based on morphological characteristics and biochemical tests, and to test their antibacterial activity against pathogenic bacteria (*Aeromonas hydrophila, Pseudomonas aeruginosa,* and *Staphylococcus aureus*). This study was conducted from September to November 2025. The method used was a survey method. The research was conducted from September to November 2025. *C. melanus* samples were taken from the Marine Station mangrove forest, Dumai City, Riau Province, and analyzed at the Marine Microbiology Laboratory, Faculty of Fisheries and Marine, Universitas Riau. The isolation and identification results yielded three bacterial isolates: C1 and C2, suspected to be Bacillus sp., and C3, suspected to be Citrobacter sp. The antibacterial activity test results of the three isolates produced inhibition zones. This indicates that the three isolates have the potential to serve as sources of promising antibiotic compounds for further development

1. INTRODUCTION

Microorganisms found in mangrove ecosystems have the potential to yield new antibiotic compounds, including those with specific antimicrobial activity that can inhibit or kill bacteria resistant to conventional antibiotics (Yulma et al., 2017). Mangrove areas such as the Marine Station mangrove forest in Dumai serve as rich microbial habitats and are home to various species, including ants. The carpenter ant *(Camponotus melanus)* is one of the dominant ant groups in mangrove ecosystems and plays a significant role.

Given the resistance of pathogenic bacteria to antibiotics, the discovery of natural antibiotic sources from microbes that live in symbiosis with *C. melanus* ants is very important in inhibiting the growth of pathogenic bacteria such as *Aeromonas hydrophila, Pseudomonas aeruginosa,* and *Staphylococcus aureus*, which are often found in water and can harm humans. Through this research, it is hoped that microbes capable of producing antibiotic compounds from the mangrove ecosystem can be discovered, particularly microbes associated with *C. melanus* ants. This finding is expected to provide a solution to the problem of antibiotic resistance.

2. RESEARCH METHODS

Time and Place

This research was conducted from September to November 2025. Samples of *C. melanus* ants were collected at the Marine Mangrove Forest Station, Dumai City, Riau Province. Sample analysis was carried out at the Marine Microbiology Laboratory, Faculty of Fisheries and Marine, Universitas Riau. The research location is shown in Figure 1.

Figure 1. Research site map

Method

The method used in this study was a survey, and bacterial isolation was performed using the streak plate method. Meanwhile, bacterial identification was performed by observing morphological characteristics and performing biochemical tests. The antibacterial activity test was carried out using the disc diffusion method.

Procedures

Sample Collection

Sampling of *C. melanus* ants in the mangrove forest, Marine Station, Dumai City, Riau, Indonesia. These ants can be found under mangrove leaf litter or on rotten wood. Ten ants were sampled. Sampling was done manually (hand collecting). The samples were placed in sample bottles and taken to the laboratory for further analysis.

Isolation of Bacteria from *C. melanus*

The isolation procedure was carried out by piercing the abdomen/stomach of the ant using a sterile syringe to extract its stomach fluid. The bacterial isolate was obtained using NA (Nutrient Agar) medium and the streak plate method. The isolated isolates were then incubated for 24 hours at 37°C. Next, the isolates were re-isolated onto NA media using a cross-scratch method, and isolates capable of inhibiting the growth of pathogenic bacteria were selected for purification until a pure culture was obtained. The isolates were then inoculated using NB (*Nutrient Broth*) media and incubated for 7 x 24 hours at 37°C. After the incubation period, the cultures were centrifuged to separate the bacterial cells from the supernatant. This supernatant was used for antibacterial activity testing.

Identification of Bacteria from *C. melanus*

Bacterial identification is carried out in several stages. First, macroscopic morphological observations are made by examining the colony's shape, surface, colour, and edges as it grows on the culture medium. Meanwhile, microscopic observations are performed to examine cellular characteristics, such as cell shape and Gram staining. Following morphological observation, biochemical tests are performed to determine the physiological properties of the bacteria. The tests performed are the Oxidase Test, Catalase Test, SIM Test (Sulphide Indole Motility), OF Test (Oxidation-Fermentation), and Sugar Fermentation Test. The final stage is identification using the 2009 edition of Bergey's Manual of Systematic Bacteriology, by Whitman, which is the standard reference for bacterial classification.

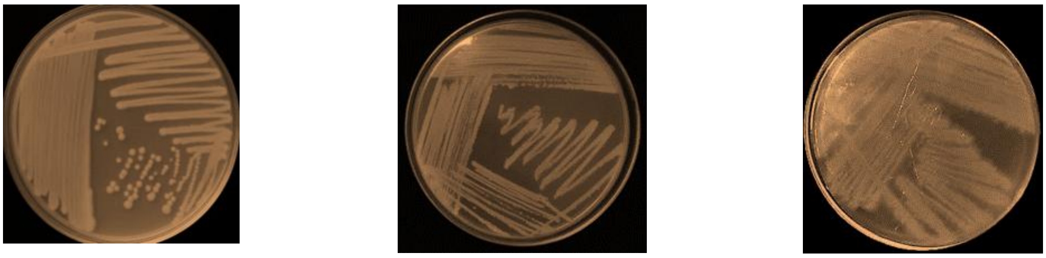
Antibacterial Activity Test

Bacterial isolates from *C. melanus* that had been purified beforehand were inoculated into NB medium and incubated for 7x24 hours at 37 °C. The culture was then centrifuged for 10 minutes at 8,000 RPM to separate the bacterial cells from the medium. Fifty microliters of the supernatant were dropped onto filter paper and placed on the surface of NA medium that had been inoculated with the test pathogenic bacteria *A. hydrophila, P. aeruginosa,* and *S. aureus*. As a comparison, tetracycline was used as a positive control and distilled water as a negative control, and the mixture was incubated at 37 °C for 24 hours. The test results were determined by measuring the diameter of the inhibition zone formed. The inhibition zone formed around the disc indicates antibacterial activity. The larger the inhibition zone diameter, the stronger the antibacterial activity.

3. RESULTS AND DISCUSSION

Bacterial Morphology of *C. melanus*

Based on cross-scraping results, three isolates were obtained. The following are bacterial colonies from the three isolates, as shown in Figure 2, and the results of bacterial morphology observations are shown in Table 1.



(a) (b) (c)

Figure 2. Bacterial colonies from *C. melanus*

Note: (a) isolate C1, (b) isolate C2, (c) isolate C3

Table 1. Bacterial Morphology

Isolate code	Colony morphology				Cell morphology	
	Colony shape	Elevation	Edge	Color	Gram	Cell shape
C1	Round	Raised	Smooth	Milky white	+	Basil
C2	Round	Raised	Wavy	Milky white	+	Basil
C3	Round	Raised	Curvy	Milky white	-	Basil

The morphology of bacterial isolates obtained from *C. melanus* ants shows several differences among the isolates. All three isolates have a milk-white colony colour, a common bacterial colour frequently observed in bacterial isolates (Putriani, 2016). Colony shape: all three isolates are round. The edges of the colonies vary. Differences in colony edges may be influenced by the bacteria's ability to produce extracellular enzymes that play a role in colony spread (Yulma *et al.,* 2019).

The elevation of the three isolates is evident in their Gram staining. Isolates C1 & C2 are Gram-positive, and isolate C3 is Gram-negative, with a bacillus cell shape. Gram-positive bacteria are purple because the crystal violet-iodine dye complex is retained even after treatment with an alcohol solution, while Gram-negative bacteria are red because the complex dissolves upon treatment with an alcohol solution, taking on the red colour of safranin (Miftahul, 2021).

Bacterial Biochemical Test

Based on the results of the biochemical tests for each bacterial isolate, further details are shown in Table 2.

Table 2. Biochemical Tests

Biochemical Test	Bacterial Isolate		
	C1	C2	C3
Oxidase	-	-	-
Catalase	+	+	+
Sulfide	-	-	+
Indole	+	+	-
Motility	+	+	-
OF	-	-	+
Glucose	+	+	+
Lactose	+	-	-
Sucrose	+	-	-
H2S	-	-	+
Gas	-	-	-

Description: Positive (+), Negative (-)

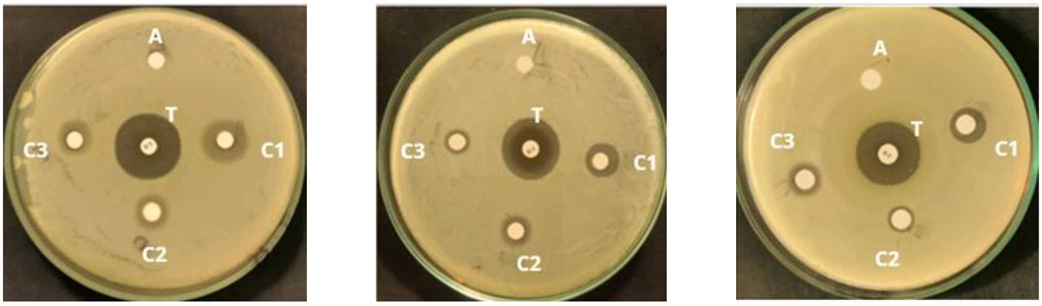
The catalase test results for the three bacterial isolates were positive, indicated by the formation of gas bubbles when a 3% H_2O_2 solution was dripped onto them. The catalase test was performed to determine whether bacterial isolates produce the catalase enzyme. The catalase enzyme catalyzes the breakdown in hydrogen peroxide into water and oxygen (Pulungan et al., 2018). The oxidase test determines whether bacteria contain the oxidase enzyme. A positive test result is indicated by a blue colour change on the oxidase paper within 10-15 seconds (Pulungan et al., 2018). This enzyme is important for protection against oxidative stress, especially in the dynamic environment of the digestive tract (Liu et al., 2019). Based on the test results, no color change occurred on the paper, indicating that all three isolates were negative.

The sulfide test results for isolates C1 and C2 were negative, as the medium did not turn black. Isolate C3 showed a positive result, marked by a black color change in the inoculated medium. This process involves enzymes such as sulfite reductase or cysteine desulfhydrase (Hardy, 2018). Based on the indole test, isolates C1 and C2 were positive, while C3 was negative. A positive result was indicated by the formation of a red ring after the medium, which had been incubated for 24 hours, was dripped with Kovacs' reagent. The motility test was conducted to assess the bacteria's motility (Yarashima & Mayasari, 2024).

In the OF (oxidative-fermentative) test, isolate C3 was positive. Isolates C1 and C2 were negative. If a yellow colour change is observed only in tubes containing paraffin, the bacteria are classified as fermentative. Conversely, if the yellow colour change occurs only in tubes without paraffin, the bacteria are classified as oxidative (Yarashima & Mayasari, 2024). The sugar fermentation test aims to determine a bacterium's ability to ferment glucose, sucrose, and lactose. If the colour on the surface of the medium (slant) does not change, it indicates a basic reaction; if it changes to yellow, it indicates an acidic reaction. The same applies to color changes in the middle of the medium (butt) (Kosasi et al., 2019).

Antibacterial Activity Against Pathogens

The results of the antibacterial activity test of three bacterial isolates, namely isolates C1, C2, and C3, against three test pathogenic bacteria, namely *A. hydrophila, P. aeruginosa,* and *S. aureus*. Tetracycline was used as the positive control, and distilled water as the negative control. The test results are shown in Figure 3.



(a) (b) (c)

Figure 3. Activity of bacterial isolates against pathogenic bacteria

Note: (a) *A. hydrophila,* (b) *P. aeruginosa,* (c) *S. aureus*

Table 3. Activity of bacterial isolates against pathogenic bacteria

Isolate	*A. hydrophila* (mm)	*P. aeruginosa* (mm)	*S. aureus* (mm)
C1	10.81	10.10	10.45
C2	12.76	10.75	10:10
C3	12.11	9.10	11.10
Aquades	0.00	0.00	0.00
Tetracycline	21.76	22.43	22.80

The antibacterial activity test results show that bacterial isolates from *C. melanus* ants can inhibit the growth of pathogenic bacteria, including *A. hydrophila*, *P. aeruginosa*, and *S. aureus*. This antibacterial activity is demonstrated by the formation of inhibition zones around the test discs, which indicate the presence of bioactive compounds produced by the isolates. The inhibition zone formed around the disc indicates antibacterial activity. The larger the inhibition zone diameter, the stronger the antibacterial activity. Based on the average value in the antibacterial test against *A. hydrophila*, isolate C2 showed the largest inhibition zone diameter of 12.76 mm. This result indicates that isolate C2 has strong antibacterial potential. In the test against *P. aeruginosa*, isolate C2 showed the largest inhibition zone of 10.75 mm. Meanwhile, in the test against *S. aureus*, isolate C3 showed the largest inhibition zone of 11.10 mm.

The difference in inhibitory ability in each isolate was due to differences in the secondary metabolite content of the isolates, which inhibited the growth of pathogenic bacteria. According to Sari et al. (2019), several factors can influence the magnitude of the inhibitory activity of antibacterial substances, including: 1) The type and age of the bacteriocin-producing bacteria and the test bacteria. 2) The concentration of the antimicrobial substance and the amount of inoculum/density of the test bacteria. 3) The resistance of bacteria to antimicrobial substances is related to differences in their cell walls. 4) The concentration of the active substance or functional group of the antimicrobial compound.

4. CONCLUSIONS

Based on the morphological characteristics and biochemical tests of the bacteria from *C. melanus*, they are suspected to be *Bacillus* sp. and *Citrobacter* sp. The results of the antibacterial activity test showed that the three isolates inhibited the growth of the pathogenic bacteria *A. hydrophila, P. aeruginosa, and S. aureus*.

REFERENCES

Hardy, H. (2018). *SIM (Sulfide, Indole, Motility) Medium*. Hardy Diagnostics, US.

Kosasi, C., Lolo, W.A., & Sedewi, S. (2019). Isolasi dan Uji Aktivitas Antibakteri dari Bakteri yang Berasosiasi dengan Alga *Turbinaria ornata* ((turner) J. Agardh) serta Identifikasi secara Biokimia. *Pharmacon*, 5(2): 321–359.

Liu, G., Greenspan, D.S., & Mao, J. (2019). Catalase Activity in Bacterial Stress Response. *Microbial Pathogenesis,* 128: 92-100.

Miftahul, K. (2021). Identifikasi Ekstrak Ubi Jalar (*Ipomea batatas poiret*) sebagai Zat Pewarna Alternatif pada Pewarnaan Gram. *Klinikal Sains Jurnal Analis Kesehatan*, 8 (2): 82-89

Pulungan, A.S., & Tumangger, D.E. (2018). Isolasi dan karakterisasi Bakteri Endofit Penghasil Enzim Katalase dari Daun Buasbuas (*Premna pubescens Blume*). *BIOLINK (Jurnal Biologi Lingkungan Industri Kesehatan*), 5(1): 71-80.

Sari, D.M., Effendi, I., & Nursyirwani, N. (2019). Identification of Antibiotic-Producing Bacteria from Extreme Microhabitat in Molecular Mangrove Ecosystems and their Activity on Pathogenic Bacteria (*Vibrio alginolyticus*). *Jurnal Perikanan dan Kelautan*, 9 (2): 137-150.

Whitman, W.B., Vos, P.D., Garrity, G.M., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., & Schleifer, K. (2009). *Bergey's Manual of Systematic Bacteriology (2nd ed., Vol. 3: The Firmicutes).* Springer.

Yarashima, S., & Mayasari, U. (2024). Eksplorasi Bakteri Kandidat Probiotik pada Sedimen Hutan Mangrove Pandan, Tapanuli Tengah. *Jurnal Biologi*, 1(4).

Yulma., Burhanudin I., Sunarti., Eka M., Neni W., & Mursyiban. (2019). Identifikasi Bakteri pada Serasah Daun Mangrove yang Terdekomposisi di Kawasan Konservasi Mangrove dan Bekantan Kota Tarakan. *Jurnal of Tropical Biodiversity and Biotechnology,* 2 (1): 28-33