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# ANTIMICROBIAL POTENTIAL OF MARINE PHOTOSYNTHETIC BACTERIA AND BIOPIGMENT EXTRACTS AGAINST AQUATIC PATHOGENS

**Hersa Javayu Defitra[1*], Ummi Mardhiah Batubara[1], Thamrin[1]**
[1]Department of Marine Science, Faculty of Fisheries and Marine,
Universitas Riau, Pekanbaru, 28293 Indonesia
*hersa.javayu6060@student.unri.ac.id

## ABSTRACT

Potential of marine photosynthetic bacteria (MPB) and their biopigment extracts as antimicrobial agents against two major aquatic pathogens, *Aeromonas hydrophila* and *Staphylococcus aureus*. The research was conducted from February to April 2025. Six MPB isolates were cultured under laboratory conditions to induce pigment production. Biopigments, natural pigments produced by microorganisms, exhibit antimicrobial and antioxidant properties. Pigment extraction was performed using acetone and ether, followed by evaporation to obtain concentrated pigment extracts. The antimicrobial activity of both MPB suspensions and their biopigment extracts was tested using the disc diffusion method on Mueller-Hinton Agar (MHA) media. The results showed that the MPB suspensions produced no inhibition zones against either pathogen. In contrast, the biopigment extracts demonstrated weak antimicrobial activity, with inhibition zone diameters ranging from 1 to 4 mm, a classification of weak. However, the ability of biopigment extracts to inhibit bacterial growth, even at low levels, suggests the presence of bioactive compounds with potential antimicrobial activity. These findings suggest that MPB-derived biopigments may serve as a natural alternative in future antimicrobial development.

**Keywords:** *Aeromonas hydrophila*, Biochemistry, Biopigments, MPB, *Staphylococcus aureus*

## 1. INTRODUCTION

Antimicrobial resistance (AMR) has emerged as one of the most pressing challenges in controlling infectious diseases in human healthcare and aquaculture. The increasing resistance of pathogens to synthetic antibiotics demands the discovery of alternative antimicrobial agents derived from natural sources. One promising candidate that has garnered growing scientific interest is marine microorganisms, particularly marine photosynthetic bacteria (MPB). These microorganisms produce secondary metabolites with unique chemical structures and potential biological activities, including antimicrobial properties[1].

MPB are a group of bacteria capable of photosynthesis and are well adapted to extreme marine environments. They produce various pigments to capture light energy and as a defence mechanism against environmental stressors, such as UV radiation and toxic substances. These pigments, known as biopigments such as carotenoids and porphyrins, have demonstrated diverse pharmacological potentials, including antioxidant, anticancer, and antimicrobial activities[2,3].

In recent years, several studies have shown that biopigments derived from bacterial genera such as *Serratia*, *Pseudomonas*, and *Pseudoalteromonas* can inhibit the growth of pathogens, including *Escherichia coli*, *Staphylococcus aureus*, and *Vibrio* spp, through mechanisms that differ from those of conventional

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antibiotics[4,5]. This aspect is particularly relevant in the context of antibiotic resistance, as it provides alternative compounds with novel modes of action.

Pathogens such as *Aeromonas hydrophila* and *Staphylococcus aureus* have developed significant resistance to a range of antibiotics, including ampicillin. *A. hydrophila*, a Gram-negative bacterium commonly found in aquatic environments, can cause opportunistic infections in both fish and humans. In contrast, *S. aureus*, a Gram-positive bacterium, can form biofilms and extracellular vesicles that hinder antibiotic penetration[6,7].

This research aims to evaluate six marine photosynthetic bacteria (MPB) isolates obtained from the Marine Microbiology Laboratory of Universitas Riau. These isolates were initially collected from seawater samples in Dumai. After culturing, the researchers extracted pigments from the isolates using organic solvents. The antimicrobial activity of the extracts was evaluated against *A. hydrophila* and *S. aureus* using the disk diffusion method. In addition to testing the biopigment extracts, this study also compared their activity with pure MPB suspensions.

The findings aim to contribute to understanding marine microorganisms as safe, eco-friendly alternatives for developing novel antimicrobial agents in aquaculture and medicine.

## 2. RESEARCH METHOD

### Time and Place

This research was conducted from October 2024 to June 2025 at the Marine Microbiology Laboratory, Faculty of Fisheries and Marine Sciences, University of Riau. The laboratory was utilized for culturing marine microorganisms, extracting bioactive compounds, and testing in vitro antimicrobial activity throughout the study.

### Method

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### Procedures

### Bacterial Isolates and Culture Conditions

The bacterial isolates used in this study included six MPB strains. These isolates were pure cultures from the Marine Microbiology Laboratory's collection, previously isolated from seawater samples collected in Dumai, Riau. The bacteria were cultured in a salt-mineral medium formulated to support the growth of photosynthetic bacteria. Incubation was carried out at room temperature for three days under continuous illumination using a 40-watt incandescent lamp to induce pigment production (Figure 1).

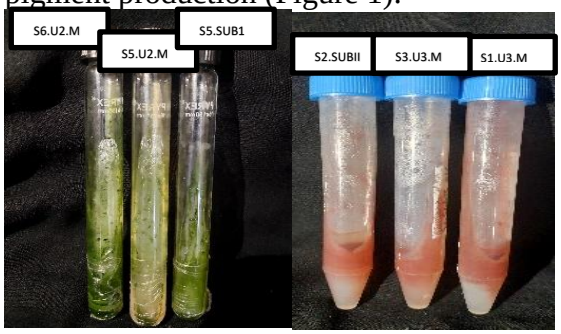


**Figure 1**. Six MPB isolates were grown on slant media after 3 days of incubation.

As model aquatic pathogens, *A. hydrophila* and *S. aureus* were used for antimicrobial testing. These pathogens were selected for their frequent occurrence in aquatic environments and their known pathogenicity toward fish and humans.

### Biopigment Extraction

After incubation, bacterial cells were separated from the culture medium by centrifugation to initiate pigment extraction. The resulting pellet was washed with sterile distilled water to remove residual media. Pigment extraction was performed by suspending the pellets in a 1:1 mixture of acetone and ether. The mixture was vortexed to ensure homogenization, followed by a second centrifugation to separate the pigment-containing supernatant from the remaining biomass. The collected supernatant was then concentrated using a

rotary evaporator at 70°C for 30 minutes to remove the solvents, yielding a concentrated biopigment extract.

### Antimicrobial Activity Assay

The antimicrobial potential of the samples was assessed using the standard disk diffusion method on Mueller-Hinton Agar (MHA). For the MPB suspensions, a standardised McFarland $10^8$ CFU/mL solution (30 µL) was applied to sterile paper disks, which were then placed on MHA plates previously inoculated with the respective test pathogens. Similarly, 30 µL of the biopigment extract was pipetted onto separate blank disks.

Sterile distilled water and the extraction solvent (acetone) were used as negative controls, while ampicillin was used as a positive control. All plates were incubated at 37°C for 48 hours. After incubation, inhibition zone diameters were measured using a calliper and classified according to the guidelines set by the Clinical and Laboratory Standards Institute[8].

### Data Analysis

The inhibition zone measurements were analyzed descriptively and presented in tables and figures. Based on the size of the inhibition zones, antimicrobial activity was categorized as strong, moderate, or weak. In addition, the effectiveness of the pure MPB suspensions against each tested pathogen was compared with that of the pigment extracts.

## 3. RESULT AND DISCUSSION

### Biopigment Extraction from MPB

Biopigment extraction was initiated from six MPB isolates cultured for 3 days on a salt-mineral medium under continuous illumination with a 40-watt incandescent lamp to stimulate pigment production. Following incubation, the bacterial cells were harvested by centrifugation, washed, and extracted with a mixture of acetone and ether.

The extraction process resulted in a significant reduction in volume, from approximately 100 mL to about 2 mL per isolate (Figure 2), indicating efficient pigment concentration. Morphological colour changes were also observed. Three initially green-pigmented isolates turned dark green to greenish-brown, while red-pigmented isolates turned orange, deep red, and reddish-brown. These variations suggest differences in pigment compound composition, which may be influenced by solvent type, extraction duration, and cell particle size[9].

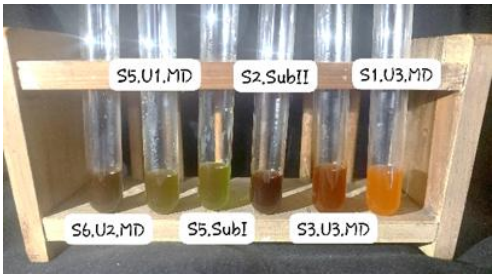


**Figure 2**. Color variation of biopigment extracts from six MPB isolates after evaporation.

### Antimicrobial Potential of MPB Suspensions

Disk diffusion assays using MPB suspensions against *A. hydrophila* and *S. aureus* showed no visible inhibition zones on MHA, indicating that the suspensions lacked antimicrobial activity against either Gram-negative or Gram-positive pathogens.

This absence of inhibition may suggest that the bioactive compounds produced by the MPB were either not present in sufficient concentrations or ineffective in their non-extracted form. These findings are consistent with previous studies, which report that antimicrobial activity is more commonly observed following the extraction and concentration of secondary metabolites, such as pigments[10,11].

### Antimicrobial Potential of MPB Biopigment Extracts

Biopigment extracts demonstrated observable antimicrobial activity, albeit weak, with inhibition zones ranging from 1 to 4 mm. Isolate S1.U3.MD produced a 4 mm inhibition zone against *A. hydrophila* during S2 isolation. SubII (2.5 mm), S5.

SubI (3 mm), and S6.U2.MD (1 mm) showed inhibition against S. aureus.

The results of this study were significantly lower than those reported by Setiyono et al.[4], who reported inhibition zones exceeding 10 mm with *Pseudoalteromonas rubra* pigments. Differences in bacterial species, geographic isolation sites, or pigment types produced could be attributed to these factors. Nevertheless, the findings suggest promising potential in underexplored sources of bioactive pigments.

According to Wayne's[8] standards, all observed inhibition zones fell into the weak category (<13 mm), indicating relatively low antimicrobial potency. However, the presence of clear inhibition zones in the biopigment extract treatments highlights the success of the extraction process in concentrating active antimicrobial compounds. Pigments such as carotenoids and porphyrins are believed to contribute to this activity through alternative mechanisms, including disruption of cell membrane integrity and interference with protein synthesis pathways[12]. A summary of the observed antimicrobial activity across all treatments is presented in Table 1.

**Table 1**. Inhibition zone diameters (mm) of MPB biopigment extracts against *A. hydrophila* and *S. aureus*

| Isolate Type | Observation of antimicrobial potential: Inhibition Zone (48 hours) | |
|---|---|---|
| | *A. hydrophilla* | *S. aureus* |
| MPB Suspension | - | - |
| MPB Biopigment Extract | + | + |
| Ampicillin (K+) | + | + |
| Aquades & acetone (K-) | - | - |

## Pathogen Resistance and Control Comparison

The positive control (ampicillin) produced an inhibition zone of approximately 7 mm against both pathogens, indicating a notable level of resistance. *A. hydrophila* has been reported to show up to 100% resistance to ampicillin[6], while *S. aureus* is known to form extracellular vesicles that help protect against antimicrobial agents[7]. These findings suggest that both pathogens possess mechanisms to evade the effects of antibiotics. The antimicrobial activity of marine photosynthetic bacteria (MPB) isolates was evaluated as an alternative, with inhibition zones compared in Figure 3.

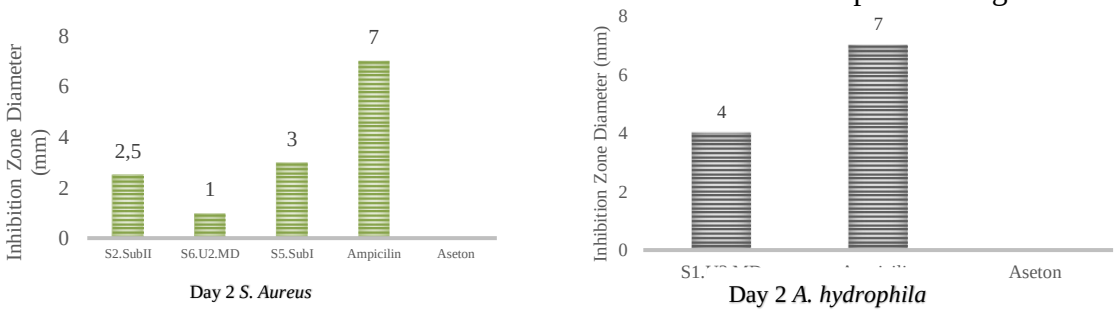


**Figure 3**. Comparison of inhibition zone diameters (mm) for each MPB isolate against *A. hydrophila* and *S. aureus*

The negative controls (distilled water and acetone) showed no inhibition zones, confirming that the observed antimicrobial activity was due to the pigment extracts and not to the solvents used[13].

## Antimicrobial Mechanisms of MPB Biopigments

Biopigments produced by MPB may inhibit pathogenic bacteria through various mechanisms. A primary mechanism is cell

membrane disruption. The lipophilic nature of these pigments allows them to penetrate the bacterial lipid bilayer, leading to ion leakage and loss of osmotic stability, ultimately resulting in cell lysis and death. Some pigments are also reported to interfere with protein and DNA synthesis. Pigments such as prodigiosin and pyocyanin can penetrate the nucleus, disrupting ribosome function and nucleic acid replication. These effects depend on the pigments' chemical structures and their affinities for molecular targets.

Carotenoids and porphyrins, commonly found in MPB, possess antioxidant properties. However, they can act as pro-oxidants at specific concentrations, generating free radicals that damage bacterial proteins and lipids. Marine microbial pigments are promising multifunctional candidates in pharmaceutical applications.

**Differential Pathogen Response: *A. hydrophila* vs. *S. aureus***

*Staphylococcus aureus* showed more consistent sensitivity to MPB pigments than A. *hydrophila*. Differences in cell wall structure may explain this. As a Gram-positive bacterium, S. aureus has a thick peptidoglycan layer but lacks an outer membrane, facilitating pigment penetration. Conversely, the Gram-negative *bacterium A. hydrophila* has an outer lipopolysaccharide membrane, which serves as an additional barrier.

However, isolate S1.U3.MD showed inhibitory activity against *A. hydrophila*, suggesting its pigments may possess more potent bioactivity or the ability to penetrate Gram-negative bacterial defences. Further chemical characterization of these pigments is recommended.

**Broader Implications of MPB Potential**

Beyond antimicrobial activity, MPB pigments also exhibit antioxidant and UV-protective properties[2], offering opportunities for further exploration in marine biotechnology and pharmaceutical applications. Previous studies have highlighted the success of microbial pigments from *S.marcescens*, *P. otitidis*, and *P. rubra* in inhibiting various pathogens[4,5].

## 4. CONCLUSION

This research concludes that biopigment extracts derived from MPB possess antimicrobial potential against *A. hydrophila* and *S. aureus*. However, the observed activity remains weak according to the Clinical and Laboratory Standards Institute. MPB suspensions exhibited no inhibitory effect, whereas the extracted pigments produced inhibition zones ranging from 1 to 4 mm. Isolate S1.U3.MD demonstrated activity against *A. hydrophila* during S2 isolation. SubII, S5. SubI, and S6.U2.MD was effective against S. aureus. The extraction process successfully concentrated secondary metabolites responsible for antimicrobial activity, though not yet at a potent level.

These findings support the use of MPB as an alternative source of natural bioactive compounds, with promising potential for further development in pharmaceuticals, aquaculture, and aquatic environmental management. Further research is needed to identify the specific active compounds, refine extraction techniques, and assess efficacy against broader pathogenic targets or in applied systems.

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