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# EXTRACTION AND PURIFICATION OF CAROTENOID PIGMENTS SYNTHESIZED BY MARINE PHOTOSYNTHETIC BACTERIA AS A SOURCE OF NATURAL ANTIBIOTICS

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## ABSTRACT

Marine photosynthetic bacteria are microorganisms with the potential to produce bioactive compounds in the form of carotenoid pigments, which can be utilized as a natural source of antibacterial agents. This study aims to characterize the carotenoid pigments produced by the marine photosynthetic bacterial isolate LD04 and to determine their potential antibacterial activity against the pathogenic bacteria *Pseudomonas aeruginosa, Escherichia coli*, and *Vibrio harveyi*. The study was conducted using an exploratory descriptive method, involving bacterial culture, antibacterial activity testing using the disc diffusion method, biomass production, pigment extraction using methanol, and purification and characterization using Thin Layer Chromatography (TLC) and High-Performance Liquid Chromatography (HPLC). LC analysis yielded a single major spot, indicating the presence of a dominant pigment component in the extract. Further characterization by HPLC at a wavelength of 450 nm produced a peak at a retention time of 6.35 minutes, whereas the astaxanthin standard was detected at 28.2 minutes. This difference in retention time suggests that the main compound in the extract is likely a xanthophyll or a more polar carotenoid derivative. Antibacterial activity testing showed that the pigment extract was able to inhibit the growth of *E. coli* and *V. harveyi*, but showed no activity against *P. aeruginosa*. The largest inhibition zone diameters were obtained for *E. coli* at 5.63 mm and *V. harveyi* at 1.23 mm, classified as weak inhibition according to CLSI standards. Based on the research results, it can be concluded that the carotenoid pigments from the marine photosynthetic bacterial isolate LD04 have potential as a source of natural antibacterial agents, particularly against *E. coli* and *V. harveyi*.

**Keywords:** Antibacterial, Carotenoids, Marine photosynthetic bacteria, Xanthophyll

## 1. INTRODUCTION

Indonesia possesses high levels of marine biodiversity, including micro-organisms that produce a range of bioactive compounds. This potential makes marine ecosystems a source of candidate natural antibiotics, supported by reports of at least 1,200 species of marine microbes in Indonesia that have the potential to produce antibiotic compounds[1]. It is important to develop this potential, given the rising incidence of antibiotic resistance driven by the overuse and misuse of synthetic antibiotics. Antibiotic resistance has become a global problem affecting both the healthcare sector and aquaculture.

Bacterial infections in Southeast Asia continue to rise, with approximately 75% of pathogenic *Vibrio* sp. bacterial isolates[2]. In Indonesia, it has been reported to be resistant to the antibiotic amoxicillin[3]. This situation has spurred the search for safer and more sustainable antibiotic sources. One promising source of bioactive compounds is marine photosynthetic bacteria. Bacterial groups such as *Kocuria rhizophila,*

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*Calidifontibacter* sp., and *Rhodococcus ruber* are known to produce carotenoid pigments that exhibit a variety of biological activities[4]. The potential of carotenoids as bioactive compounds is becoming increasingly attractive for development, as marine photosynthetic bacteria that produce these pigments have been successfully identified in the waters off Dumai[5]. In addition to serving as natural pigments, carotenoids have been reported to exhibit antibacterial activity against several pathogenic bacteria, such as *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhi*[6].

Although the antibacterial potential of carotenoids has been widely reported, the characterization of pigments produced by marine photosynthetic bacteria from the waters of Dumai and their activity against pathogenic bacteria have not yet been extensively studied. Therefore, this study was conducted to characterize the carotenoid pigments produced by marine photosynthetic bacteria and to evaluate their potential antibacterial activity against *P. aeruginosa*, *E. coli*, and *V. harveyi*, with these organisms serving as candidates for natural antibiotic sources.

## 2. RESEARCH METHOD

### Time and Place

The research was conducted from September 2025 to February 2026 at the Marine Microbiology Laboratory of the Department of Marine Sciences, Faculty of Marine Fisheries, Universitas Riau, and the School of Pharmacy (STIFAR).

### Method

This study employs an exploratory descriptive method to examine and describe the characteristics of carotenoid pigments produced by marine photosynthetic bacteria, as well as their antibacterial activity against pathogenic bacteria. The research stages include bacterial cultivation, biomass production, extraction and characterization of carotenoid pigments, and antibacterial activity testing.

### Procedures

### Preparation of Isolates and Media

Marine photosynthetic bacterial isolates were obtained from the Marine Microbiology Laboratory collection at the Universitas Riau, while the pathogenic bacteria used as test organisms were *P. aeruginosa*, *E. coli*, and *V. harveyi*. All equipment and media were sterilized using an autoclave at 121°C for 15 minutes[7]. The mineral salt medium was prepared in 1 L of distilled water, consisting of 1 L of distilled water containing Na-asetat 2,5 g, $(NH_4)_2SO_4$ 1,25 g, $K_2HPO_4 \cdot 3H_2O$ 0,9 g, $KH_2PO_4$ 0,6 g, yeast extract 0,5 g, $MgSO_4 \cdot 7H_2O$ 0,2 g, $CaCl_2 \cdot 2H_2O$ 0,07 mg, $FeSO_4 \cdot 7H_2O$ 0,003 mg, dan EDTA 0,002 mg. It was then used as a growth medium for marine photosynthetic bacteria[8].

### Bacterial Culture

Marine photosynthetic bacteria were cultured in a liquid mineral salt medium, while *P. aeruginosa* and *E. coli* were cultured on Nutrient Agar (NA), and *V. harveyi* on Thiosulfate Citrate Bile Salts Sucrose (TCBS). Pathogenic bacterial cultures were incubated aerobically at 37°C for 18–24 hours until optimal growth was achieved, then used for antibacterial activity testing.

### Antibacterial Activity Test

Antibacterial activity was tested using the disk diffusion method. The supernatant of the marine photosynthetic bacterial culture was obtained by centrifugation at 4000 rpm for 20 minutes, then impregnated onto sterile disks[9]. The discs containing the supernatant were placed on Mueller-Hinton Agar (MHA) media inoculated with *P. aeruginosa*, *E. coli*, and *V. harveyi*. Chloramphenicol was used as a positive control, and distilled water as a negative control. After incubation at 37°C for 24 hours, antibacterial activity was determined based on the diameter of the inhibition zone formed around the discs. The inhibition zone values were then calculated and classified in accordance with the 2023 Clinical and

Laboratory Standards Institute (CLSI) standards[10].

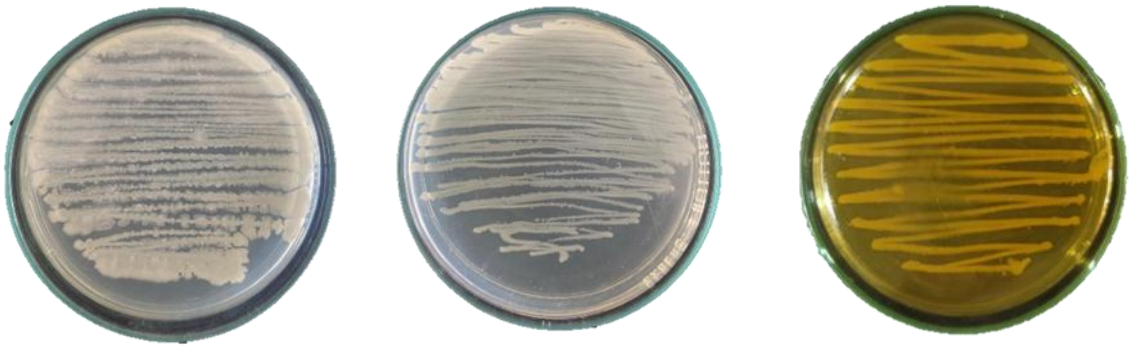
## 3. RESULT AND DISCUSSION

### Characteristics of Marine Photosynthetic Bacterial Cultures and Pathogenic Bacteria

Isolates of marine photosynthetic bacteria (MPB) successfully grew on mineral salt media, forming round colonies with smooth surfaces, flat edges, and flat to slightly convex elevations. After 2–3 days of incubation under 40 W incandescent lighting, the colonies changed color from pale to deep red, indicating the accumulation of intracellular carotenoid pigments. Changes in the morphology and pigmentation of MBIs are shown in Figure 1.

**Figure 1.** Isolates of marine photosynthetic bacteria on agar medium after pigmentation has developed.

Marine photosynthetic bacteria grew well on mineral salt media, characterized by uniform colony growth across the media surface. These results indicate that the media can support the growth of marine bacteria because their composition resembles their natural habitat11.



A B C

**Figure 2.** Pathogenic bacterial isolates. A) *P. aeruginosa*, B) *E. coli*, (C) *V.harveyi*

The test bacteria used in the antibacterial activity assay were *E. coli*, *P. aeruginosa*, and *V. harveyi*. All three bacteria exhibited good growth on the culture medium, with varying colony morphological characteristics (Figure 2). These morphological variations reflect the physiological differences and adaptive capabilities of each bacterium to the growth medium. Furthermore, all three are Gram-negative and exhibit relatively high resistance due to the presence of a lipopolysaccharide layer in their cell walls.

### The Antibacterial Potential of Marine Photosynthetic Organisms Against Pathogenic Bacteria

The antibacterial activity of the BFL isolate was tested using the disk diffusion method against *E. coli*, *P. aeruginosa*, and *V. harveyi*. The test results showed that the metabolites produced by the isolate exhibited varying degrees of inhibition against each pathogenic bacterium. These differences indicate variations in the sensitivity of the test bacteria to the bioactive compounds produced during the isolate's growth inhibitory zone diameters were obtained for each treatment. The tests were conducted in triplicate (P1–P3) with

chloramphenicol as the positive control (K+) and distilled water as the negative control (K−). The results of the inhibitory zone diameter measurements are presented in Table 1.

**Table 1.** Data on the inhibition zone diameters of the extracts against *P. aeruginosa*, *E. coli*, and *V. harveyi*

| No | Bacteria | Treatment | Result (mm) | R (mm) | Category |
|---|---|---|---|---|---|
| | | $P_1$ | 0,00 | | |
| | | $P_2$ | 0,00 | 0,00 | There are no obstacles |
| 1 | *P. aeruginosa* | $P_3$ | 0,00 | | |
| | | K+ | 31,1 | - | Strong |
| | | K- | 0,00 | - | There are no obstacles. |
| | | $P_1$ | 4,60 | | |
| | | $P_2$ | 7,25 | 5,63 | Weak |
| 2 | *E. coli* | $P_3$ | 5,05 | | |
| | | K+ | 18 | - | Strong |
| | | K- | 0,00 | - | There are no obstacles |
| | | $P_1$ | 2,85 | | |
| | | $P_2$ | 0,7 | 1,23 | Weak |
| 3 | *V. harveyi* | $P_3$ | 0,15 | | |
| | | K+ | 20,4 | - | Strong |
| | | K- | 0,00 | - | There are no obstacles |

The positive control (chloramphenicol) produced inhibition zones of 31.1 mm against *P. aeruginosa*, 18.0 mm against *E. coli*, and 20.4 mm against *V. harveyi*, whereas the negative control showed no inhibition. These results confirm that the antibacterial activity stems from metabolites produced by the BFL isolate.

The highest sensitivity observed in *E. coli* indicates that the bioactive compounds produced by isolate LD04 remain capable of inhibiting its growth. Conversely, the absence of an inhibition zone in *P. aeruginosa* indicates the high intrinsic resistance of this bacterium, which is associated with low membrane permeability and the presence of an efflux pump system[12]. The observed antibacterial activity is thought to originate from a combination of secondary metabolites, such as carotenoids, bacteriocins, organic acids, and other antimicrobial compounds produced during the growth of marine photosynthetic bacteria.

**Biomass Production and Carotenoid Pigment Separation**

Isolate LD04, which exhibited antibacterial activity, was then cultured on a larger scale to produce biomass as a source of carotenoid pigments. During incubation, the culture exhibited increased turbidity and a color change to a deep red, indicating cell growth and the accumulation of intracellular pigments (Figure 3A).

After centrifugation, a clear separation formed between the supernatant and the red-colored biomass pellet (Figure 3B). The pellet was then extracted using methanol, yielding a red pigment extract and a paler pellet, indicating that the pigment had successfully dissolved in the solvent16. From approximately ±13 mL of biomass, ±2 mL of pigment extract was obtained, demonstrating the ability of isolate LD04 to synthesize carotenoids.

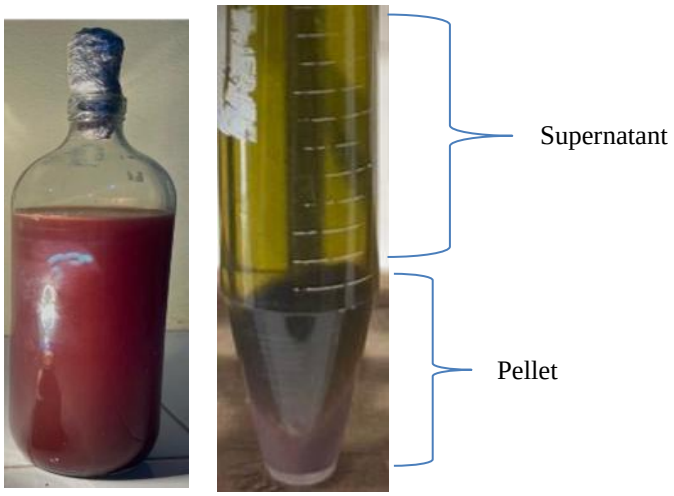



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**Figure 3.** Biomass production of the marine photosynthetic bacterial isolate LD04. (A) The LD04 culture is deep red in color. (B) The centrifugation result showing the separation of the supernatant and pellet

**Purification of Carotenoid Pigments**

The purification of carotenoid pigments from isolate LD04 was performed using Thin-Layer Chromatography (TLC) followed by High-Performance Liquid Chromatography (HPLC). The TLC results showed pigment spots separated from the solvent front, with migration positions close to the astaxanthin standard (Figure 4). As a comparison, the astaxanthin standard showed the separation of several fractions, namely astaxanthin diester, astaxanthin monoester cis-isomer, astaxanthin monoester, and free astaxanthin[13].

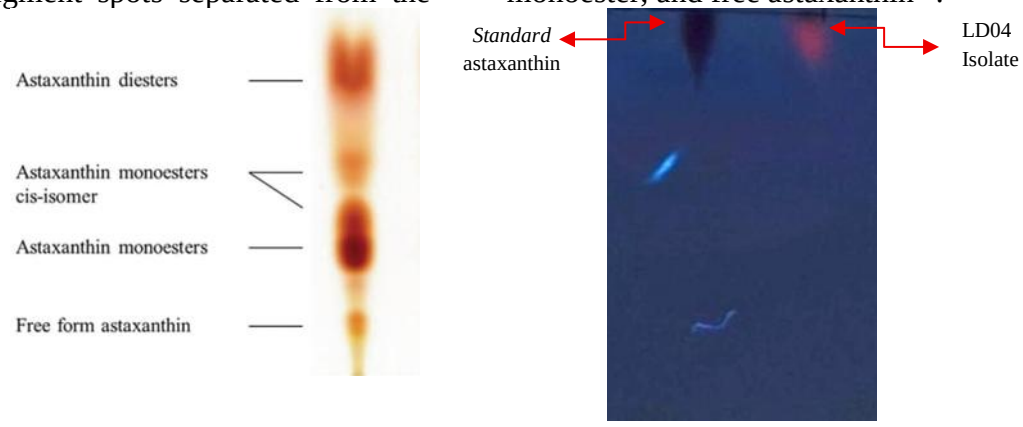


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**Figure 4.** LC-MS visualization of astaxanthin using the LD04 bacterial test sample. A) Chromatographic results of the sample analysis by Miyakawa[13]. B) Carotenoid pigment extract from LD04 isolate

The appearance of colored spots and fluorescence under 366 nm UV light indicates the presence of compounds with a conjugated double bond system, which is a key characteristic of the carotenoid group. Tailing was also observed during TLC separation, preventing accurate determination of the Rf value.

Further analysis by HPLC at 450 nm yielded a chromatogram with a main peak at a retention time of approximately 6.35 minutes. In contrast, the astaxanthin standard showed a peak at approximately 28.2 minutes (Figure 5). This difference in retention time indicates that the dominant compound in the LD04 extract is not pure

astaxanthin but rather another, more polar carotenoid. Detection at a wavelength of 450 nm confirmed the presence of carotenoid pigments, as this wavelength corresponds to the region of maximum absorption commonly found in compounds with a conjugated double-bond system[14]. The shorter retention time compared to the astaxanthin standard indicates that the dominant compound in the LD04 extract is likely a xanthophyll, a group of oxygenated carotenoids with higher polarity that elute more rapidly in a reverse-phase HPLC system.

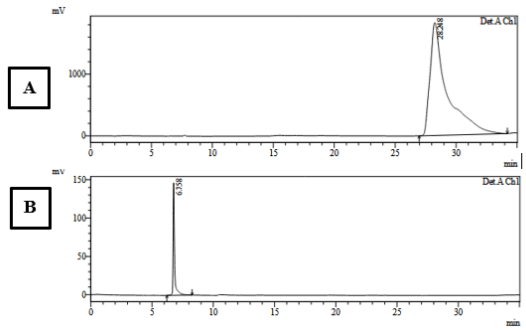


**Figure 5**. HPLC chromatograms of the LD-04 isolate extract and the astaxanthin standard. (A) Astaxanthin standard at 450 nm; (B) LD-04 extract at 450 nm

## 4. CONCLUSION

The marine photosynthetic bacterial isolate LD04 produces carotenoid pigments, which TLC and HPLC successfully characterized. The TLC results indicated the presence of a dominant pigment component in the extract. At the same time, HPLC analysis at a wavelength of 450 nm yielded a major peak at a retention time of 6.35 minutes, which differed from that of the astaxanthin standard (28.2 minutes). This difference indicates that the dominant compound produced is not pure astaxanthin but is suspected to belong to the xanthophyll group or to a more polar carotenoid derivative. The carotenoid pigment also shows potential as a natural antibacterial agent against Gram-negative pathogens, as evidenced by the formation of inhibition zones against *Escherichia coli* and *Vibrio harveyi*. The highest inhibitory activity was observed against *E. coli*, with an average inhibition zone diameter of 5.63 mm, followed by *V. harveyi* at 1.23 mm, while Pseudomonas aeruginosa showed no inhibition. These results suggest that isolate LD04 has potential as a source of bioactive carotenoid pigments with antibacterial activity, although its inhibitory efficacy is still relatively weak.

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