

## EXTRACTION OF CAROTENOID PIGMENTS FROM BACTERIA LD07 ISOLATED FROM THE COASTAL WATERS OF DUMAI

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### ABSTRACT

Carotenoids are natural pigments with significant potential that are widely synthesized by photosynthetic bacteria. In addition to playing a role in photosynthesis, carotenoids also function as natural antioxidants capable of inhibiting the formation of free radicals and providing protection against oxidative stress. This study aims to characterize and to identify carotenoid pigments synthesized by marine photosynthetic bacteria, to determine the types of pigments, as well as to prove their antioxidant activity. The method used in this study was experimental, conducted to test the ability of marine photosynthetic bacteria to synthesize carotenoid pigments as producers of antioxidant compounds. The results of the study indicate that isolate LD07, a photosynthetic marine bacterium, contains carotenoid pigments. UV-Vis spectrophotometer analysis revealed a maximum absorption peak at a wavelength of 490 nm, a value within the characteristic absorption region of carotenoid pigments

**Keywords:** Carotenoid, Free radical, Marine bacteria

### 1. INTRODUCTION

A global issue currently receiving widespread attention is the danger of free radicals to the human body. The World Health Organization reported that, in 2019, degenerative diseases such as cardiovascular disease, diabetes mellitus, hypertension, and Alzheimer's disease accounted for 76.32% of the top ten causes of death in Indonesia. These degenerative diseases are caused by oxidative stress triggered by free radicals.

Oxidative stress is a state of imbalance between the levels of free radicals and antioxidants in the body. To counteract these damaging effects, the body needs antioxidants. Antioxidants are molecules that can prevent or inhibit the oxidative processes caused by free radicals, which can lead to cellular damage. Antioxidants can be obtained from a group of natural compounds known to be rich in antioxidants, namely carotenoids.

Carotenoids are believed to enhance immune responses, possess anticancer and

antioxidant properties, and serve as a treatment for conditions sensitive to UV radiation<sup>1</sup>. Carotenoids are pigments typically found in various organisms, such as plants, algae, photosynthetic bacteria, and fungi. However, the use of bacteria as carotenoid producers is more promising due to their shorter regeneration time. Photosynthetic bacteria living in marine waters are highly promising candidates due to their ability to synthesize carotenoid pigments as a protective mechanism against intense sunlight.

To date, there has been limited research on the specific characteristics and antioxidant potential of carotenoid pigments produced by marine photosynthetic bacteria. Therefore, this study aims to characterize and identify the carotenoid pigments synthesized by marine photosynthetic bacteria and evaluate their antioxidant potential.

## 2. RESEARCH METHOD

### Time and Place

This research was conducted from August 2025 to February 2026 at the Marine Microbiology Laboratory, Faculty of Fisheries and Marine Sciences, University of Riau.

### Method

The method used in this study was an experimental method, conducted to test the ability of marine photosynthetic bacteria to synthesize carotenoid pigments. This study was carried out in several stages, namely: sterilization of equipment and materials, preparation of culture media, bacterial culture, morphological and physiological characterization of photosynthetic bacteria, and extraction of carotenoid pigments.

### Procedures

#### Sterilization of Tools and Materials

Instruments must be sterilized to ensure they are free of pathogenic organisms not originating from the sample being used. According to Afia<sup>2</sup>, instruments are sterilized by wrapping them in HVS paper and placing them in an autoclave filled with distilled water. The temperature is set to 121°C at 1 atm for 15 minutes. Once the alarm sounds, turn off the autoclave and open the steam vent. Wait 10–15 minutes to ensure that the steam has fully escaped and the autoclave has cooled down.

#### Preparation of Salt Mineral Media

The growth medium for photosynthetic marine bacteria is a mineral salt medium containing several types of ingredients, including Na-asetat 2,5 g, NH<sub>4</sub>SO<sub>4</sub> 1,25 g, K<sub>2</sub>HPO<sub>4</sub> 0,9 g, KH<sub>2</sub>PO<sub>4</sub> 0,6 g, Yeast Extract 0,5 g, MgSO<sub>4</sub> 7H<sub>2</sub>O 0,2 g, CaCl<sub>2</sub> 0,07 mg, FeSO<sub>4</sub> 7H<sub>2</sub>O 0,003 mg and EDTA 0,002 mg. All ingredients are mixed and dissolved in 1 L of distilled water using a hotplate. Once the media composition is homogeneous, it is sterilized in an autoclave at 121°C<sup>3</sup>. To prepare the mineral salt medium in agar form, unflavored agar-agar

can be added to the mineral medium solution at a concentration of 2g/100 mL.

#### Marine Photosynthetic Bacteria Culture

Bacterial samples were obtained from the Marine Microbiology Laboratory of the Faculty of Fisheries and Marine Sciences, University of Riau, and then diluted from 10<sup>-1</sup> to 10<sup>-5</sup> using 9 ml NaCl. The bacterial samples were then added to a 10<sup>-1</sup> dilution solution in 1 mL increments up to a 10<sup>-5</sup> dilution, and 0,1 ml of the suspension from the 10<sup>-5</sup> dilution was placed into Petri dishes containing mineral salt agar medium. The bacterial samples on the medium were then spread and smoothed using an L-shaped rod aseptically near a bunsen burner. After growth occurs on the mineral salt agar medium, the isolates are then pure cultured using the streak plate method and incubated under a 40-watt incandescent lamp at a room temperature of approximately 25°C to observe the natural pigments of the marine photosynthetic bacteria that appear.

After obtaining a pure culture, it is necessary to increase the cell biomass to obtain the biopigments required for the extraction stage. To increase the biomass, prepare a bottle containing 1 liter of liquid mineral salt medium, then use a sterile inoculation needle to transfer the pure bacterial culture into the bottle and incubate it under a 40-watt incandescent lamp. During incubation, shake the flask occasionally to ensure that nutrients and oxygen are distributed evenly.

#### Morphological and Physiological Characterization of Marine Photosynthetic Bacteria

The morphological characterization of the observed marine photosynthetic bacteria included colony shape, color, surface, elevation, and edges. Meanwhile, the physiological characteristics of marine photosynthetic bacteria were determined using several biochemical tests, namely differential staining (Gram), catalase test, indole test, motility test, sulfide test, citrate test, and sugar fermentation tests (glucose,

lactose, sucrose). The results of the characterization analysis were then compared against identification keys for marine photosynthetic bacteria.

### Extraction of Carotenoid Pigments

Bacterial pigment extraction is performed by separating the pellets from the supernatant. Bacterial pigments are produced by inoculating pigment-producing isolates into mineral salt medium; the culture is then centrifuged at 4000 rpm for 15 minutes. The resulting pellets are then extracted using various organic solvents with specific compositions and ratios of solvent to pellet. The centrifugation results with the solvents are separated, then transferred to bottles and stored at freezer temperature.

### Data Analysis

The data obtained were then presented in the form of tables and figures, which were subsequently analyzed descriptively. This analysis was conducted to determine the potential of marine photosynthetic bacteria

to synthesize carotenoid pigments, thereby providing new information regarding bacterial species and natural products (carotenoids) from Indonesian waters.

## 3. RESULT AND DISCUSSION

### Growth of Marine Photosynthetic Bacteria

Cultures of marine photosynthetic bacteria were purified using a spread plate method on solid mineral media to obtain single colonies free of contaminants. The purification results revealed characteristic red-colored colonies, consistent with the characteristics of marine photosynthetic bacteria that produce carotenoid pigments. Isolate LD07 exhibited good growth on mineral salt medium, both in liquid and solid forms. In liquid medium, growth was characterized by a uniform red discoloration of the medium, whereas in solid medium, red colonies with uniform morphology formed. Incubation was conducted under light using an incandescent lamp as an energy source to support the photosynthesis process.



**Figure 1.** The process of culturing marine photosynthetic bacteria under a 40-watt incandescent light as a source of energy for photosynthesis. A) Bacterial growth on solid mineral salt medium, B) Bacterial growth on liquid mineral salt medium

Carotenoid pigment production by marine photosynthetic bacteria generally reaches an optimal level after approximately three days of incubation, when the culture enters the stationary phase<sup>4</sup>. During this phase, the production of secondary metabolites such as carotenoids tends to increase due to changes in cellular physiological conditions that trigger the activation of pigment biosynthetic pathways

in response to environmental conditions. These results indicate that isolate LD07 is capable of adapting well to mineral salt media and possesses the ability to produce carotenoid pigments.

### Characterization of Photosynthetic Bacteria

Based on the bacterial culture performed, the morphological

characteristics and physiological properties of isolate LD07 need to be examined. The

results of the morphological examination of bacterium LD07 are shown in Table 1.

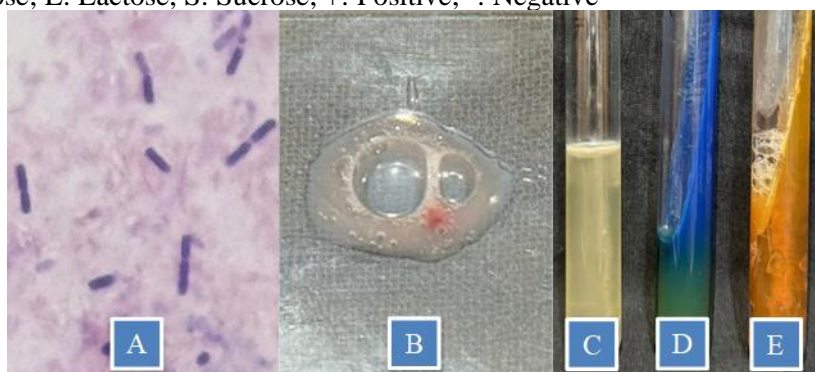
**Table 1.** Morphological characteristics of bacterium LD07

Isolate Code	Morphological Characteristics				
	Shape	Color	Surface	Elevation	Edge
LD07	Irregular	Red	Smooth and glossy	Flat	Irregular

**Table 2.** Characterization of the physiological properties of bacterium LD07

Isolate Code	Physiological Characteristics							Sugar Test		
	Shape	Gram	Catalase	Indole	Motility	Sulfide	Citrate	G	L	S
	LD07	Rod	+	+	-	-	-	+	-	+

Notes: G: Glucose; L: Lactose; S: Sucrose; +: Positive; -: Negative



**Figure 2.** Biochemical tests for bacterium LD07. A) Gram stain, B) Catalase test, C) SIM test, D) Citrate test, E) Sugar test.

The results of physiological tests indicate that isolate LD07 is a Gram-positive bacterium. According to Safika<sup>5</sup>, Gram-positive bacteria are those that retain the violet stain during the Gram staining process.

A positive result in the catalase test indicates that isolate LD07 is capable of producing the catalase enzyme, which breaks down hydrogen peroxide into water and oxygen<sup>6</sup>. This ability is important for photosynthetic bacteria because it helps protect cells from damage caused by oxidative compounds formed during metabolic processes and exposure to light.

A negative result in the indole test indicates that the isolate is unable to break down tryptophan into indole, while a negative result in the motility test indicates that the bacteria lack the ability to move actively. A negative sulfide test indicates that the isolate does not produce H<sub>2</sub>S gas from available substrates.

A positive result in the citrate test indicates that the isolate is capable of utilizing citrate as a carbon source<sup>7</sup>. Additionally, positive reactions in the lactose and sucrose tests demonstrate the bacterium's ability to ferment both sugars as energy sources. Meanwhile, a negative result in the glucose test indicates that glucose utilization is not the primary metabolic pathway under these testing conditions.

### Extraction of Carotenoid Pigments

The extraction process is a critical initial step that will ultimately influence the compounds to be analyzed. The extraction method used must be tailored to the properties and stability of the compounds to be analyzed. The LD07 bacterial biomass culture in liquid medium was first centrifuged to separate the bacterial cells (pellet) from the culture medium (supernatant). This step aims to concentrate the cell biomass as the primary source of intracellular pigments.

Solvent variation experiments in the extraction of LD07 bacterial pigments were conducted to determine the most effective solvent combination and ratio for extracting pigments from bacterial cell biomass. Differences in solvent type and solvent ratio

affect the solvent's ability to dissolve pigment compounds, which is highly influenced by the solvent's polarity and its interaction with bacterial cell wall and membrane components.

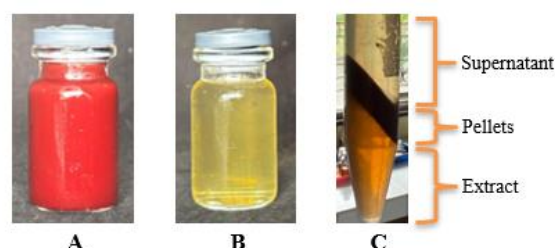
**Table 3.** Solvent extraction experiments for bacterium LD07

No	Solvent	Ratio	Remarks
1	Methanol	1 : 1	Green
2	Dichloromethane : n-hexane	1 : 1	Green
3	Chloroform : Methanol	3 : 2	Green
		1 : 3	Green
		1 : 2	Green
		3 : 1	Red
4	Dichloromethane : Methanol	4 : 1	Red
		5 : 1	Reddish orange
		7 : 3	Yellow

In the methanol solvent experiment (1:1 ratio), the resulting green-colored extract indicates that polar methanol was unable to optimally dissolve the non-polar carotenoid pigments, resulting in the target pigments not being predominantly extracted.

The dichloromethane: n-hexane combinations (1:1 and 3:2) also produced green-colored extracts. Although both of these solvents are non-polar, the absence of a polar solvent in the mixture results in less effective solvent penetration into the cell structure, so that pigments bound to the cell membrane are not maximally released. Similarly, with the chloroform:methanol combination (1:3), the more dominant proportion of polar solvent was still unable to balance the dissolution process of non-polar pigments.

Experiments using the dichloromethane: methanol combination yielded the most significant results. At ratios of 1:2, 3:1, and 4:1, the extract was red, and at a 5:1 ratio, the extract was reddish-orange, indicating that the pigments were beginning to be extracted effectively. The peak occurred at a 7:3 ratio, where the extract was bright yellow, a characteristic typical of carotenoid pigments. This color indicates that the pigment has dissolved more purely and dominantly compared to other compound mixtures.

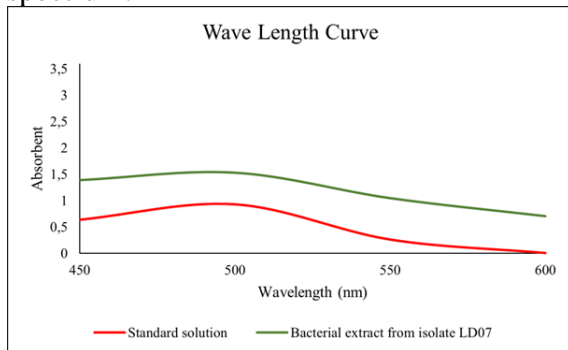


**Figure 3.** Results of carotenoid pigment extraction. A) collected biomass pellets, B) extraction results, C) pellets after the addition of a solvent

These results indicate that the balance between the non-polar solvent (dichloromethane) and the polar solvent (methanol) is crucial. Dichloromethane dissolves the non-polar carotenoid pigments, while methanol helps disrupt the cell membrane structure, allowing the bound pigments to be released into the solution<sup>8</sup>. This combination of polar and non-polar solvents is commonly used in the extraction of microbial secondary metabolites because it enhances the efficiency of bioactive compound extraction.

This suggests that isolate LD07 has the potential to produce carotenoid pigments as secondary metabolites, which in microorganisms generally function as a protective mechanism against oxidative stress.

The characterization of the pigments was further confirmed through UV-Vis spectrophotometric analysis to determine the absorption spectrum characteristics of the pigments produced by the LD07 bacterium. This method was used because carotenoid pigments possess a conjugated double bond system capable of absorbing light in the visible wavelength region, thereby producing a characteristic absorption spectrum.



**Figure 4.** UV-Vis spectrophotometer results at wavelengths of 450–600 nm

Based on the UV-Vis spectrophotometer measurements shown in Figure 4, the absorption curve of the LD07 bacterial extract exhibits a maximum absorption peak at a wavelength of 490 nm.

This value falls within the characteristic absorption region of carotenoid pigments, which generally lies in the 400–500 nm range<sup>9</sup>. The spectral curve also indicates that the absorption pattern of the LD07 bacterial extract resembles that of the standard solution, although the absorbance values of the extract tend to be higher.

The similarity in the absorption peak between the LD07 bacterial extract and the standard solution at a wavelength of 490 nm indicates that the pigment produced by LD07 shares characteristics with the standard carotenoid pigment used as a reference. This suggests that the LD07 isolate has potential as a source of carotenoid pigments with biological activity.

#### 4. CONCLUSION

The LD07 bacterial isolate is capable of synthesizing carotenoid pigments, as evidenced by red-colored colonies and a change in the extract's color to yellowish-orange following the extraction process. UV-Vis spectrophotometer analysis results show a maximum absorption peak at a wavelength of 490 nm, consistent with the characteristics of carotenoid pigments.

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