

## Screening and characterization of marine carotenoid-pigmented bacteria in Dumai waters, Riau Province, Indonesia

<sup>1</sup>Ummi M. Batubara, <sup>1</sup>Nursyirwani, <sup>2</sup>Irvina Nurrachmi, <sup>1</sup>Naomi H. Br Simanjuntak, <sup>1</sup>Hersa J. Defitra, <sup>1</sup>Jonathan R. George, <sup>2</sup>Arisandi Hasibuan

<sup>1</sup> Marine Microbiology Laboratory, Faculty of Fisheries and Marine Sciences, University of Riau, Pekanbaru, Indonesia; <sup>2</sup> Chemical Oceanography Laboratory, Faculty of Fisheries and Marine Sciences, University of Riau, Pekanbaru, Indonesia. Corresponding author: U. M. Batubara, [ummimardhiah@lecturer.unri.ac.id](mailto:ummimardhiah@lecturer.unri.ac.id)

**Abstract.** The marine environment represents an aquatic ecosystem that serves as one of the largest sources of genetic diversity in nature. In Indonesia, one approach to harnessing the potential of marine resources involves the exploration of indigenous bacteria capable of producing carotenoid pigments. This study aimed to screen for carotenoid-producing bacteria isolated from the coastal waters of Dumai. The research employed a survey method, utilizing water and sediment samples. Sample analysis was conducted at the Marine Microbiology Laboratory of the Faculty of Fisheries and Marine Sciences. Detection of carotenoid-producing bacteria was performed using mineral salt media. The screening process yielded seven isolates demonstrating the ability to produce carotenoid pigments, as evidenced by the production of red-colored substances observed in the bacterial growth media.

**Key Words:** aquatic, carotenoid, marine, natural pigment, photosynthetic bacteria.

**Introduction.** Carotenoids are natural pigments that play a crucial role in absorbing sunlight. Based on their chemical structure, carotenoids are polyene isoprenoid compounds that range in color from yellow to orange and red (Maoka 2020). They are a dominant group of pigments that are abundant in nature. They are produced naturally by various organisms, from prokaryotes to higher plants, in diverse quantities and characteristics (Nawaz et al 2020).

More than 700 sources of carotenoids have been identified with different structures (Ye et al 2019). One of the primary sources of carotenoid pigments is pigmented marine bacteria. Ecologically, marine bacteria produce pigments that play a vital role as biomarkers and photoprotective pigments in the ocean (Silvia et al 2021). The carotenoid pigments produced by marine bacteria are compounds with specific molecular structures that play an important role in the physiology of these microorganisms, such as protecting cells from solar radiation and adapting to extreme environments (Batubara et al 2024). Due to their unique properties and characteristics, carotenoids have been utilized in various applications, including pharmaceuticals, agriculture, fisheries, and food industries (Corinaldesi et al 2017).

Currently, the commercial production of carotenoids is largely based on plant extracts or chemical synthesis. However, the development of biotechnology in the global market presents an opportunity. Microbial production of compounds has significant potential in terms of production efficiency and the diversity of carotenoid structures. Therefore, exploring and isolating carotenoids from pigmented marine bacteria will reveal diverse structures and interesting properties (Silva et al 2021).

In this paper, we explore the coastal waters of Riau, Indonesia, to obtain new bacterial potential isolates that can produce carotenoid pigments. Carotenoid pigments offer several advantages over those obtained from other organisms. In addition to their stable production, microorganisms require a relatively short growth period and can yield

high biomass. Utilizing the isolated and characterized pigmented marine bacteria is expected to increase species diversity and provide valuable information for other researchers. Exploring pigmented marine bacteria as a source of carotenoids is particularly promising due to their unique characteristics. Sea environments are known for their extreme conditions, including high salinity, varying temperatures, and exposure to intense UV radiation. Bacteria that thrive in these environments have developed specialized metabolic pathways and adaptations to cope with these stressors. One such adaptation is the production of carotenoid pigments, which can serve as potent antioxidants, protecting cells from oxidative damage caused by environmental factors (Maoka 2020).

Furthermore, the diversity of marine bacterial species, especially in unexplored or understudied regions, presents a vast untapped potential for novel carotenoid-producing strains. These newly isolated bacteria may harbor unique carotenoid structures or exhibit enhanced production capabilities, potentially leading to the identification of valuable compounds with applications in some industries. The exploration of pigmented marine bacteria also helped to develop sustainable and efficient production for carotenoids. This approach holds promise for expanding our understanding of carotenoid diversity and enabling the development of novel, environmentally friendly, and economically viable sources of these valuable natural pigments (Ye 2019). Therefore, this study aims to screen marine carotenoid-pigmented bacteria in the Dumai waters, Riau Province, Indonesia, and to characterize their ecology for optimal growth to produce the carotenoid pigment.

## Materials and Methods

**Sampling sites.** The surface water was sampled in the Dumai waters, Riau Province, Indonesia. Samples were taken from six different sites with a distance of around 2-5 km. Then, water samples were put into sterile bottles containing mineral salt medium at a depth of  $\pm 10$ -15 cm from the sea surface. Measurement of environmental conditions was carried out using *in situ* methods based on physical and chemical aspects, such as temperature, pH, salinity, water brightness, dissolved oxygen, nitrate, phosphate, and water color.

**Media preparation.** The growth media for marine pigmented bacteria is mineral salt media, with salinity around 25‰ and pH 7 had the following composition ( $\text{g L}^{-1}$ ): Na-acetate, 2.5;  $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ , 0.9;  $\text{KH}_2\text{PO}_4$ , 0.6;  $(\text{NH}_4) \text{SO}_4$ , 1.25;  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.07;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.2;  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.003; EDTA, 0.002; and yeast extract, 0.5 (Merck). All ingredients are mixed and dissolved in 1000 mL of distilled water. After the media became homogenous, it was then put into a glass bottle and sterilized using a 121°C autoclave (Batubara et al 2022).

**Enrichment, isolation, and screening of pigmented bacteria.** About 1 mL seawater sample was placed into a test tube containing a sterile liquid-mineral salt Media for the enrichment process. The tube with the water sample was taken to the Marine Microbiology Laboratory, Faculty of Fisheries and Marine Sciences, Riau University, for further analysis. In the isolation process, the water sample was inoculated on a new mineral salt media and then incubated at room temperature ( $\pm 25^\circ\text{C}$ ) using the 40-watt fluorescent lamp. To enhance light absorption, all culture media were placed in a circle around the lamp. The incubation time for marine-pigmented bacteria was seven days (168 hours). The growth of pigmented bacteria is characterized by the color change in the liquid media from clear to orange or red. Then, all isolates that grow and form color are re-inoculated on a mineral salt agar media. This media is made by adding 10 g of agar powder to 500 mL of liquid mineral salt media (Batubara et al 2022).

**Morphological and physiological characterization.** After all bacterial isolates have been pure cultured, the next stage is to characterize their morphological and physiological characteristics. Morphological characterization of pigmented carotenoid bacteria includes observing colony shape, size, color, surface texture, elevation, and edges. Furthermore, physiological characterization is carried out through several biochemical tests such as differential staining (Gram staining), catalase test, gelatin test, sugar fermentation test, indole test, sulfide test, citrate test, and bacterial motility test.

**Bacterial ecological analysis.** Determination of the ecological characteristics of pigmented bacteria, such as temperature, pH, salinity, brightness (light), and oxygen demand, was carried out. About 1 mL (McFarland Standard) bacterial suspension was inoculated into 10 mL of the mineral salt media. Next, the media containing the bacterial culture was incubated for 24 hours with a 40-watt fluorescent lamp under different environmental conditions, including a) Temperature, consisting of three treatments, such as 10, 25 and 45°C; b) pH, consisting of three treatments, such as pH 4, 7 and 10; c) Salinity, consisting of three treatments, such as 0, 5 and 10‰; brightness, consisting of three treatments, such as bright, slightly bright and dark; and e) Oxygen Requirements, consisting of three treatments, such as aerobic, microaerophilic, and anaerobic. Furthermore, the ability of bacteria to live in different conditions was characterized by turbidity or color changes in the previous Media.

**UV-VIS spectrophotometer.** Bacterial cells were analyzed using the UV-VIS spectrophotometry method. For each experiment, 20 mL (McFarland Standard) bacterial suspension was entered into a 200 mL mineral salt Media. Then, the bacterial culture was incubated for 96 hours with a 40-watt incandescent lamp at room temperature ( $\pm 25^\circ\text{C}$ ). Cell number measurements were analyzed for 0, 24, 48, 72, and 96 hours by inoculating 5 mL of the cultured liquid suspension into a spectrophotometer cuvette. Bacterial cell growth can be seen from the optical density (OD) at a wavelength of 600 nm and can be expressed in absorbance units (Jeong et al 2022).

**Carotenoid pigment analysis.** Carotenoid pigments are one of the secondary metabolite products produced by marine pigmented bacteria. The color formed from bacterial metabolism is then analyzed using a UV-VIS spectrophotometer. For each experiment, 25 mL (Mc Farland Standard) bacterial suspension was inoculated into 250 mL mineral salt Media. Next, the media containing bacterial culture was incubated for 96 hours under a 40-watt incandescent lamp at room temperature ( $\pm 25^\circ\text{C}$ ). The color formed in the bacterial culture media was centrifuged at 8000 rpm for 10 minutes. The supernatant solution separated from the bacterial cells was extracted using methanol. The bacterial pigment extract was then analyzed at various wavelengths ranging from 350-650 nm (Setiyono et al 2020).

## Results and Discussions

**Sampling and characterization of marine pigmented bacteria.** Based on an analysis of physical and chemical factors in Dumai Waters, Riau Province, Indonesia. Several environmental characteristics were found to support the growth of marine-pigmented bacteria. The results showed a correlation between sampling locations and complex coastal ecosystem dynamics. The data of water temperature measurements ranging from 29.3-30.6°C is optimal for the growth of photosynthetic bacteria in tropical waters. Water temperature is the main factor that supports increased enzyme activity in bacterial photosynthesis, especially in the group of purple non-sulfur bacteria. The gradient of water brightness from a distance of 98-180 cm shows significant variations in light penetration. Mangrove areas and river estuaries show locations with the lowest brightness but have the highest organic material and sediment content. According to (Batubara et al 2021), variations in light penetration can drive vertical stratification of photosynthetic bacterial communities, where species with different pigment adaptations dominate at various depths.

On the other hand, the environmental parameter analysis showed the pH in the six locations in the range of 6.20-6.75 (slightly acidic conditions). The settlement area (Site 1) had the highest pH (6.75), while the harbor and river estuary (Sites 3 and 6) showed more acidic conditions (sequentially, 6.30 and 6.20). At the time of sampling, thermal conditions at all locations were relatively stable, ranging between 29.3-30.6°C. Salinity measurements (28-30.3‰) indicated a moderate salty environment typical for coastal transition zones. The harbor (Site 3) showed slightly higher salinity (30.3‰), possibly due to reduced freshwater input and higher evaporation rates. Water transparency varied

significantly (98-180 cm), with the fish auction site showing the highest clarity (180 cm) and the mangrove the lowest (98 cm). These variations likely reflect differences in suspended solids, biological activity, and local hydrodynamics (Table 1).

Table 1

An analysis of physical and chemical factors in Dumai Waters at six sampling sites

<i>Categories</i>	<i>Site 1</i>	<i>Site 2</i>	<i>Site 3</i>	<i>Site 4</i>	<i>Site 5</i>	<i>Site 6</i>
Specific location	Settlement	Fish auction	Harbor	Purnama beach	Mangrove	River estuary
Sampling depth (m)	0.20	0.20	0.20	0.20	0.20	0.20
pH	6.75	6.76	6.30	6.20	6.40	6.20
Temperature (°C)	30	30.3	30.6	30.6	29.3	30.6
Salinity (‰)	28	29.6	30.3	28.6	29.3	28
Water brightness (cm)	137.5	180	115	135	98	102.5
Dissolved oxygen (mg L <sup>-1</sup> )	2	1.1	1.2	1.3	1.2	1.2
Nitrate (mg L <sup>-1</sup> )	0.0125	0.0083	0.0167	0.0125	0.0229	0.0188
Phosphate (mg L <sup>-1</sup> )	0.0619	0.0398	0.0796	0.3407	0.0553	0.4226
Water color	clear reddish	clear greenish	brownish yellows	clear greenish	brownish green	brownish green

Analysis of chemical parameters also showed various results. Dissolved oxygen (DO) levels range from 1.1 to 2.0 mg L<sup>-1</sup>, which is relatively low compared to coastal waters. The settlement (Site 1) showed the highest DO (2.0 mg L<sup>-1</sup>), while other sites had levels between 1.1 to 1.3 mg L<sup>-1</sup>. Nitrate concentrations (0.0083-0.0229 mg L<sup>-1</sup>) were relatively low at all locations, with the mangrove (Site 5) showing the highest concentrations. This pattern suggests efficient nutrient cycling or limited nitrogen input from terrestrial sources. Phosphate levels showed striking variations (0.0619-0.4226 mg L<sup>-1</sup>), with higher concentrations in the river estuary (0.4226 mg L<sup>-1</sup>) and Purnama beach (0.3407 mg L<sup>-1</sup>). Variations in watercolor from clear to brownish-greenish indicated different levels of biological activity and suspended material at each location. The transition from clear water in settlement areas and fish auctions to brownish-greenish colors in mangrove zones and river estuaries reflected natural gradients in coastal ecosystems (Table 2).

Another environmental parameter that supports the growth of marine pigmented bacteria is dissolved oxygen concentration. Based on direct measurements in the field, the dissolved oxygen concentration at the six sampling locations is still in the low category (1.1-2 mg L<sup>-1</sup>). These indicate microaerophilic conditions support the growth of photosynthetic bacteria, and these conditions are also optimal for purple sulfur and green sulfur bacteria, which use H<sub>2</sub>S as an electron donor in photosynthesis (Batubara et al 2022). Nutrient distribution also shows attractive patterns. The highest phosphate concentrations were found at the river mouth (0.4226 mg L<sup>-1</sup>) and Purnama beach (0.3407 mg L<sup>-1</sup>). This pattern reflects the influence of land input and local upwelling potential. According to (Ibrahim et al 2023), the N:P ratio in waters will affect the pigment composition and photosynthetic efficiency of marine bacteria. Furthermore, the color of the water at the sampling location can also indicate the presence of photosynthetic pigments and bacterial biomass. Thus, locations that are brownish/greenish indicate the potential for high levels of bacteriochlorophyll and carotene.

Table 2

Morphological and biochemical characterization of marine pigmented bacteria

<i>No.</i>	<i>Stations</i>	<i>Colony color</i>	<i>Shape</i>	<i>Edge</i>	<i>Elevation</i>	<i>Colony count</i>
	St.1.10-3	Milk white	Circular	Entire, undulate, filamentous	Flat, raised, convex	258
1	St.1.10-4	Milk white	Circular, spindle, irregular	Entire, undulate	Flat, raised, convex, Umbonate	190
	St.1.10-5	Milk white	Circular, irregular, rhizoid	Entire	Flat, raised, convex	39
	St.2.10-3	Milk white	Circular, spindle, irregular	Entire	Flat, raised	93
2	St.2.10-4	Milk white	Circular, spindle, irregular	Entire	Flat, raised	66
	St.2.10-5	Milk white	Circular, spindle	Entire, undulate	Flat, raised	37
	St.3.10-3	Milk white	Circular, irregular	Entire, undulate	Flat, raised, convex	54
3	St.3.10-4	Milk white	Circular, irregular, spindle	Entire, undulate	Flat, raised, convex, umbonate	24
	St.3.10-5	Milk white	Circular, irregular	Entire, undulate	Flat, raised, convex	7
	St.4.10-3	Milk white	Circular	Entire, undulate	Flat, raised	17
4	St.4.10-4	Milk white	Circular	Entire	Flat	21
	St.4.10-5	Milk white	Circular	Entire	Flat	9
	St.5.10-3	Milk white	Circular, irregular, spindle	Entire	Flat, raised, convex	24
5	St.5.10-4	Milk white	Circular, irregular	Entire, undulate	Flat, convex, raised, umbonate	28
	St.5.10-5	Milk white	Circular, spindle	Entire, undulate	Flat	6
	St.6.10-3	Milk white	Circular	Entire, rhizoid	Flat	18
6	St.6.10-4	Milk white	Circular, irregular	Entire, undulate	Flat, convex	37
	St.6.10-5	Pale white	Circular, filamentous	Entire, lobate	Flat, raised	8

There were six sampling locations with a total of 18 bacterial isolates found. Based on morphological characteristics, 17 isolates had milk-white colonies, with only one isolate being pale white (St.6.10-5). The characteristic shapes of the colony were dominated by a circular shape with several variations, such as spindle, irregular, and rhizoid. Regarding edges, most of the isolates showed entire and undulate characteristics. Some isolates also have shapes like rhizoids and lobates. The colony elevations generally showed some characteristics, such as flat, raised, convex, and combination (Table 2).

Morphologically, most bacteria have the same colony color, namely milk white, but their shapes vary, which are circular, irregular, spindle, and filamentous. This significant variation shows the diversity of adaptation to environmental conditions. Generally, the morphological characteristics of the colony correlated with the photosynthetic ability and ecological adaptation. Based on several colonies, there were variations between the isolates. The highest number of colonies was found in Site 1 ( $10^3$ ), with 258 colonies, while the lowest number was found in Site 3 ( $10^5$ ), with seven colonies. The distribution of colony numbers did not show a consistent pattern between stations, indicating the possible influence of different environmental factors at each sampling site's location. Overall, the data showed relatively high morphological diversity in marine pigmented bacterial populations, although there are some dominant characteristics such as milk-white colony color and circular shape. Variations in colony numbers and morphological characteristics reflect specific environmental conditions at each sampling site.

**Ecological characteristics, growth, and pigment production.** Another factor that supports screening for marine pigmented bacteria is the ecological properties and bacterial growth in producing pigments. The eight isolates produced red color (Figure 1) after being cultured in a mineral salt medium, and then were analyzed for ecological properties in several environmental conditions. Five ecological characteristics were assessed, such as temperature, pH, salinity, brightness (light), and oxygen requirements (Table 3). The environmental condition of bacteria related to sampling sites and complex dynamics of coastal ecosystems (Table 3). The temperature and pH showed that most pigmented bacterial isolates were mesophilic groups that grow optimally at a temperature of 25°C. Only one isolate (S1U3MD) showed thermophilic (45°C), and no one classified as psychrophilic bacteria. Furthermore, all isolates showed the ability to grow in the neutral to alkaline pH (pH 7-10), with optimum conditions in neutral. This pH adaptation is a general characteristic of marine bacteria (Falaise et al 2019).

Table 3

The ecological characteristics of pigmented bacteria against several environmental parameters

Isolate Code	Environmental Condition of Bacteria Growth														
	Temperature			pH			Salinity			Brightness (light)			Oxygen requirement		
	T	M	P	A	N	Al	H	Md	L	Sb	L	D	Ae	M	An
S1U3MD	+	+	-	+	+	+	+	+	-	+	-	-	+	-	+
S1 SUB II	-	+	-	+	+	-	+	+	-	+	+	+	+	-	+
S2 SUB II	-	+	-	+	+	+	+	+	-	+	-	-	+	+	+
S3 U2 MD	-	+	-	+	+	-	+	+	+	+	-	-	-	+	+
S3 U3 MD	-	+	-	-	+	+	+	+	+	+	+	-	+	+	+
S3 SUB III	-	-	-	-	+	+	+	+	-	-	+	+	+	-	-
S4 U1 MD	-	+	-	+	+	+	+	+	+	-	+	+	-	-	+
S5 SUB II	-	+	-	+	+	+	+	+	+	-	-	+	-	+	+

T (Thermophiles, 45°C); M (Mesophiles, 25°C); P (Psychrophiles, 10°C); A (Acidophiles, pH 4); N (Neutrality, pH 7); Al (Alkaliphiles, pH 10); H (High, 10 ‰); Md (Media, 5 ‰); L (Low, 0 ‰); B (Bright); Sb (Slightly bright); D (Dark); Ae (Aerobes); M (Microaerophiles); An (Anaerobes).

The ecological characteristics analysis of marine pigmented bacteria revealed that most pigmented bacterial isolates are mesophilic groups that grow optimally at a temperature of 25°C. Only one isolate (S1U3MD) showed thermophilic (45°C), and no one classified as psychrophilic bacteria. Furthermore, all isolates showed the ability to grow in the neutral to alkaline pH (pH 7-10), with optimum conditions in neutral. This pH adaptation is a general characteristic of marine bacteria. Salinity tolerance showed that all isolates grow optimally at moderate salinity (5‰), indicating moderate halotolerant characteristics. Several isolates (S3U2MD, S3U3MD, S4U1MD) could live at low salinity (0‰), indicating high osmotic adaptation. Analysis of the response to light showed that the most isolates had positive results. This means the preference for light intensity is moderate. Several isolates (S1SUBII, S3U3MD, S3SUBIII, S4U1MD) also showed they can grow in the light (L) condition. Furthermore, the oxygen requirements showed the characteristics of facultative anaerobes, where all isolates could grow in aerobic and anaerobic conditions. Several isolates (S2SUBII, S3U2MD, S3U3MD) showed microaerophilic conditions, which means the bacteria can survive volatile oxygen in the marine environment (Table 3).

**Growth and pigment production.** The screening process for carotenoid pigment-producing bacteria was conducted in seven days. Bacteria produce the carotenoid pigment after being cultured in a mineral salt media incubated with light. The capability of pigmented bacteria to produce carotenoid pigments is detailed in Figure 1.



Figure 1. Carotenoid pigments are produced by marine-pigmented bacteria.

Furthermore, an analysis of growth patterns and pigment production was carried out. The optical density (OD<sub>600</sub>) observations showed variations in growth patterns between isolates during 96 hours of incubation. The S5SUBII isolate showed the highest pigment production with a peak OD of 3.2 at 48 hours but experienced a significant decline subsequently. Furthermore, most isolates represent a lag phase of up to 24 hours, except S1SUBII, which showed faster initial growth. However, the stability of pigment production occurred after the bacteria were incubated for 72 hours. All isolates showed stable pigment production with OD ranging from 1.8 to 2.2 (Figure 2).

Bacteria produce the carotenoid pigment after being cultured in a mineral salt medium incubated with light. The eight strains exhibited varying growth patterns during the 96-hour incubation period. Carotenoid pigment production occurred during the exponential phase, specifically from the 24th to the 48th hour. Optical density (OD) measurements indicated that isolate S5SUBII produced the highest pigment, achieving a peak OD of 3.2 at the 48th hour; however, this production experienced a significant decline afterward. Most isolates displayed a lag phase of up to 24 hours, except isolate S1SUBII, which demonstrated faster initial growth. Between the 24th and 48th hours, the exponential (logarithmic) phase likely occurred, indicating active cell division and optimal pigment production. From the 72nd to the 96th hour, most isolates entered a stationary phase, with OD values ranging from 1.8 to 2.2. During this phase, the bacteria continued to produce pigment, as evidenced by the absence of the death phase. This suggests that the growth pattern and mass production of carotenoid pigments could potentially be scaled up for industrial applications. The previous ecological analysis data showed that isolates tolerant to variations in light intensity, such as S1SUBII and S3SUBIII, demonstrated more adaptive pigment production patterns (Wibowo et al 2023) explained that the ability to adapt to changes in light is closely related to the regulation of carotenoid biosynthesis, which acts as a photoprotection mechanism.

The growth graph in Figure 2 illustrates that the eight strains exhibited varying growth patterns during the 96-hour incubation period. Carotenoid pigment production occurred during the exponential phase, specifically from the 24th to the 48th hour. Optical density (OD) measurements indicated that isolate S5 SUB II produced the highest pigment, achieving a peak OD of 3.2 at the 48th hour, however, the number of cells decreased. Most isolates displayed a lag phase of up to 24 hours, except isolate S1 SUB II, which demonstrated faster initial growth.

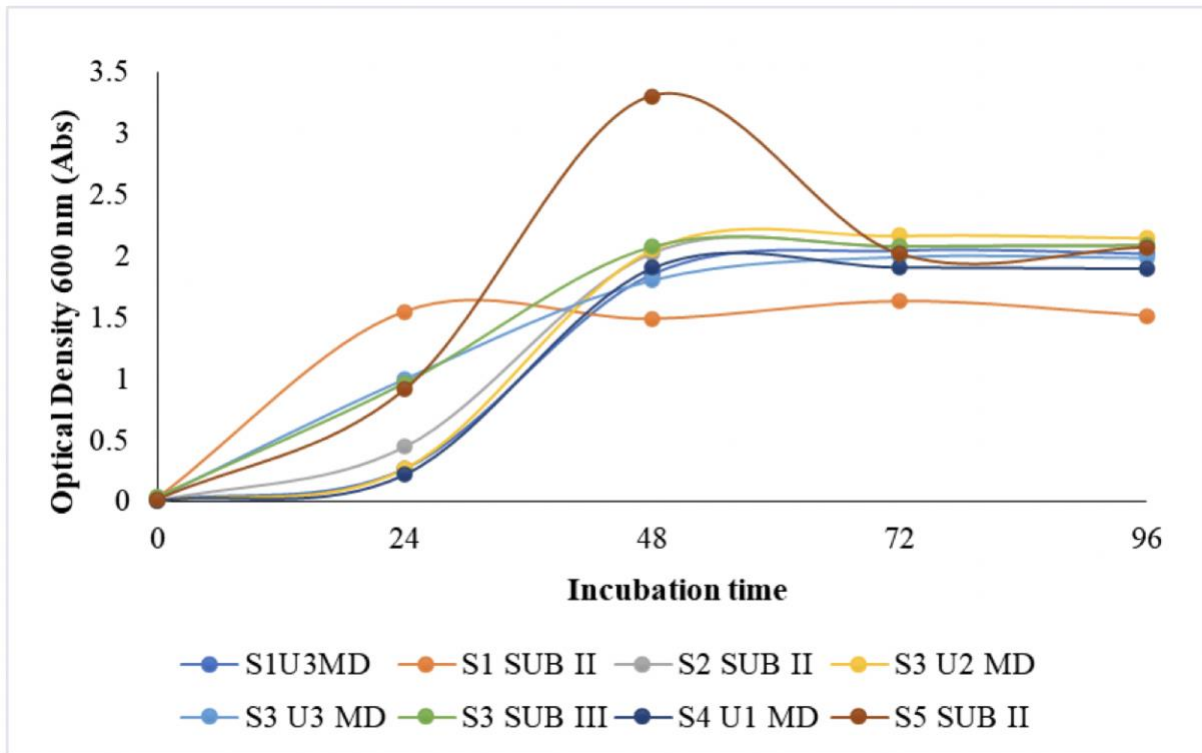


Figure 2. Carotenoid pigments from marine pigmented bacteria.

After obtaining the spectrophotometer calculation results for analyzing the growth of pigmented bacteria, the red color produced by the bacteria was analyzed using a UV-VIS spectrophotometer. The results showed a maximum absorption peak at a wavelength of around 400 nm for all isolates, with isolate S3U2MD showing the highest absorption (1.7). The isolate (S3U2MD) had the highest absorption and showed stable growth with an OD600 of around 2.0 to 2.2 in the stationary phase. Variations in absorption intensity between isolates (0.8 to 1.7 at 400 nm) reflected differences in pigment biosynthesis capacity (Figure 3).

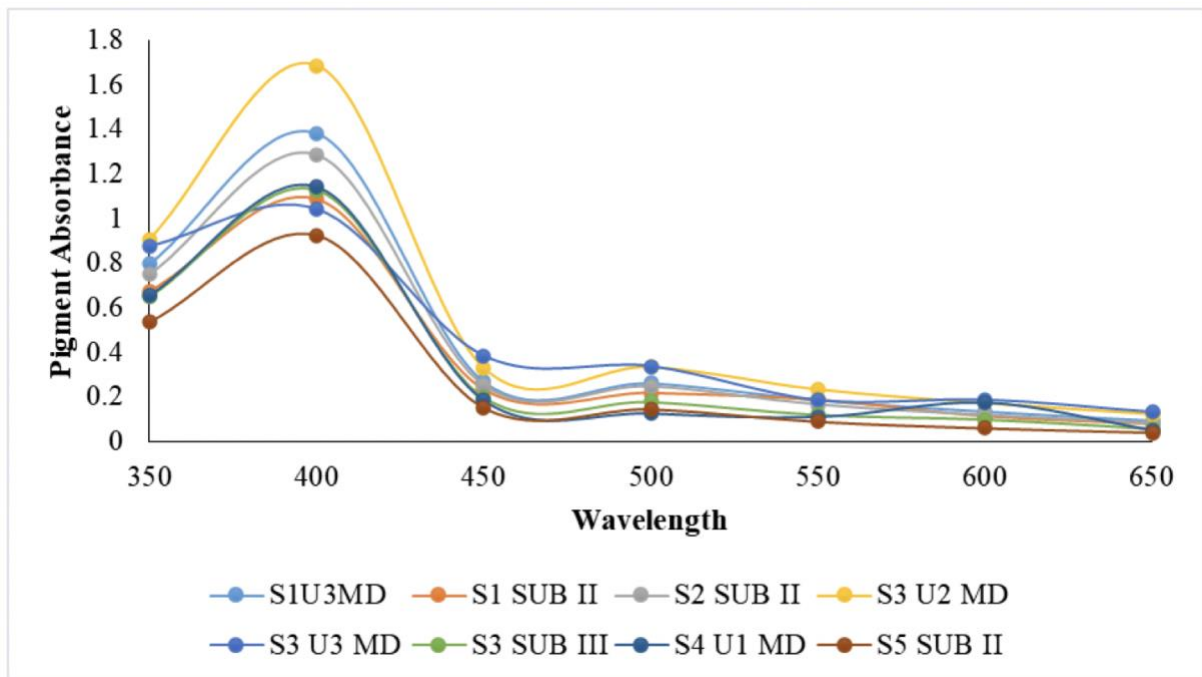


Figure 3. Carotenoid pigments from marine pigmented bacteria.



The results of bacterial pigment analysis using the spectrophotometric method showed that the maximum absorption peak occurred at a wavelength of 400 nm for all isolates. The absorbance at a wavelength of 400 nm is by the carotenoid spectrum, which shows maximum absorption in the 400-450 nm range (Ahmad et al 2021). Analysis of pigments produced by all bacterial isolates illustrates the consistent characteristics of carotenoid compounds. The absorption peak in the range of 380-500 nm is a typical characteristic of carotenoid pigments, especially the astaxanthin (Ahmad et al 2021); prodigiosin (Ramesh 2020);  $\beta$ -carotene and zeaxanthin groups (Narsing Rao et al 2017). (Batubara et al 2024), reported that the carotenoid pigment group has the advantage of producing antioxidant compounds, which can ward off free radicals and protect cells. Carotenoids also are found in many marine bacteria, such as astaxanthin (Srinivasan et al 2021). Several types of carotenoids were reported to have antimicrobial activity against Gram-positive pathogenic bacteria (MIC=31.25-62.5 mg mL<sup>-1</sup>), such as *Paracoccus haeundaensis* and *Bacillus indicus*. Several types of carotenoids are reported to have antimicrobial activity against food spoilage pathogenic bacteria, such as *Salmonella typhimurium* and *Staphylococcus aureus* and have been recommended as natural antibacterial preservatives in various food ingredients (Naisi et al 2023).

Variations in absorbance peak values among bacterial samples indicate differences in carotenoid concentrations. The isolate S3U2MD exhibited the highest absorbance peak, measuring around 1.6, suggesting that it has potential for carotenoid pigment production. In contrast, samples with lower absorbance peaks, such as S5SUBII, are likely to contain lower concentrations of carotenoids. These differences may arise from factors such as growth conditions, biosynthetic capacity, or the adaptive responses of the bacterial strains. Consistent absorption results across all isolates suggest a uniform dominance of carotenoid types. Therefore, all pigmented bacterial strains show great potential for further development in various applications, including bio-pharmacology and the food industry.

**Conclusions.** In this research, eight isolates of marine pigmented bacteria were found in the Dumai waters, Riau Province, Indonesia. All the isolates produced carotenoid pigments after being cultured on mineral salt media and delivered the red natural pigment. Based on growth pattern and UV-VIS spectrophotometry analysis, the optimum biomass produced from the eight pigmented marine bacterial isolates occurred in the logarithmic (exponential) growth phase with a time range between 48 and 72 hours.

**Acknowledgements.** The author would like to gratitude the Institute for Research and Community Service of the University of Riau and the Directorate of Research, Technology, and Community Service, who have provided research grants under the Basic (Fundamental) Research Scheme with contract number 083/E5/PG.02.00.PL/2024 and No: 20660/UN19.5.1.3/AL.04/2024.

**Conflict of interest.** The authors declare that there is no conflict of interest.

## References

- Ahmad I., Malak H. A., Abulreesh H. H., 2021 Environmental antimicrobial resistance and its drivers: a potential threat to public health. *Journal of Global Antimicrobial Resistance* 27:101-111.
- Batubara U. M., Nursyirwani N., Nurrachmi I., Simanjuntak N. H. B., Defitra H. J., George J. R., Hasibuan A., George J. R., 2024 Diversity of carotenoid pigmented marine photosynthetic bacteria in the Dumai sea waters. *Bio Web of Conferences* 136:04005.
- Batubara U. M., Sibagariang R. D., Siregar S. S., Maelina T., Ginting T. Y., Pratama M. R., Jaboro M. R., 2022 Determination of anoxygenic photosynthetic bacteria from water and sediment in Dumai coastal waters, Indonesia. *IOP Conf. Series: Earth and Environmental Science* 1118(1):012027.
- Batubara, U. M., Aini, F., & Manurung, M. M., 2021 Screening and characterization of anoxygenic photosynthetic bacteria as carotenoid pigments producer from palm liquid sewages. *Jurnal Pembelajaran Dan Biologi Nukleus* p-ISSN 2442:9481.

- Corinaldesi C., Barone G., Marcellini F., Dell'Anno A., Danovaro R., 2017 Marine microbial-derived molecules and their potential use in cosmeceutical and cosmetics product. *Marine Drugs* 15(4):118.
- Falaise C., James A., Travers M. A., Zanella M., Badawi M., Mouget J. L., 2019 Complex relationships between the blue pigment marennine and marine bacteria of the genus *Vibrio*. *Marine drugs* 17(3):160.
- Ibrahim W. M., Olama Z. A., Abou-elela G. M., Ramadan H. S., Hegazy G. E., El Badan D. E. S., 2023 Exploring the antimicrobial, antiviral, antioxidant, and antitumor potentials of marine *Streptomyces tunisiensis* W4MT573222 pigment isolated from Abu-Qir sediments, Egypt. *Microbial Cell Factories* 22(1):94.
- Jeong S. W., Yang J. E., Choi Y. J., 2022 Isolation and characterization of a yellow xanthophyll pigment-producing marine bacterium, *Erythrobacter sp.* SDW2 Strain, in coastal seawater. *Marine Drugs* 20(1):73.
- Maoka T., 2020 Carotenoids as natural functional pigments. *Journal of Natural Medicines* 74:1-16.
- Naisi S., Bayat M., Salehi T. Z., Zarif B. R., Yahyataeyat R., 2023 Antimicrobial and anti-biofilm effects of carotenoid pigment extracted from *Rhodotorula glutinis* strain on food-borne bacteria. *Iranian Journal of Microbiology* 15(1):79.
- Narsing Rao M. P., Xiao M., Li W. J., 2017 Fungal and bacterial pigments: secondary metabolites with wide applications. *Frontiers in Microbiology* 8:1113.
- Nawaz A., Chaudhary R., Shah Z., Dufosse L., Fouillaud M., Mukhtar H., Haq I., 2020 An overview on industrial and medical applications of bio-pigments synthesized by marine bacteria. *Microorganisms* 9(1):11.
- Ramesh C., Vinithkumar N. V., Kirubakaran R., Venil C. K., Dufosse L., 2020 Applications of prodigiosin extracted from marine red pigmented bacteria *Zooshikella sp.* and *Actinomycete Streptomyces sp.* *Microorganisms* 8(4):556.
- Setiyono E., Adhiwibawa M. A. S., Indrawati R., Prihastyanti M. N. U., Shioi Y., Brotosudarmo P., 2020 An Indonesian marine bacterium, *Pseudoalteromonas rubra*, produces antimicrobial prodiginine pigments. *ACS OMEGA* 5(9):4626-4635.
- Silva T. R. E., Silva L. C. F., de Queiroz A. C., Alexandre Moreira M. S., de Carvalho Fraga C. A., de Menezes G. C. A., Rosa L. H., Bicas J., Oliveira V. M., Duarte A. W. F., 2021 Pigments from antarctic bacteria and their biotechnological applications. *Critical Reviews in Biotechnology* 41(6):809-826.
- Srinivasan R., Kannappan A., Shi C., Lin X., 2021 Marine bacterial secondary metabolites: A treasure house for structurally unique and effective antimicrobial compounds. *Marine Drugs* 19(1):530.
- Wibowo J. T., Bayu A., Aryati W. D., Fernandes C., Yanuar A., Kijjoa A., Putra M. Y., 2023 Secondary metabolites from marine-derived bacteria with antibiotic and antibiofilm activities against drug-resistant pathogen. *Microorganisms* 21(1):50.
- Ye K. X., Fan T. T., Keen L. J., Han B. N., 2019 A review of pigments derived from marine natural products. *Israel journal of chemistry* 59(5):327:338.

Received: 06 January 2025. Accepted: 28 February 2025. Published online: 28 February 2025.

Authors:

Ummi Mardhiah Batubara, Marine Microbiology Laboratory, Faculty of Fisheries and Marine Sciences, University of Riau, Pekanbaru, Indonesia, email: [ummimardhiah@lecturer.unri.ac.id](mailto:ummimardhiah@lecturer.unri.ac.id)

Nursyirwani, Marine Microbiology Laboratory, Faculty of Fisheries and Marine Sciences, University of Riau, Pekanbaru, Indonesia, email: [nursyiwani@lecturer.unri.ac.id](mailto:nursyiwani@lecturer.unri.ac.id)

Irvina Nurrachmi, Chemical Oceanography Laboratory, Faculty of Fisheries and Marine Sciences, University of Riau, Pekanbaru, Indonesia, email: [irvina.nurachmi@lecturer.unri.ac.id](mailto:irvina.nurachmi@lecturer.unri.ac.id)

Naomi Hiberin Br Simanjuntak, Marine Microbiology Laboratory, Faculty of Fisheries and Marine Sciences, University of Riau, Pekanbaru, Indonesia, email: [naomi.heberin2815@student.unri.ac.id](mailto:naomi.heberin2815@student.unri.ac.id)

Hersa Javayu Defitra, Marine Microbiology Laboratory, Faculty of Fisheries and Marine Sciences, University of Riau, Pekanbaru, Indonesia, email: [hersa.javayu6060@student.unri.ac.id](mailto:hersa.javayu6060@student.unri.ac.id)

Jonathan R. George, Marine Microbiology Laboratory, Faculty of Fisheries and Marine Sciences, University of Riau, Pekanbaru, Indonesia, email: [jonathan.ramadhanu1625@student.unri.ac.id](mailto:jonathan.ramadhanu1625@student.unri.ac.id)

Arisandi Hasibuan, Chemical Oceanography Laboratory, Faculty of Fisheries and Marine Sciences, University of Riau, Pekanbaru, Indonesia, email: [arisandi.hasibuan1651@student.unri.ac.id](mailto:arisandi.hasibuan1651@student.unri.ac.id)

This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

How to cite this article:

Batubara U. M., Nursyirwani N., Nurrachmi I., Simanjuntak N. H. B., Defitra H. J., George J. R., Hasibuan A., 2025 Screening and characterization of marine carotenoid-pigmented bacteria in Dumai waters, Riau Province, Indonesia. *AACL Bioflux* 18(1):535-545.