BIO Web of Conferences **136**, 04005 (2024) https://doi.org/10.1051/bioconf/202413604005
ISFM XIII 2024

Diversity of Carotenoid Pigmented Marine Photosynthetic Bacteria in the Dumai Sea Waters

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Abstract. Marine photosynthetic bacteria represent autotrophic microorganisms capable of synthesizing natural pigments. This study aims to explore the diversity of carotenoid-pigmented marine photosynthetic bacteria in the Dumai Sea waters, Riau Province, Indonesia. The research method was a survey using marine surface water as samples from six locations. The research was conducted in four primary phases: 1) measurement of environmental conditions; 2) enrichment and isolation of marine photosynthetic bacteria; 3) enumeration of total bacteria; and 4) analysis of pigments colour production. Isolation results and population density measurements of photosynthetic bacteria revealed fascinating patterns. The highest cell count was observed at fish auctions (sampling site 2), while the lowest cell count was recorded near a river estuary (sampling site 6). Detailed characterization studies identified ten isolates capable of producing natural carotenoid pigments exhibiting colours ranging from pink to deep red. These findings contribute to the understanding of marine microbial diversity and the potential of bacteria for biotechnology products, aquaculture, and medical applications.

1 Introduction

The marine ecosystem holds great potential for biodiversity in national development strategies. The management of aquatic biological resources can be achieved through innovation and research in biotechnology. The Dumai sea waters, located in Riau Province, is a strategic area with a diverse range of biodiversity, including marine photosynthetic bacteria. These bacteria are pigmented and produce natural colours through their metabolism. Generally, marine photosynthetic bacteria are photoautotrophs, as they contain pigments that capture sunlight for the photosynthesis process [1]. The pigments in marine photosynthetic bacteria include bacteriochlorophyll and various carotenoids, resulting in colours ranging from purple, red, brown, to orange. Marine photosynthetic bacteria are abundant in nature as

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producers of carotenoid pigments [2]. These photosynthetic bacteria are widely distributed in aquatic ecosystems such as seas, rivers, lakes, sediments, moist soil, and habitats with high salinity. Photosynthetic bacteria can survive and carry out photosynthesis in a wide range of environmental conditions due to their flexible adaptation [3].

A group of photosynthetic bacteria species has been discovered, including *Rhodobacter sphaeroides*, *Rhodopseudomonas palustris*, *Serratia marcescens*, *Cordyceps militaris*, and *Streptomyces echinoruber* [4]. One of the advantages of photosynthetic bacteria is their ability to grow in both aerobic and anaerobic environments and their capacity to utilize organic and inorganic materials to supply N_2 and CO_2 [1]. The marine photosynthetic bacteria can be used to produce some biotechnology product such as foods, feeding fish, cosmetics, fashion and medical ingredients [5]. Photosynthetic bacteria are capable of producing a variety of valuable substances, including single-cell proteins, coenzyme Q10, 5-aminolevulinic acid (ALA), carotenoids, bacterio-chlorines, and polyhydroxyalkanoates (PHAs). They have the potential to serve as a source of raw materials for producing a range of industrial and biomedical products that are valuable for various applications [6].

The production of Pigment occurs both inside and outside cells, and it has different characteristics and functions. Various studies have shown that numerous bacteria, fungi, and microalgae can produce natural pigments like chlorophyll, carotenoids, iridoids, flavins, flavonoids, melanin, anthocyanins, etc [7]. Marine photosynthetic bacteria are a relatively untapped source of pigments with potential applications in industries such as textiles, foods, medicines, furniture, and others. Using bacteria for biotechnological purposes has several advantages, including ease of culture, relatively fast generation time, genetic manipulability, and the ability to be extracted and produced in large quantities [7][8]. Consequently, it is imperative to conduct an exploration of marine photosynthetic bacteria originating from the Dumai waters as their natural habitat. Subsequently, potential isolates obtained from the waters can be subjected to further analysis for development into diverse biotechnological applications, particularly in the marine sector.

2 Material and Method

2.1 Sampling Sites

Surface water samples were collected from the Dumai waters, Riau Province, Indonesia. The samples were taken from six different sites with a distance of around 2-5 km. Then, the samples were put into sterile bottles containing modified mineral media at a depth of ± 10-15 cm from the sea surface.

2.2 Measurement of environmental conditions

Measurement of environmental conditions is carried out using in-situ methods based on physical and chemical aspects, such as temperature (water thermometer), pH (pH meter), salinity (hand-refractometer), water transparency (Secchi disk).

2.2 Medium preparation

The growth medium for marine photosynthetic bacteria is a modified mineral medium containing several types of ingredients including Na-acetate, K_2HPO_4 $3H_2O$, KH_2PO_4, (NH_4) SO_4, $CaCl_2$ $2H_2O$, $MgSO_4$ $7H_2O$, $FeSO_4$ $7H_2O$, Yeast Extract, and Ethylene Diamine Tetraacetic Acid Tetrasodium (EDTA). All ingredients are mixed and dissolved in 1000 ml of distilled water. After all the media composition is homogeneous, the media is then put into an Erlenmeyer bottle and sterilized using a 121°C autoclave.

2.3 Enrichment and isolation

About 1 mL seawater sample was placed into a test tube containing sterile liquid-modified mineral media for the enrichment process. The tube with the water sample was then taken to the marine microbiology laboratory of Fisheries and Marine Sciences at Riau University for further analysis. In the isolation process, the water sample was inoculated on a newly modified mineral medium and then incubated at room temperature using a 40-watt fluorescent lamp. To enhance light absorption, all insulating media were placed in a circle around the lamp. The cultivation time for marine photosynthetic bacteria was seven days.

2.4 Enumeration of total bacteria

The density of marine photosynthetic bacterial cells was determined using the Total Plate Count (TPC) method with Serial Dilution Agar Plating. A total of 1 ml of seawater sample was mixed with 9 ml sterile physiological salt solution (0.8%) and homogenized. The sample solution was then gradually diluted until the dilution reached 10^{-5}. The diluted sample was spread in 1 ml portions on the surface of Plate Count Agar (PCA Merck) media and then incubated at room temperature for approximately 48 hours. Bacterial cells that formed colonies on the media surface were then counted using a colony counter. The calculation results were then converted into CFU (Colony Forming Units) per mL using the dilution factor and inoculation volume.

2.5 Analysis of pigments production

Bacterial pigment production analysis was conducted following the isolation of pure bacterial cultures [3]. The isolates were inoculated into modified liquid mineral media and incubated for seven days. Bacterial cultivation was performed at ambient temperature (20-25°C) provided by a 40-watt fluorescent lamp. The identification of carotenoid-synthesizing bacteria was based on the observed colour transformation of the culture medium from initially clear or colourless to shades ranging from brown to dark red. This colour change is indicated by carotenoid production, as these pigments typically exhibit colours in the yellow-to-red spectrum.

3 Result and Discussion

3.1 Field conditions

The research location map focuses on the coastal area of the Dumai City that borders the Malacca Strait. There are six sampling sites spread along the coastline, from south to north (Fig.1.). Based on data from measurements of environmental factors at the six sampling locations, it can be seen that four parameters were carried out in situ such as pH, temperature, salinity, and brightness. Water pH ranged from 6.0 to 7.15, with the highest value recorded at sampling site 1 (7.15) and the lowest at sampling site 3 (6.0). Generally, locations have a pH slightly below neutral, indicating a tendency for slightly acidic water conditions. These pH variations can be influenced by various factors such as biological activity, freshwater input, or anthropogenic pollution.

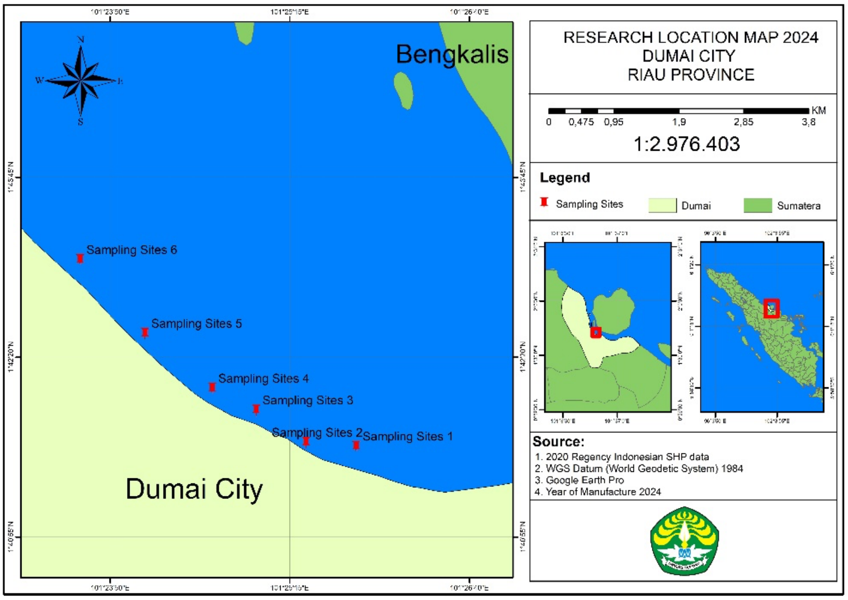

Fig. 1. Sampling sites and measuring process of environmental aspects

Water temperatures are relatively consistent at all locations, ranging from 29°C to 31°C. Sampling sites 3 and 4 recorded the highest temperature (31°C), while sampling site 1 had the lowest temperature (29°C). This temperature stability indicates uniform climatic conditions throughout the study area, although small differences can be caused by variations in air depth, current patterns, or sampling time. The salinity of the waters varies from 28 to 31 ppt (parts per thousand). The highest value was recorded at sampling location 6 (31 ppt), while the lowest was found at sampling sites 2 and 4 (28 ppt). These salinity variations may reflect the influence of freshwater input from rivers, marine air circulation patterns, or meteorological factors such as rainfall and evaporation (Table 1.).

Table 1. Field condition of sampling site

No	Sampling sites	Environmental Parameters			
		pH	Temperature (°C)	Salinity (ppt)	Water transparency (m)
1	Sampling site 1	7.15	29	30	1.80
2	Sampling site 2	6.07	30	28	1.95
3	Sampling site 3	6.0	31	30	1.35
4	Sampling site 4	6.20	31	28	1.02
6	Sampling site 5	6.32	30	29	0.98
7	Sampling site 6	6.27	30	31	1.15

Water transparency, measured in meter units, shows significant variation between locations. The highest value was recorded at sampling site 2 (1.95), while the lowest was at sampling site 5 (0.98). These variations in water transparency can be caused by differences in depth, suspended sediment content, or biological activity such as plankton blooms. Variations in salinity indicate the influence of land and complex marine air circulation patterns. Significant differences in water transparency between locations indicate variations in air quality and may reflect differences in sediment input or biological activity [9]. Further research is needed to understand the factors that influence variations in these parameters, as well as their activation on coastal ecosystems and marine biota in the Dumai waters area.

pH is a factor influencing the physiology and metabolism of marine photosynthetic bacteria. The positive correlation between pH and high bacterial density is that many marine photosynthetic bacteria have optimal growth at pH above neutral [10]. Water temperature between 29-31°C is optimal for tropical photosynthetic bacteria growth. These variations can affect the metabolic rate and generation time of the bacteria [11] [12]. Salinity, which varied from 28 to 31 ppt at the sampling sites, affected the osmoregulation and photosynthetic efficiency [13]. Water transparency is correlated with bacteria and is crucial in marine bacterial photobiology. Deeper light penetration favors a euphotic zone and allows a more extensive vertical distribution of photosynthetic bacteria. These complex interactions between environmental factors create a mosaic of microhabitats that support functional diversity in photosynthetic bacterial communities [14].

3.2 Spatial distribution of Marine Photosynthetic bacteria

Research on the density of marine photosynthetic bacteria in the Dumai waters shows significant variations between sampling locations. This variation reflects the complexity of marine ecosystems and the importance of environmental factors in shaping microbial communities. Marine photosynthetic bacteria play a crucial role in biogeochemical cycles and food chains.

Table 2. Total bacterial count in each of sampling sites

Total bacteria	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
CFU (cells mL^{-1})	93×10^3	258×10^3	18×10^3	54×10^3	24×10^3	17×10^3
CFU (cells mL^{-1})	66×10^4	190×10^4	37×10^4	24×10^4	28×10^4	21×10^4
CFU (cells mL^{-1})	37×10^5	39×10^5	8×10^5	7×10^5	6×10^5	9×10^5

Based on the data in Table 2. shows that there are differences in the density of photosynthetic bacteria found at each sampling location. The counting of total bacteria using the TPC method shows that the highest density of photosynthetic bacteria was at sampling location 2 for all dilution levels. It indicated that sampling site 2 has more optimal environmental conditions for the growth of photosynthetic bacteria compared to another sampling site, such as nutrient availability, pH, temperature, and light intensity. In contrast, at sampling site 6, the total number of bacteria for all dilution levels showed a relatively lower density. It may be caused by limiting factors such as competition with other organisms or unfavorable physicochemical conditions.

Variations in bacterial density between locations can be explained through ecological “niche partitioning” in marine microbes. According to [15][16], marine microbial communities are strongly influenced by environmental gradients such as temperature, salinity, and nutrient availability. In the context of the Dumai waters, differences in the density of photosynthetic bacteria may reflect variations in the microhabitat at each sampling location. It is important to note that the observed bacterial density varies depending on the dilution level used. Based on data analysis, the density of photosynthetic bacteria in the

Dumai waters reveals the complexity of interactions between microbial communities and their environment. Coastal marine ecosystems, such as those found in the Dumai, are very dynamic environments where abiotic factors play an important role in determining the structure and function of microbial communities. Variations in the density of photosynthetic bacteria observed at the six sampling locations reflect the spatial heterogeneity commonly encountered in marine ecosystems. This phenomenon is often associated with "uneven distribution" in microbiology, where microorganisms tend to be seen in nutrient-rich aggregates or micro-zones [17].

It is important to note that the CFU counting method used in this study may only capture a small portion of the total photosynthetic bacterial community. Many marine bacteria are "viable but non-culturable" (VBNC) or have specific growth requirements under standard laboratory conditions. Therefore, molecular integration techniques such as qPCR, FISH, or 16S rRNA gene amplicon sequencing are for further research to obtain a more comprehensive picture of community structure and dynamics. Spatial variations in the density of photosynthetic bacteria in the Dumai waters reflect the complexity and dynamics of marine microbial ecosystems. Further research with multi-omics approaches and time series analysis will be valuable to the mechanisms underlying the distribution and ecological function in dynamic coastal environments.

3.3 Carotenoid pigment identification result

Carotenoid pigments synthesized by bacteria are a group of isoprenoid compounds that have an important role in bacterial physiology and have broad application potential in various industries (Fig.2.). The main functions of carotenoids in bacteria include photoprotection, membrane stabilization, antioxidant activity, and inside some species, a role in photosynthesis [18]. Types of carotenoids commonly found in bacteria include β-carotene, astaxanthin, canthaxanthin, and zeaxanthin, with their production influenced by environmental factors such as temperature, pH, light, and nutrient availability [2][19].

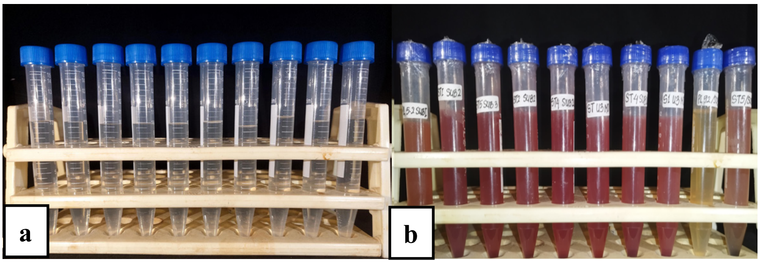

Fig. 2. Natural pigment produced by marine photosynthetic bacteria. a) media conditions before adding the marine photosynthetic bacteria and b) media after adding the marine photosynthetic

According by [10], revealed the potential of carotenoid-producing bacteria in bioremediation, where these pigments play a role in protecting bacteria from oxidative stress during the pollutant degradation process. Genomic studies conducted by [13] in *Rhodonellum psycrophylum* GL8 detected a new regulatory pathway that controls carotenoid biosynthesis, opening the opportunity to engineer bacterial metabolism to increase pigment production. [7][20] reported the discovery of marine bacterial strains capable of producing carotenoids with superior antioxidant activity, indicating potential applications in the nutraceutical and pharmaceutical industries.

Industrial applications of bacterial carotenoids continue to grow and focus on use as natural colorants in the food industry, nutritional supplements, and active ingredients in cosmetics [5]. Advances in fermentation techniques and genetic engineering have enabled increased bacterial carotenoid production on an industrial scale. [21] have succeeded in optimizing astaxanthin production by *Paenibacillus* sp using a co-culture strategy. Challenges that still face the production of carotenoid bacteria include optimizing breakdown conditions, developing super-producing strains, and increasing production scale [22].

Aspects of the ecology of carotenoid bacteria have also received attention. [17] revealed that pigments play an important role in the adaptation process to extreme environments such as Antarctica. Additionally, [20] demonstrated the potential of carotenoid bacteria as biomarkers in studies of marine microbial ecology, providing new insights into microbial population dynamics in aquatic ecosystems. With rapid advances in the understanding of bacterial carotenoid biosynthesis, regulation, and applications, this field continues to offer exciting opportunities for research and innovation. The integration of multi-omics approaches, metabolic engineering, and advanced fermentation technologies is expected to unlock more potential for carotenoid bacteria in the future, both in scientific contexts and industrial applications [23][24].

4 Conclusion

4.1 The analyzing photosynthetic bacteria isolated from the Dumai sea waters using the TPC method, it was found that the highest number of bacteria was located in sampling site 2, which is the fish auction, and the lowest number was found in sampling site 6, the Sungai Masjid estuary.

4.2 The analysis using liquid-modified mineral media revealed ten isolates of photosynthetic bacteria from the Dumai waters capable of synthesizing natural carotenoid pigments. It was indicated by a color change in the bacterial media to brown and dark red.

Acknowledgement.

The author would like to gratitude to 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.

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