

Antibacterial Activity and Potential of Natural Textile Dyes from Sea Water Bacteria

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Abstract

This research aimed to determine the potential of bacterial isolates from Marina beach seawater to be used as natural textile dyes and to test their antibacterial ability. The media employed for culturing bacteria was a Zobel synthesis medium with a mixture of seawater. The results of the isolation and extraction of color pigments obtained a red color with a characteristic wavelength of 535 nm and a variety of chemical content results. Pigment immersion trials utilized three fabric types: cotton, primisima, and doobby fabric. The findings of immersion with ethanolic solvent achieved optimum results for 12 hours. With the addition of mordant ($\text{Fe.H}_2\text{O}_4\text{S.7H}_2\text{O}$), the fabric's color was strong enough to withstand washing with detergent and sunlight. From the results of the study, data revealed that the dyed primisima fabric gave a reduction or inhibition of the growth of *E. coli* bacteria by 9%. In comparison, the inhibition of *S. aureus* bacteria was 116%. The pigment could be used as a dye for batik cloth with the provisions of optimizing to find suitable reinforcement or mordant. This study has implications for discovering red pigment-producing bacteria from the isolation of seawater at Marina Semarang beach.

Keywords: Antibacterial; Color resistance; GCMS; Marine bacteria; Natural dyes; Textile dyes.

Introduction

The batik textile industry in Indonesia consists of large, medium, and small industrial scales carried out on a household scale. The extent of the distribution of batik industry players affects social and environmental conditions, including ecosystem conditions (Venil et al., 2021). On a large scale, the batik industry generally has a batik wastewater treatment unit in the form of synthetic dye waste. However, this is different from the case of medium or small industrial scales, where waste is often dumped into the environment (Manzoor & Sharma (2019) stated that Batik textile dye waste could cause environmental pollution

in the form of pungent odors, the death of aquatic species and plants, and the emergence of various diseases that infect humans because dye waste was toxic and carcinogenic (Pujilestari, 2016); (Manzoor & Sharma, 2019). Batik synthetic dye waste contained non-biodegradable materials such as iron (II) sulfate (FeSO_4), aluminum ($\text{K}_2\text{SO}_4\cdot\text{Al}_2(\text{SO}_4)_3\cdot 24\text{H}_2\text{O}$), calcium oxide (CaO), and wax or wax wax (Handayani et al., 2020). The presence of chemicals in synthetic batik dyes had been proven to cause changes in water quality with parameters in the form of levels of BOD, COD, heavy metal content, color, odor, and taste in batik industry centers, for example, in Solo and Yogyakarta (Kusumawati et al.,

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2021). This pollution could be seen from the appearance of the color and smell of water from the Bengawan Solo river (Prajoko, 2018).

The use of synthetic textile dyes was an unavoidable environmental problem due to the limitations of waste processing equipment. Various efforts had been used to reduce textile dye wastewater pollution, for example, by supervising companies to use wastewater treatment plants (Sastrawidana et al., 2015), neutralizing waste that had been scattered in the environment, and reducing the use of synthetic textile dyes (Naimah et al., 2014). Natural textile dyes were one solution that could be applied to reduce environmental pollution. The use of natural dyes could be directly applied in the eco-printing process, namely, printing patterns with natural materials (Enrico, 2019). Natural dyes could be obtained from natural pigment sources, for example, from plants, animals, algae, fungi, and bacteria that could be used in the textile industry and even the food industry (Zulfikar et al., 2017).

Natural pigments produced by microorganisms were still limited to a few primary colors, such as yellow, red, and blue. Based on its existence, blue was a color that was rarely found by microorganisms (L. Dufossé, 2016); (Gupta et al., 2011). Several studies have been carried out to extract natural pigments from microorganisms, including the extraction of red pigment from fungi found in the soil where milk waste is dumped. Natural pigments were constant and robust (Sastrawidana et al., 2015). Extraction of natural pigments from *Serratia marcescens* derived from the digestive system of insects produced three colors that had good resistance to fabric fibers (Venil et

al., 2021). The use of dyes natural products derived from the latest microorganisms has the development of 12 species of microorganisms in the industrial production stage, 12 species of microorganisms in the research development stage, and 12 species of microorganisms in the research stage (Laurent Dufossé, 2018), extraction of natural pink pigment from the remains of the eruption of Mount Vesuvius in dry conditions, high salinity, and moderate lighting conditions were included in the actinobacteria group (Tescari et al., 2018), and natural color studies of microorganisms that produced various pigments such as carotene, melanin, flavin, quinone, prodigiosins, monascin, and violacein or indigo (Gupta et al., 2011).

Research on the extraction of natural color pigments in Indonesia is limited. It is rare for research to produce products in the form of natural color pigments themselves. This triggers researchers to extract pigments from the marine environment of Marina Semarang beach to find various pigments that can be used as alternative natural dyes for making batik and eco-printing that are environmentally friendly and have good resistance.

Material and Methods

This research was descriptive qualitative research conducted in a laboratory (laboratory research) using the Completely Randomized Design (CRD) method. This research is included in descriptive qualitative research that uses numbers, begins to collect data, interpret data, display results and draw conclusions

by analyzing color visuals and descriptive results of GCMS. The research methods include.

Isolation of Bacteria

Isolate samples were obtained from seawater at Marina beach, Semarang city. Water sampling and bacterial isolation were carried out using the method (Setiyono et al., 2020). Water samples were taken from the sea surface, put in a 50 mL sterile plastic tube, and then stored in a container filled with ice. 35 mL of seawater samples were cultured using the Spread Plate method directly on Zobell marine agar (Himedia) in a petri dish and incubated for three days at 35°C. Growing colonies were observed and selected based on different colors.

Pigment Extraction and Purification

Pigment extraction was carried out using the method of Setiyono et al. (2020). Pure colonies from seawater and soil samples were cultured on fresh medium for isolation for 24 hours at 32°C. The Data Analysis Techniques were used to determine the character of seawater bacterial pigments, resistance to detergents, color absorption, resistance to exposure to sunlight, and antibacterial activity. Growing bacterial cells were taken and placed in a 25 mL plastic tube and then precipitated by centrifugation at 10000 rpm for 10 minutes at 4°C. 95% methanol solution was added to the bacterial cells as much as 1 mL/0.1 gram of cells and homogenized using a vortex five times, and then the cells were lysed using sonication. CaCO₃ and sodium ascorbate were added to keep the pigment from oxidizing. The mixture was centrifuged at 20000 g for 5 min at four °C to separate the pigment and cell debris. The pigment extract from the supernatant was taken and dried

using an oven. The dry extract of the pigment was stored at -20°C for further analysis.

Identification of Pigments

The purified pigment solutions were identified using spectrophotometric methods at a wavelength of 200-1100 nm and Gas Chromatography-Mass Spectroscopy (GC-MS) (Venil et al., 2021).

Evaluation of Color Resistance

Cotton cloth with a size of 2 x 2 cm was washed with a solution containing detergent with a concentration of 2 g/l at 50°C for 30 minutes. The cloth was then rinsed with tap water and dried at room temperature. Staining was carried out under optimized conditions, then rinsed with water and dried at room temperature. Color strength was analyzed by spectrophotometry using computer color-matching software. The cloth was treated as an acid solution with a pH of 5, an alkaline solution with a pH of 8, a detergent solution with a detergent-water ratio of 1:1, rubbing, and sunlight for 1 hour. The color resistance of the fabric after treatment was observed qualitatively (Agha et al., 2019).

Data Analysis Techniques

Analysis Techniques used to determine the character of seawater bacterial pigments, resistance to detergents, color absorption, resistance to exposure to sunlight, and antibacterial activity.

Research Results

Pigment Extraction and Purification

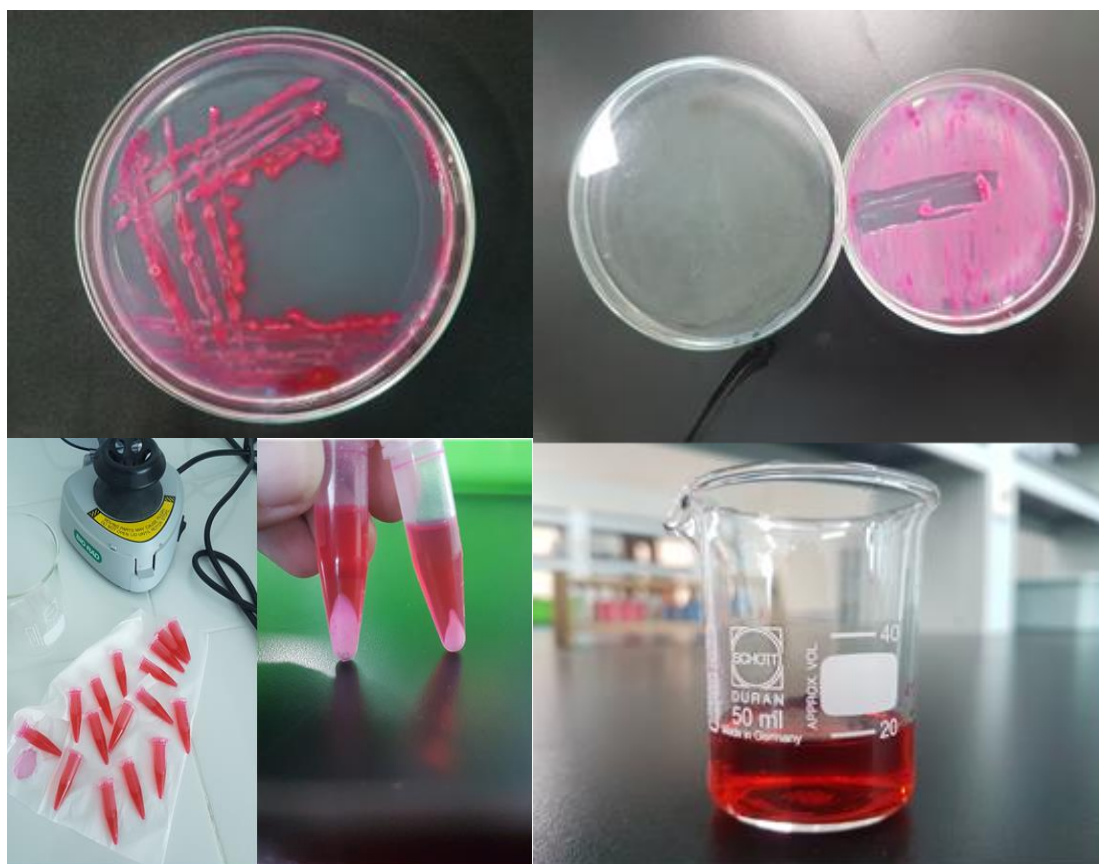
The condition of water samples taken from 3 different points had the same criteria: a water pH of 7, a water temperature 23° C, and the appearance of clear water with the presence of waste fragments and dissolved

algae. The results of the culture of water samples obtained isolates with various colors like red, white, cream, and gray with different shapes of isolate edges. The isolate

pigments that are interesting to be used as natural dyes are isolates with a red pigment. The isolate cultures were then re-cultured on Zobel media to obtain a single isolate.

Figure 1

A. the result of isolation of seawater with Zobel media; B. Harvesting bacteria by scraping the isolates; C, after adding methanol, the homogenization process was carried out; D. The stock of pigments ready to be baked is obtained.



The result of this seawater bacterial pigment extraction process was still in the form of an extract solution mixed with a solvent, so it needed to be evaporated to separate the solvent (95% methanol) from the seawater bacterial pigment extract. The separation process between the solvent and the extract used an oven to get the results of really extract. The evaporated extract was in the form of a dark red jam-like powder. The

spectrophotometer results of the bacterial pigment extract showed that the maximum wavelength was 535 nm. Several wavelength peaks were detected in the spectrophotometric process, 535 nm and 456 nm, with absorbances of 3221 nm and 1335 nm, respectively. The largest absorbance of 3221 was detected at 535 nm, so the maximum wavelength was 535 nm.

Identification of Pigments

The pigment extract obtained in this study had a maximum absorbance value of 535 nm. This absorbance value indicated that the pigment extract measured was prodigiosin, in accordance with the results of previous studies, which showed that prodigiosin had an absorbance peak at 535 nm (De Araujo et al., 2010 & Lins et al., 2014). Identification using the GC-MS method showed that the constituent compounds of the pigment extract consisted of 231 types of compounds. However, prodigiosin was not detected in the pigment extract. This could be caused by the inaccurate GC-MS method, so the results are inappropriate. Optimization of the GC-research of Venil et al. (2021),

The gas MS method needed to be done to detect compounds accurately. The chromatogram of prodigiosin pigment extract showed a peak at 323 m/z. The molecular mass of the prodigiosin pigment from a study by Lapenda et al. (2020) at GC-MS is 323 Da, equivalent to 323 m/z. Silva et al. (2012) also obtained the same thing which showed that the red pigment from *S. marcescens* had a molecular weight of 323 m/z and was characterized as prodigiosin.

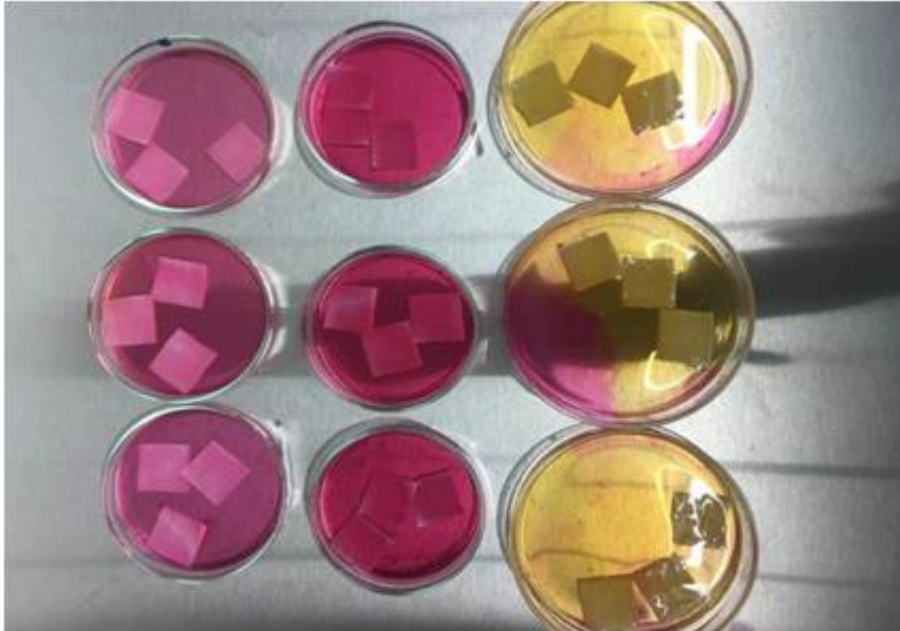
Evaluation of Color Resistance

One factor that affected the strength or absorption of color was the immersion time of the fabric. This research used three fabrics generally used as batik materials: primisima cloth, cotton cloth, and doobby cloth. Selection of the type of fabric was based on the ability of the fabric to absorb dye pigments. The immersion stage was carried out using the extracted material in powder or solid mixed with a solvent (ethanol). The dye solution was manufactured by calculation to obtain the dye concentration at 1000 ppm.

Soaking the fabric was divided into two different time variables to determine the optimum absorption. The time used was 1 hour of immersion and 12 hours of immersion. Each fabric was subjected to a documentation process to determine the absorption capacity. An exciting thing happened to the doobby type of fabric because the originally red pigment solution turned yellow. This happened both in the 1-hour immersion process and the 12-hour immersion stage.

Furthermore, the dye solution was checked for pH so that it was known that the pH on the doobby cloth had increased. This could happen because the process of making doobby cloth, bleaching, aimed to make the cloth whiter. The addition of these chemicals made the pH condition increase. After washing the doobby cloth and soaking it with bacterial pigments, the color of the pigment solution did not turn yellow and remained red.

The results of dyeing pigment immersion showed different results for each fabric material. Primisima fabric had good absorption, especially at 12 hours of soaking time, which was visually clear pink compared to pigment-soaked fabric for 1 hour. Pigment absorption was not only affected by the fabric's absorbency, but the fabric's texture might also influence it. Primisima fabric had a thin texture and a smooth surface. As a control, it was seen that the primisima fabric that was not pigmented had a pure white color. While cloth soaked for 1 hour has a color that tends to fade after drying. Optimization had not been carried out in this immersion process, and no mordant or color enhancer had been added.

Figure 2*Test results on bacterial pigment extract samples*

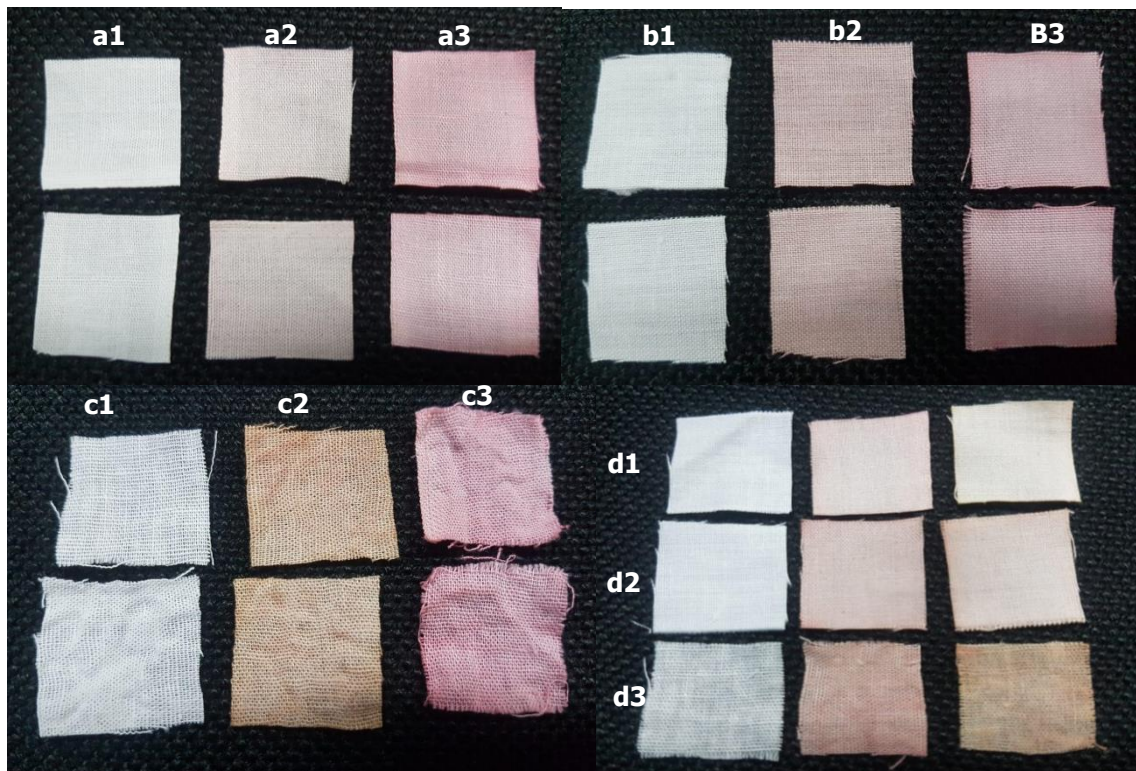
Soaking in cotton cloth had better absorption than primisima. It could be seen from the visual appearance of immersion of pigment for 1-hour that cotton cloth had a pink color which was more visible when compared to primisima cloth which was also soaked for 1 hour (Figure 4.13). While the cloth soaked for 12 hours had almost the same color as the primisima cloth. Dobby fabric had a different texture from cotton and primisima fabrics. The doobby cloth had as well as a large one, and was quite rough. This allowed more pigment absorption to occur. After soaking the pigment for 12 hours, the color that appeared after the dry cloth had a better depth than cotton and primisima fabrics.

An immersion test using detergent determines the extent to which the durability of the pigment was attached to the

fabric material. This test used one color enhancer or mordant, iron (II) sulfate heptahydrate ($\text{Fe} \cdot \text{H}_2\text{O}_4 \cdot 7\text{H}_2\text{O}$). The use of mordant was done to prevent the color of the pigment from being released from the fabric. The research showed that without adding mordant, cotton cloth washed with detergent for 5 minutes experienced color fading. the primisima cloth material, which also experienced fading, and to the doobby cloth. All fabrics used in the detergent test were discolored. The results of the test on resistance to sunlight, After being dried for 2 hours, it was visually compared with the control and cloth samples soaked for 12 hours. The results for primisima and doobby fabrics had the same color as the fabric samples soaked for 12 hours, while the color slightly changed for cotton fabrics. This

Figure 4.

a. Primisima cloth immersion, a1. Primisima method without soaking; a2. Soaking for 1 hour; a3. Immersion for 12 hours; b. Cotton cloth immersion, b1. Cotton method without soaking; b2. Soaking for 1 hour; b3. Immersion for 12 hours; c. Dobby cloth immersion, c1. Dobby method without soaking; c2. Soaking for 1 hour; c3. Immersion for 12 hours; d. Results of drying cloth. d1. Cotton not soaked in color pigments; Pigment soaked cotton 12 hours; Cotton is dried for 2 hours; d2. Primisima is not soaked in color pigments; Primisima is soaked in pigment for 12 hours; Primisima is dried in the sun for 2 hours; d3. Dobby is not soaked in color pigments; Pigment soaked Dobby for 12 hours; Dobby is dried in the sun for 2 hours



The drying test process was essential to determine the durability of the pigment attached to the fabric. Using fabrics with different fibers allowed the absorption of pigments also to differ. Apart from the absorbed color, which had been tested by checking the absorbance of the remaining dye pigment with a spectrophotometer, it turned out that the fabric fiber also had an effect.

Discussion

In the process of isolating bacteria from seawater, the water contained many

fragments of dirt, which were thought to be the result of contamination from surrounding waste. After the culture was successfully grown on Zobel media, the cells were harvested by scraping. Bacterial cell scraping produced results that varied between petri dishes. The thickness of the isolated culture was influenced by the thickness of the Zobel media and the distance between scratches, or the spread was more evenly distributed on the surface of the media, the more and thicker the culture isolates obtained from the tip of the spatula. Scraping was carried out carefully

so that no Zobel media was taken and added to the original weight of the bacterial isolate. After obtaining the results of the pigment stock (Ragunathan et al., 2019).

The pigment extract obtained in this study had a maximum absorbance value of 535 nm. This absorbance value indicated that the pigment extract measured was prodigiosin, in accordance with the results of previous studies, which showed that prodigiosin had an absorbance peak at 535 nm (De Araujo et al., 2010 & Lins et al., 2014). Identification using the GC-MS method showed that the constituent compounds of the pigment extract consisted of 231 types of compounds. However, prodigiosin was not detected in the pigment extract. This could be caused by the inaccurate GC-MS method, so the results were inappropriate—optimization of the GC-MS method needed to be done so that it could detect compounds accurately. In the study of Venil et al. (2021), the Gas chromatogram of prodigiosin pigment extract showed a peak at 323 m/z. The molecular mass of the prodigiosin pigment from a study by Lapenda et al. (2020) at GC-MS is 323 Da, equivalent to 323 m/z. The same thing was obtained by Silva et al. (2012), who showed that the red pigment from *S. marcescens* had a molecular weight of 323 m/z and was characterized as prodigiosin. In their research, Yang et al. (2013) showed that prodigiosin from *Micocystis aeruginosa* had a molecular weight of 323 m/z. In another study by Lin et al. (2019), prodigiosin from *Serratia marcescens* showed a significant peak at a molecular weight of 323.9.

Furthermore, the pigments were tested on three fabrics to determine their absorption and resistance. Of the three types

of fabrics, the most robust absorbing colors were Dobby, Cotton, and Primissima fabrics. The research results showed that the three types of fabrics could be optimized by adding materials that bind color pigments called mordant. Mordant application was a crucial phase in the fixation process by immersion method because for better dye fixation, and natural dyes require metal ions to form insoluble precipitates on the fiber surface. Mordant was a substance added to a textile substrate to change the interaction of dye and fabric fibers to provide better absorption and resistance to fading and subtle discoloration (Yusuf et al., 2017).

Different types of mordant with certain natural dyes can lighten, darken, or change the color of the dye significantly, causing a subtle discoloration of the final result, which was desirable or undesirable depending on the purpose of the stain. Copper sulfate and ferrous sulfate were categorized as mordant. Both copper sulfate and ferrous sulfate were soluble in water and were known as blue and green vitriol, respectively. The effect was observed in discoloration, darkening/browning, and blackening of color shades (Samanta & Konar, 2011). Baking soda and mordant with lemon juice of different colors. This might result from variations in the baking soda's alkalinity and the acidity of the lemon juice. In textile immersion, the mordant and its concentration were essential selection criteria. The mordant could cause an increase in the intensity of the shadow or a significant change in the final dyeing result (Morales-Oyervideset al., 2017).

The use of natural dyes was still quite limited because the suitability of color pigments, fabric materials, and mordant or

color enhancers had yet to be found. However, in addition to this, exposure to sunlight also had an adverse effect on fabrics that use natural dyes. Applying natural dyes for textile dyeing usually involved problems with the dyed textiles' lower fastness properties and limited color range (Morales-Oyervides et al., 2017). The fading of dyed fabrics after exposure to light was an essential issue in the textile industry that needed a solution. When dyed textiles were exposed to visible and ultraviolet light, often in the presence of oxygen and moisture, fading occurs (Forster et al., 2017). Generally, poor light stability occurs in most natural dyes compared to the most effective synthetic dyes. UV radiation's energy caused the dye's brittle bonds to break or re-form. Oxygen and atmospheric moisture also reacted with weak bonds that can change the composition of the dye, which in turn affected its color. The tendency of the dye to fade during washing was reduced because the fabric had formed a more vital bond interaction with the dye (Ahmad et al., 2012).

The pigment extract in this study showed antibacterial activity against the test bacteria, *E. coli*, and *S. Aureus*. Growth inhibition of *S. aureus* was higher than that of *E. coli*, with growth reduction values of 116% and 9%, respectively. The antimicrobial ability of a pigment was closely related to the structure of the pigment, especially the presence of functional groups (Singh et al., 2005). Many studies showed that bacteria have potential for clinical applications. Their pigments have been used in treating several diseases and had specific properties such as antibiotics, anti-cancer, and immunosuppressor compounds. The bacterial genus *Serratia* is

capable of producing a red-colored compound called prodigiosin. This compound had been widely known to have antibiotic and antimalarial activity and immune suppressive activity (Kim et al., 2003).

Another study showed prodigiosin stored in the cellulose matrix effectively removed *E. coli* and *B. cereus* from contaminated water. Recently the induction of autolysins in *Bacillus subtilis* and other *Bacillus* species had been recognized to have a robust antibacterial mechanism. A different study (Alihossein et al., 2008) reported the inhibitory effect of prodigiosin on *E. coli*. On the other hand, several studies showed no effect of prodigiosin on *E. coli* cells. Since the mechanism of action of prodigiosin on *E. coli* is unknown, conflicting data on the antibacterial action of prodigiosin was challenging to find a precise solution. As several ecophysiological roles of prodigiosin had been proposed for bacteria, such as airborne bacterial dispersal upstream metabolic for NAD(P)H or proline, light energy storage, anion exchange, energy spill function, and UV protection (Boric et al., 2011), there were possibilities that antimicrobial activity was not a result of prodigiosin targeting single cells, but may, in turn, had a pleiotropic effect on *E. coli*. Many antimicrobial agents were known to have multiple effects on microorganisms (Jenssen et al., 2006).

Prodigiosin is a secondary metabolite compound with no apparent function in the pigment-producing cells. Some physiological functions that prodigiosin pigments might have were host protection or pigment production from environmental stresses and facilitating the spread of bacteria in their ecological environment (Song et al., 2006). Another possible physiological role was in

an interspecies competition where prodigiosin inhibited the growth of a broad spectrum of gram-positive and gram-negative bacteria living in similar environments (Ibrahim et al., 2014).

Conclusions

Based on the series of activities in the research program, it is concluded that the results of the isolation of seawater from the marina beach obtained isolates with red pigment. Based on the endurance test, the extraction of marine bacteria can be used as an alternative to natural textile dyes by optimizing the strengthening of the dye or mordant and having antibacterial activity.

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