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

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Abstract

This research aims 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 used for culturing bacteria is 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 has a variety of chemical content results. Pigment immersion trials using 3 types of fabric, cotton, primisima and dobby fabric. The results of immersion with ethanolic solvent obtained optimum results for 12 hours and with the addition of mordant (Fe.H2O4S.7H2O), the color of the fabric is strong enough to withstand washing with detergent and sunlight. From the results of the study, data showed that the dyed primisima fabric gave a reduction or inhibition of the growth of *E. coli* bacteria by 9% while the inhibition of *S. aureus* bacteria was 116%. The pigment has the potential to be used as a dye for batik cloth with the provisions of optimizing to find the right reinforcement or mordant. This study has implications for the discovery of 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 industrial scale, medium industrial scale and also small industrial scale which is carried out on a household scale. The extent of distribution of batik industry players affects social and environmental conditions, including ecosystem conditions (Venil et al., 2021). The batik industry on a large scale 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). Batik textile dye waste can 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 are toxic and carcinogenic (Pujilestari, 2016); (Manzoor & Sharma, 2019).

Batik synthetic dye waste contains non-biodegradable materials such as iron (II) sulfate (FeSO4), aluminum (K2SO4.Al2 (SO4)3.24H2O), calcium oxide (CaO), and wax or wax wax (Handayani et al., 2020). The presence of chemicals in synthetic batik

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dyes has 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., 2021). This pollution can clearly 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 is an unavoidable problem for the environment due to the limitations of waste processing equipment. Various efforts have 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 has been scattered in the environment and reducing the use of textile synthetic dyes (Naimah et al., 2014). Natural textile dyes are one solution that can be applied to reduce environmental pollution. The use of natural dyes can be directly applied in the ecoprinting process, namely printing patterns with natural materials (Enrico, 2019). Natural dyes can be obtained from natural pigment sources, for example, from plants, animals, algae, fungi, and bacteria that can be used in the textile industry and even the food industry (Zulfikar et al., 2017).

Natural pigments produced by microorganisms are currently still limited to a few main colors, for example, yellow, red and blue. Based on its existence, blue is the color that is 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 found to be constant and strong (Sastrawidana et al., 2015), extraction of natural pigments from Serratia marcescens derived from the digestive system of insects produced 3 colors that have good resistance to fabric fibers (Venil et al., 2021), the use of dyes natural products derived from the latest microorganisms have 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 are included in the actinobacteria group (Tescari et al., 2018), and natural color studies of microorganisms that produce 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. In fact, it is rare for research to produce products in the form of natural color pigments themselves. This makes researchers want to carry out the process of extracting pigments obtained from the marine environment of Marina Semarang beach in an effort to find various pigments that have the potential to be used as alternative natural dyes for making batik and ecoprinting that are environmentally friendly and have good resistance.

Material and Methods

This research is a 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, descriptive results of GCMS. The research methods include.

Isolation of Bacteria

Isolate samples were obtained from sea water 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 and put in a 50 mL sterile plastic tube and then stored in a container filled with ice. A total of 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 3 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 as used for isolation for 24 hours at 32°C. The data analysis techniques 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 min 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 5 times and then the cells were lysed using sonication. $CaCO_3$ and sodium ascorbate are added to keep the pigment from oxidizing. The mixture was then centrifuged at 20000 g for 5 min at 4°C to separate the pigment and cell debris. The pigment extract in the form of 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

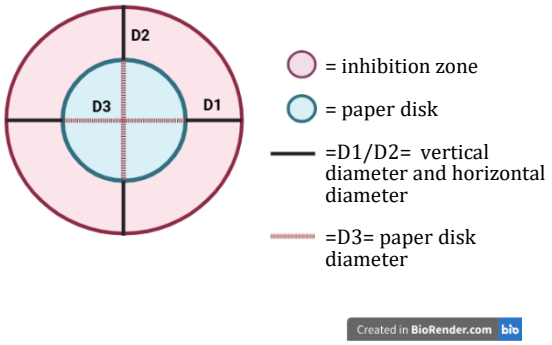
Cotton cloth with a size of 2 x 2 Cm was washed with a solution containing detergent with a concentration of 2 g/l at 50°C for 30 minutes. The cloth was then rinsed with tap water and dried at room temperature. Staining was carried out under optimized conditions, then rinsed with water and dried at room temperature. Color strength was analyzed by spectrophotometry using computer color matching software. The cloth was treated in the form of 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).

Antibacterial activity

Antibacterial activity was evaluated using the Kirby–Bauer disk diffusion assay on Mueller–Hinton agar. Briefly, bacterial suspensions were grown to mid-log phase, adjusted to a 0.5 McFarland standard (~1–2 × 10^8 CFU/mL), and lawn-inoculated with a sterile swab. Sterile 6 mm paper disks were loaded with 10 µL of the test solution at the indicated concentrations; dimethyl sulfoxide (DMSO) served as the vehicle negative control, and a chloramphenicol disk (30 µg) was used as the positive control. Plates were allowed to pre-diffuse for 10–15 min at room temperature, then incubated inverted at 35 ± 2 °C for 16–18 h. Inhibition zones were measured in millimeters with a digital caliper and reported as mean ± SD from ≥3 independent experiments; where relevant, qualitative susceptibility was interpreted against accepted breakpoints. This procedure follows recent implementations of Kirby–Bauer methodology reported in the literature (e.g., Mojica et al., 2020, Journal of Clinical Microbiology).

Figure 1

Measurement of Inhibition Zone



$$\text{Inhibition Zone} = \frac{(D1 - D3)(D2 - D3)}{2}$$

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.

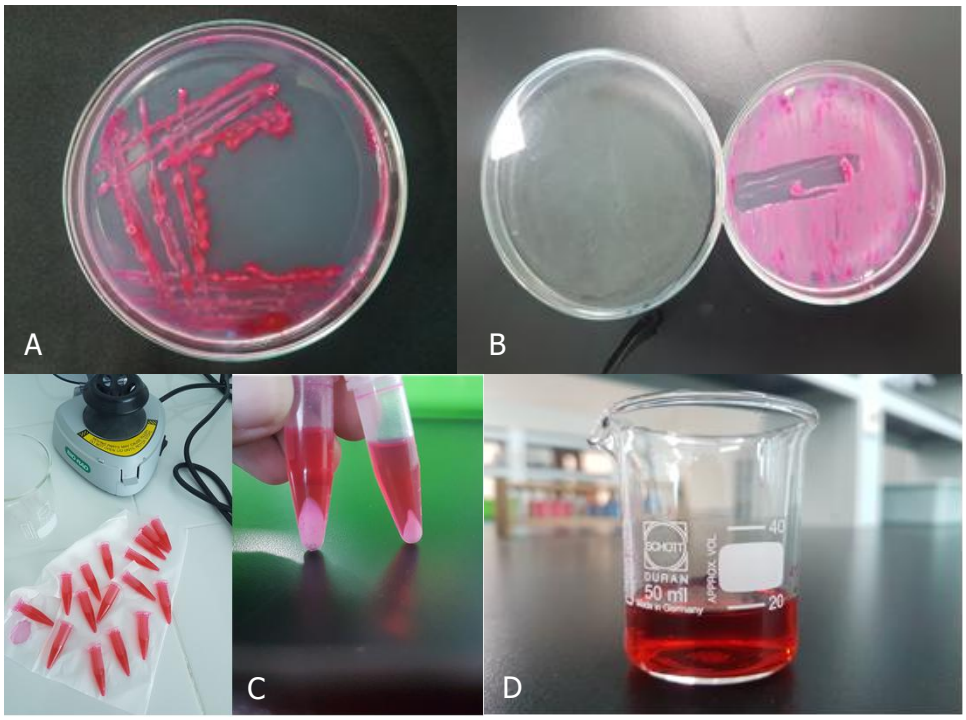
Research Results

Pigment Extraction and Purification

The condition of water samples taken from 3 different points had the same criteria, namely having a water pH of 7, water temperature 23° C, 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 that have a red pigment. The isolate cultures were then re-cultured on Zobel media to obtain a single isolate.

Figure 2

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 is still in the form of an extract solution mixed with a solvent, so it needs to be evaporated to separate the solvent (95% methanol) from the sea water bacterial pigment extract. The separation process between the solvent and the extract uses an oven to get the results really extract. The evaporated extract is 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 a wavelength of 535 nm so that 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 indicates that the pigment extract measured is prodigiosin, in accordance with the results of previous studies which showed that prodigiosin has 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 can be caused by the inaccurate GC-MS method so that the results obtained are not appropriate. Optimization of the GC-research of Venil et al. (2021),

Gas MS method needs to be done so that it can detect compounds accurately. In 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 which is equivalent to 323 m/z. The same thing was also 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.

Evaluation of Color Resistance

One of the factors that affect the strength or absorption of color is the immersion time of the fabric. This research uses 3 types of fabrics that are generally used as batik materials, including primisima cloth, cotton cloth and dobby cloth. Selection of the type of fabric is based on the ability of the fabric to absorb dye pigments. The immersion stage is carried out using the extracted material in the form of powder or solid mixed with a solvent (ethanol). The manufacture of the dye solution was carried out by calculation so that the dye concentration was obtained 1000 ppm.

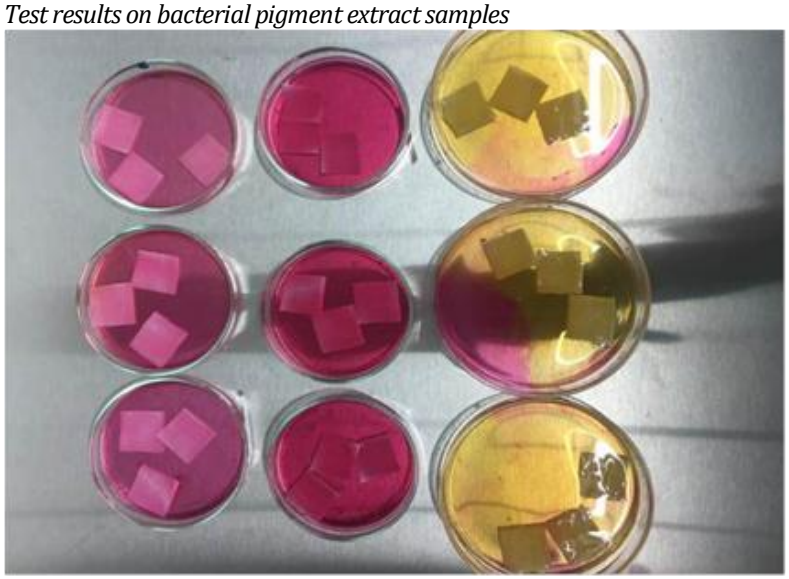
The process of soaking the fabric is divided into 2 different time variables to determine the optimum absorption. The time used is 1 hour of immersion and 12 hours of immersion. Each fabric is subjected to a documentation process to determine the absorption capacity. An interesting thing happened to the dobby type of fabric because the pigment solution which was originally red turned yellow. This happened both in the 1 hour immersion process and the 12 hour immersion stage. Furthermore, the dye solution is checked for pH so that it is known that the pH on the dobby cloth has increased. This can happen because during the process of making dobby cloth, bleaching which aims to make the cloth whiter. The addition of these chemicals made the pH condition increase, after washing the dobby

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 from each fabric material. Primisima fabric has good absorption, especially at 12 hours soaking time, which is visually clear pink in color when compared to pigment soaked fabric for 1 hour. Pigment absorption is not only affected by the absorbency of the fabric, but it may also be influenced by the texture of the fabric. Primisima fabric has a thin texture and has a smooth surface. As a control, it was seen that the primisima fabric

Figure 3

Test results on bacterial pigment extract samples



that was not pigmented had a clean white color. While cloth soaked for 1 hour has a color that tends to fade after drying. In this immersion process, optimization has not been carried out and no mordant or color enhancer has been added.

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

Immersion test using detergent serves to determine the extent to which the durability of the pigment attached to the fabric material. This test uses one kind of color enhancer or mordant, namely iron (II) sulfate heptahydrate ($Fe.H_2O_4S.7H_2O$). The use of mordant is done to prevent the color of the pigment from being released from the fabric. The use of mordant still has to be done through optimization efforts so that the color remains intact. Based on the research, it is known that without the addition of mordant, cotton cloth washed with detergent for 5 minutes experienced color

fading. The same thing happened to the primisima cloth material which also experienced fading as well as to the dobby 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 dobby fabrics have the same color as the fabric samples soaked for 12 hours, while for cotton fabrics the color slightly changes.

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

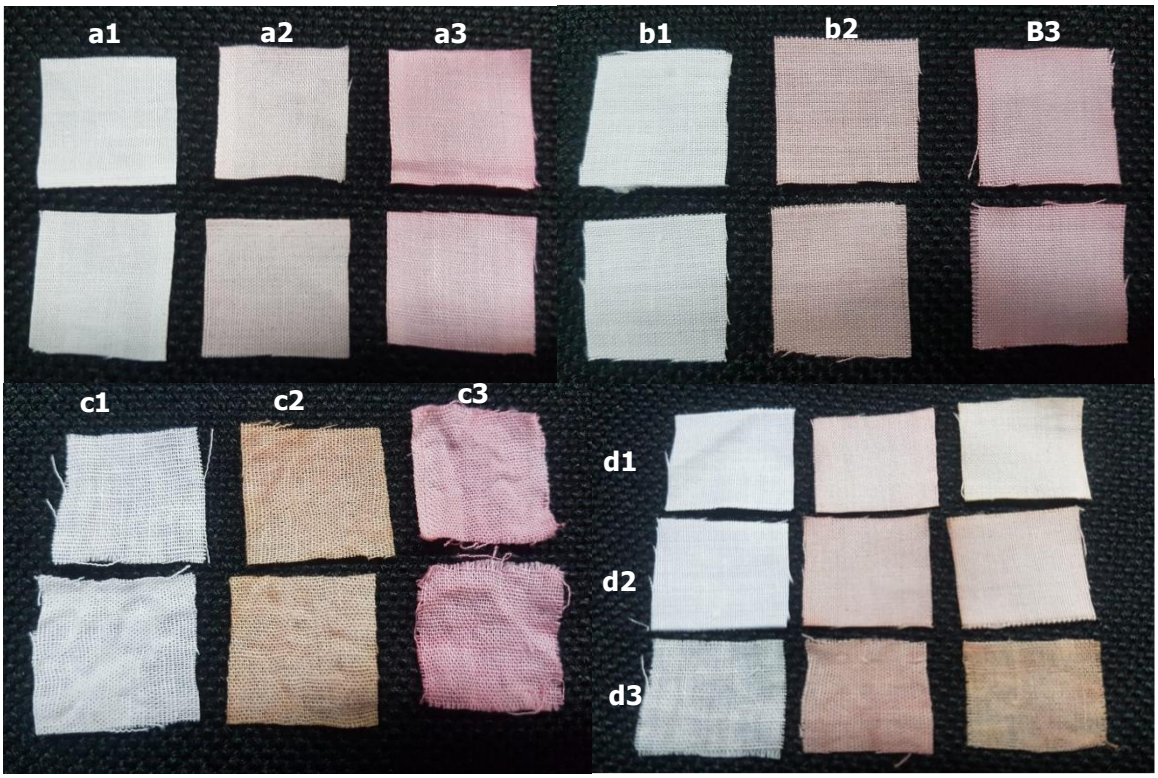


Table 1. Antibacterial Activity Test Results of Pigment Extract from Marine Bacteria

Bacteria	Treatment	Rep 1 (mm)	Rep 2 (mm)	Rep 3 (mm)	Mean (mm)
E. coli	Pigmen extract from Sea Bacteria	7	8	6	7±1
E. coli	Chloramphenicol (positive control)	24	25	23	24±1
E. coli	DMSO (negative control)	0	0	0	0
S. aureus	Pigmen extract from Sea Bacteria	26	27	25	26±1
S. aureus	Chloramphenicol (positive control)	23	22	24	23±1
S. aureus	DMSO (negative control)	0	0	0	0

This drying test process is important to do to determine the durability of the pigment attached to the fabric, the use of fabrics that have different fibers allows the absorption of pigments to also differ. Apart from the absorbed color which has been tested by checking the absorbance of the remaining dye pigment with a spectrophotometer, it turns out that the fabric fiber also has an effect.

Discussion

In the process of isolating bacteria from seawater, the water taken contains many fragments of dirt which are 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 produces results that vary between petri dishes. The thickness of the isolate 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 indicates that the pigment extract measured is prodigiosin, in accordance with the results of previous studies which showed that prodigiosin has 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 can be caused by the inaccurate GC-MS method so that the results obtained are not appropriate. Optimization of the GC-MS method needs to be done so that it can detect compounds accurately. In the study of Venil et al. (2021), 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 which is 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. Yang et al. (2013) in his research 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 major peak at a molecular weight of 323.9.

Furthermore, the pigments were tested on 3 types of fabrics to determine their absorption and resistance. Of the three types of fabrics, the strongest absorbing colors are Dobby, Cotton, and Primisima fabrics. Based on the research results, it is known that the three types of fabrics can be optimized by adding materials that can bind color pigments called mordant. Mordant application is a necessary phase in the fixation process by immersion method, because for better dye fixation, natural dyes require metal ions to form insoluble precipitates on the fiber surface. Mordant is a substance added to a textile substrate to change the interaction of dye and fabric fibers to provide better absorption, better

resistance to fading, and/or subtle discoloration (Yusuf et al., 2017).

The use of 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 is desirable or undesirable depending on the purpose of the stain. Copper sulfate and ferrous sulfate are categorized as mordant. Both copper sulfate and ferrous sulfate are soluble in water and are known as blue and green vitriol, respectively. The effect is observed in discoloration, darkening/ browning and blackening of color shades (Samanta & Konar, 2011). Baking soda and mordant with lemon juice of different colors. This may be as a result of variations in the alkalinity of the baking soda and the acidity of the lemon juice. In textile immersion, both the type of mordant and its concentration are important selection criteria, because the mordant can 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 is still quite limited because the suitability of color pigments, fabric materials and mordant or color enhancers has not been found. However, in addition to this, exposure to sunlight also has an adverse effect on fabrics that use natural dyes. Applying natural dyes for textile dyeing usually involves problems with the lower fastness properties and limited color range of the dyed textiles (Morales-Oyervides et al., 2017). The fading of dyed fabrics after exposure to light is an important issue in the textile industry that needs a solution. When dyed textiles are 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. The energy of UV radiation causes the brittle bonds of the dye to break or re-form. Oxygen and atmospheric moisture also react with weak bonds that can change the composition of the dye, which in turn affects its color. The tendency of the dye to fade during washing is reduced because the fabric has formed a stronger 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 pigment extract stopped both bacteria from growing, but it worked much better on *S. aureus* than on *E. coli.* Inhibition zone were large for *S. aureus* (26 mm) and small for *E. coli* (7 mm). Chloramphenicol made big rings (23–24 mm); DMSO showed none. Averages from three tests (disk included).

The antimicrobial ability of a pigment is closely related to the structure of the pigment, especially the presence of functional groups (Singh et al., 2005). Many studies show that bacteria have potential in clinical applications and their pigments have been used in treating several diseases and have certain properties such as antibiotics, anti-cancer and immunosuppressor compounds. The bacterial genus *Serratia* capable of producing a red colored compound called prodigiosin. This compound has been widely known to have antibiotic and antimalarial activity as well as immune suppressive activity (Kim et al., 2003).

Another study showed that prodigiosin stored in the cellulose matrix was effective in removing *E. coli* and *B. cereus* from contaminated water. Recently the induction

of autolysins in *Bacillus subtilis* and other *Bacillus* species has been recognized to have a strong 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 is difficult to find a precise solution. As several ecopysiolo-roles of prodigiosin have 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 are possibilities that antimicrobial activity is not a result of prodigiosin targeting single cells, but may in turn have a pleiotropic effect on *E. coli*. Many antimicrobial agents are known to have multiple effects on microorganisms (Jenssen et al., 2006).

Prodigiosin is a secondary metabolite compound that has no clear function in the pigment-producing cells. Some of the physiological functions that prodigiosin pigments may have are host protection or pigment production from environmental stresses and/or facilitating the spread of bacteria in their ecological environment (Song et al., 2006). Another possible physiological role is in interspecies competition where prodigiosin inhibits the growth of a fairly 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 was 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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