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Diffusible Pigment of Actinomycete as A Source of Textile Dye

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ABSTRACT

Natural dyes from microorganisms offers as an alternative to synthetic dyes due to the negative impacts caused by synthetic dyes to the environment. This study focused on the ethyl extraction of blue pigment from actinomycete, *Micromonospora sp.* K7-06 previously isolated from Kuantan mangrove forest. *Micromonospora sp.* K7-06 was found to produce blue diffusible pigment when cultured on ISP4. Several solvents were used to extract the blue pigment namely, distilled water, ethanol, methanol, dichloromethane (DCM), ethyl acetate and hexane. Optimum extraction of the blue pigment was achieved when using distilled water. The pigment obtained was characterized using UV-Spectrophotometer and FTIR Spectroscopy. The blue pigment has λ_{max} of 263 nm and four functional groups i.e. amines, nitriles, nitro, ester and ether identified through FTIR spectroscopy. Cotton and wool were used to assess the blue pigment potential as textile dye. The blue pigment showed textile dyeing ability as it was able to withstand for three consecutive normal wash treatment. Agar well diffusion method was used to evaluate the antibacterial potential against *S. aureus* and *B. cereus*. No inhibition zone was observed indicating that the pigment does not have an antibacterial property against *S. aureus* and *B. cereus*. Nevertheless, the blue pigment has textile dye potential due to its retentive property.

Keywords: *Micromonospora sp.* K7-06, natural dye, textile, blue pigment, antibacterial

ABSTRAK

Pewarna semulajadi daripada mikroorganisma adalah sebagai pilihan alternatif kepada pewarna sintetik disebabkan impak negatif pewarna sintetik terhadap persekitaran. Kajian ini memfokuskan pengekstrakan pigment biru menggunakan etil asetat daripada aktinomiset Micromonospora sp. K7-06 yang dipencilkan dari hutan paya bakau di Kuantan. Micromonospora sp. K7-06 didapati menghasilkan pigmen biru apabila dikultur di atas ISP4. Beberapa pelarut iaitu air suling, etanol, metanol, diklorometana (DCM), etil asetat dan heksana digunakan untuk mengekstrak pigmen biru. Pengekstrakan pigmen biru secara optimum diperolehi dengan menggunakan air suling dan diikuti dengan pencirian menggunakan spektrometer UV dan spektroskopi FTIR. Pigmen biru tersebut mempunyai λmax pada 263 nm dan terdapat empat kumpulan berfungsi iaitu amina, nitril, nitro, ester dan eter yang dikenalpasti melalui spektroskopi FTIR. Kapas dan wul digunakan untuk menguji kobolehupayaan pigmen biru sebagai pewarna tekstil. Pigmen biru didapati menunjukkan kebolehupayaan sebagai pewarna tekstil kerana ketahanannya terhadap perawatan tiga kali cucian normal secara berturut-turut. Kaedah telaga resapan agar digunakan untuk menilai potensi antibakteria terhadap S. aureus dan B. cereus. Tiada zon perencatan diperolehi dan ini menunjukkan pigmen biru tersebut tidak bersifat antibakteria terhadap S. aureus dan B. cereus. Walau bagaimanapun, pigmen biru mempunyai potensi sebagai pewarna tekstil berdasarkan sifat muatan tahanannya.

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Kata Kunci: *Micromonospora sp.* K7-06, pewarna semulajadi, tekstil, pigmen biru, anti-bakteria

1. INTRODUCTION

Conventional sources of pigments range from inorganic metals and metal oxides to organic molecule. Over the course of the 20th century, naturally occurring organic pigments have been almost completely displaced by synthetic molecules. The discovery of synthetic dyes by Perkins in 1856 has provided a wide range of dyes that are colour fast and come in a wider colour range and brighter shades. Most of textile industry nowadays used synthetic dyes rather than natural dyes. However, textile dyeing and finishing industry has created a huge pollution problem as it is one of the most chemically intensive industries on earth [1].

Serious environmental and safety problems caused by synthetic pigments have triggered intense research on the green production of safe natural pigments from natural resources in the food, textile, and pharmaceutical industries [2]. Microbial dyes are environmentally friendly and non-toxic, and they are a good natural alternative to synthetic dyes in foods, medicines, textiles and cosmetics. Moreover, they are easily cultured coupled with high stability of the pigments produced make them more ideal candidate for natural pigments over plants pigments. Examples of microbial pigments includes carotenoids, astaxanthin, canthaxanthin, flavins and quinones [3].

Actinomycetes are Gram positive fungus like bacteria, and they are known for their potential in producing compounds with wide biological activities. Genus *Streptomyces* alone accounted for ~70% of the currently available antibiotics. Actinomycetes also synthesize and excrete dark pigments, melanin or melanoid, which are considered to be a useful criterion for taxonomical studies [4]. Other colours that usually produced by actinomycetes are blue, purple, pink, black, green, and brown. Although, actinomycetes produced many pigments, extraction of pigment from actinomycetes on an industrial level is not much practiced due to the unavailability of protocols for large scale fermentation and downstream processing [5]. Genus *Micromonospora* have been recognized as one of the most important sources of new antibiotics [6]. Members of *Micromonospora* genus are widely distributed in diverse geographical habitats – freshwater, marine ecosystem, plants. *Micromonospora* K7-06 used in this study was previously isolated from Kuantan mangrove forest [7]. This isolate was found to produce blue diffusible when growing on ISP4. Hence, this study attempts to extract the blue diffusible pigment released by *Micromonospora* K7-06 followed by cotton and wool dyeing to assess its potential as textile dye. Antibacterial activity of this pigment against *S. aureus* and *B. cereus* was also evaluated in this study.

2. MATERIAL AND METHODS

2.1 Pigment Extraction from *Micromonospora* K7-06

Micromonospora K7-06 was cultured on ISP4 agar and incubated at 28-30^{o}C for 7-14 days. Several solvents namely ethanol., methanol ethyl acetate DCM, distilled water, hexane, and butanol were used in this study to determine which solvent is the best to extract the blue diffusible pigment. Spent agar was extracted three times with each solvent followed by evaporation to dryness using rotary evaporator.

2.2 Characterization of Pigment Using UV-Vis and FTIR Spectroscopy

The blue pigment extracted was characterized using spectrophotometer was scanned at 200-800 nm. The functional groups present in the pigment was determined using FTIR spectroscopy.

2.4 Textile Dyeing

Wools and cotton as substrates for textile dyeing using the extracted blue pigment. In pigments dyeing, insoluble colorant is fixed on the fibre surface using binders. In this study, 5% (w/v) of ferrous sulphate and copper sulphate were used as premordant prior to dyeing. Next, the samples (wools and cotton) were thoroughly washed with warm distilled water, rinsed with tap water and air dried. Textile dyeing without the addition of premordant was also performed. The samples were treated with three consecutive normal wash to observe the dyeing properties.

2.5 Antibacterial Test

Agar well diffusion was used in this experiment. Four wells were made on Mueller Hinton Agar using sterile borer and sterile cotton swab was used to inoculate the agar surface with *S. aureus* and *B. cereus.* About 50 µl of blue pigment extract (1mg/ml) was added into the well followed by incubation at 37ºC for 24 hours. Sterile nutrient broth was used as negative control while chloramphenicol (10µg) was used as control positive. Experiment was conducted in triplicate and a positive result of antimicrobial test is depicted by a clear zone of inhibition surrounding the well.

3. RESULTS AND DISCUSSION

Micromonospora K7-06

At 5 days of incubation period, *Micromonospora* K7-06 formed orange colonies and as they aged, black spores formed on the colony surface (Figure 1). Blue diffusible pigment was produced after day 8 and harvested after day 14. The production of diffusible pigment in actinomycetes are useful for taxonomical studies, but some coloured diffusible pigments are related to the production of antibiotics. For example, actinomycin, a yellow-coloured antibiotic is released into the agar by certain strains of *Streptomyces*. Many factors influence the production of diffusible pigment - pH, media composition, salinity, temperature etc. Previous study showed that *Micromonospora* K7-06 produced blue diffusible pigment when growing on ISP4, Starch yeast extract (SYE) agar, Starch casein agar (SCA) but not in Nutrient agar [8].

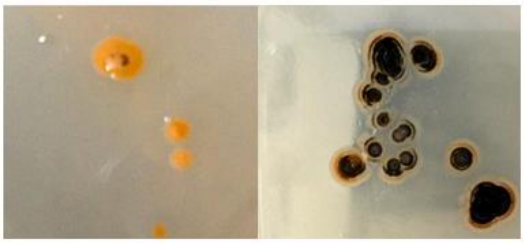

Figure 1*: Micromonospora* K7-06 colonies at 5 days (A) and 8 days (B)

Characterisation of Blue Pigment

Several solvents were used to extract the blue pigment based on their polarity. Distilled water, ethanol, methanol, dichloromethane, ethyl acetate are polar solvents while butanol and hexane are non-

polar solvents. Extraction of the blue pigment using organic solvents (polar and non-polar) was found to be unsuccessful. Warm distilled water showed to be the best solvent to extract the blue pigment. λ_{max} of the blue pigment was determined at 263 nm and four functional groups – amines, nitriles, nitro and ester and ether characterized by FTIR as depicted in Figure 2.

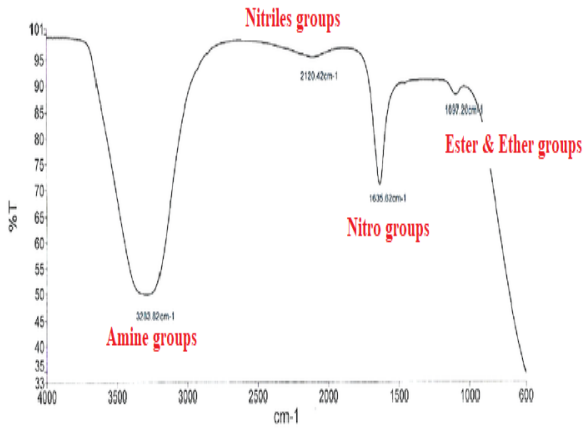

Figure 2: FTIR characterization of blue pigment

Textile Dyeing of the Blue Pigment

Cotton and wools were selected for to assess the textile dyeing capacity of the blue pigment. The colouring capacity of the extracted pigment was determined after three consecutive normal water wash treatment. The results showed that the color imparted was retentive for both materials as the colour remained after three treatments of normal wash, though the blue colour imparted was not vibrant and dark as expected (Figure 3). Any sort of dye requires a fixative or a mordant, which helps in the attachment of the dye to the material. The blue pigment failed to retain after first normal wash when premordant was not included. In another similar study, pink pigment of actinomycete did not require any fixative to incorporate the colours on the materials. Different pigment may have different set of compounds which will influence its dyeing capability [9].

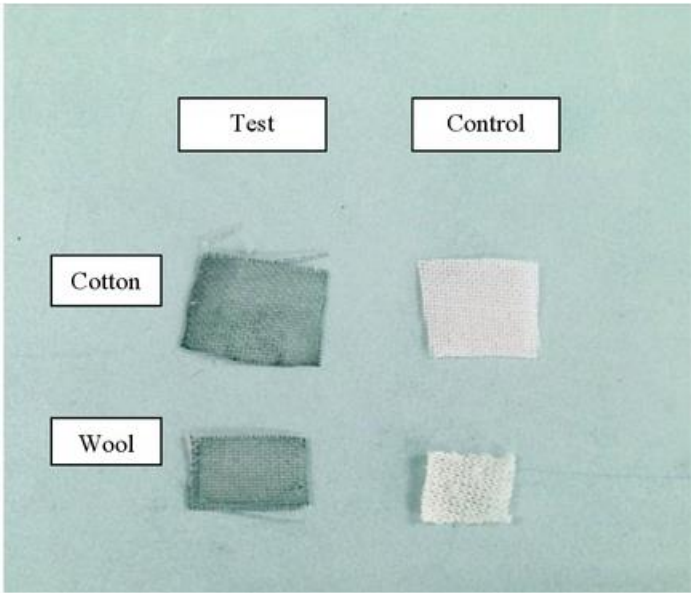

Figure 3: Textile dyeing of the blue pigment from *Micromonospora sp.* K7-06

Evaluation of Antibacterial Activity of the Blue Pigment

The blue pigment of *Micromonospora* K7-06 did not demonstrate any antibacterial activities against *S. aureus* and *B. cereus* as no inhibition zones were recorded in all triplicates. Genus *Micromonospora* is next to the genus *Streptomyces* in terms in superiority in producing antibiotics. Moreover, actinomycetes are usually more active against Gram positive bacteria than Gram negatives. *S. aureus* and *B. cereus* are both Gram-positive bacteria, but blue pigment failed to exhibit any antibacterial activity. It is important to note that not all

diffusible pigments exhibit antimicrobial property. Some of the pigments are useful for taxonomical characteristic only. Pigment of various colours are synthesized to protect the cells of microorganisms from injurious effect of light rays of visible and near ultraviolet range [10]. Nevertheless, pigments produced by actinomycetes showed potential to be explored and exploited as natural dyes to be utilized in many applications including food colouring, textile dyeing and others.

4. CONCLUSION

Findings of this study indicated the potential of blue diffusible pigment produced by *Micromonospora* K7-06 as natural colorant for textile dyeing. Further studies especially on the optimization of dyeing capacity is required to ensure strong retentive of the pigment on textiles. This study also demonstrated that natural pigments from bacteria particularly actinomycetes could be exploited for colorants resources that can be used for many applications.

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