Faculty of Resource Science and Technology

Isolation and Characterization of Pigmented Bacteria for Dye Sensitized Solar Cell Application

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(Resource Biotechnology)
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Isolation and Characterization of Pigmented Bacteria for Dye Sensitized Solar Cell Application

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A final project report submitted in partial fulfillment of the requirement for the degree of
Bachelor of Science with Honours
(Resource Biotechnology)

Supervisor: Dr. Azham Zulkharnain

Department of Molecular Biology
Faculty of Resource Science and Technology
University Malaysia Sarawak
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ACKNOLEDGMENT

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LIST OF ABBREVIATIONS

DSSC	Dye sensitized solar cell
TiO_2	Titanium dioxide
SEI	Semiconductor electrolyte interface
UV	Ultraviolet rays
TLC	Thin layer chromatography
FTIR	Fourier transform infrared spectroscopy
NMR	Nuclear magnetic resonance spectroscopy
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
EDTA	Ethylenediaminetetra-acetate
CTAB	Centyl trimethy ammonium bromide
SDS	Sodium dodecyl sulphate
KOH	Potassium hydroxide
PBS	Phosphate buffered saline
SnO_2	Tin (IV) oxide
ZnO	Zinc oxide
SEM	Scanning electron microscopy
Nacl	Sodium chloride
PCI	Phenol chloroform isoamyl alcohol
CI	chloroform isoamyl alcohol
TE	Tris ethylenediaminetetra-acetate
EtBr	Ethidium bromide
DCM	Dichloromethane
FTO	Flourine doped Tin oxide

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ABSTRACT

Presently, the burning of fuel for electricity production contributes greatly to global warming and hence the need for clean, renewable source of electricity has never been greater. Dye sensitized solar cells (DSSCs) are amongst the newest and promising technologies that have great potential to be an alternative for the development of a new generation of photovoltaic devices. Bacterial pigments due to their biodegradability and higher compatibility with the environment offer promising avenues for various applications such as electricity production. In this project, natural bacterial pigments were applied as sensitizers in the DSSCs and their performances were studied. Pigment producing bacteria species such as *Serratia maercescens, Chromobacterium violaceum* and *Bacillus cibi* were isolated and their pigments extracted and characterized prior to their application in the DSSCs.

Key words: Dye sensitized solar cells (DSSCs); Photovoltaic devices; Pigment producing bacteria

ABSTRAK

Pada masa kini, pembakaran bahan api untuk tujuan pengeluaran elektrik banyak menyumbang kepada pemanasan global. Bukan itu sahaja, malah hal ini adalah sukar bagi memperoleh sumber tenaga elektrik yang bersih dan boleh diperbaharui. Pewarna sensitif sel solar (PSSS) merupakan satu teknologi yang terbaru dan menjanjikan potensi besar untuk menjadi alternatif peranti fotovoltaik kepada pembangunan generasi baru. Oleh kerana ciri-ciri pigmen bakteria yang biodegradasi serta mempunyai keserasian yang lebih tinggi dengan persekitaran, ia menawarkan peluang yang cerah dalam pelbagai aplikasi pengeluaran elektrik. Dalam kajian ini, pigmen bakteria semula jadi telah digunakan sebagai zat memeka dalam PSSS dan prestasi bakteria dikaji. Bakteria penghasilan pigmen seperti *Serratia maercescens, Chromobacterium violaceum* dan *Bacillus cibi* telah diasing dan pigmen yang diekstrak tersebut telah dikenalpasti sebelum diaplikasikan dalam PSSS.

Kata kunci: Pewarna sensitif sel solar (PSSS); Peranti fotovoltaik; Bakteria penghasilan pigmen

I. INTRODUCTION

Consumption of energy is said to be at its highest peak in the 21st century. As the population of the world is increasing rapidly, the demand for energy in the form of electricity has sky rocketed. Many developing countries lack adequate electricity supply and the main source of electricity which is obtained from fossil fuels is an exhaustible resource which will not last long looking at the current rate of consumption. Currently, burning of fuel for the production of electricity has contributed greatly in global warming and hence the need for clean, renewable source of electricity has never been greater. It has been accepted that fossil fuel combustion is responsible for much of the increase in atmospheric CO_2 concentration above the preindustrial values and that this increased CO_2 level results in an increased long wave (infrared) radiative forcing of the troposphere that leads to a warming of the earth's surface (Schwartz, 1993). Therefore, Scientists and concerned environmentalists have been looking for alternative clean resources of electricity with the aim to substitute fossil fuels.

Many technologies have been developed with the aim of substituting fossil fuels but there still exists a great room left for improvement. Solar cells have been developed and are currently in use worldwide. However the use of solar cells has not been increasing as expected mainly due to the high cost of manufacturing associated with silicon based solar cells. As the result researchers have been looking into alternative solar cells such as the Dye Sensitized Solar Cell. Dye Sensitized Solar Cell (DSSC) is amongst the newly developed technologies that have showed a great potential for future use. They are a promising alternative for the development of a new generation of photovoltaic devices. DSSC is the only solar cell that can offer both flexibility and transparency with its efficiency being comparable to amorphous silicon solar cells but with a much lower cost

(Wei, 2010). They are third generation solar cells developed by O' Regan and Gratzell in 1991. DSSCs convert inexpensive photon from solar energy to electrical energy based on sensitization of wide band gaps semiconductor dyes and electrolytes (Kimpa et al., 2012).

DSSCs involve several subsystems which work in tandem, interlaced with the adsorbed dye on a semiconductor surface. These dyes on the semiconductor surface are responsible for the adsorption of the visible near-IR photons and the electrons are pumped into the conduction band of the semiconductor which is the electron-mediator for the whole conduction and the counter electrode catalytic material. This movement of electrons from one band to another is associated with the production of electricity. Naturally occurring pigments have been reported to be capable of serving as electron mediators since they can absorb light in the wavelength range of visible region (Vargas et al., 2000). Pigments can be natural, synthetic and inorganic. Natural pigments are produced by living organisms such as plants, animals and microorganisms.

In this project, the main objective was to replace the synthetic dyes used in DSSC with pigments extracted from pigment producing bacteria. The project was divided into three phases which aimed to achieve the objective of isolating pigmented bacteria from the natural environment, extraction of their pigments and lastly the application of the pigments in DSSC. As the result several species of pigment producing bacteria such as *S. maercescens*, *C. violaceum* and *B. cibi* were isolated from water samples and their pigments were extracted and used as sensitizers in DSSC to test for the production of electricity.

II. LITERATURE REVIEW

1. Photo Electrochemical Solar Cells

Photoelectrochemical solar cell is a cell that is composed of a photoactive semiconductor working electrode and counter electrode made of either metal such as Pt or semiconductors (Wei, 2010). These electrodes are immersed in the electrolyte containing suitable redox couples to complete the circuit. When the semiconductor-electrolyte interface is illuminated with a light having energy greater than the band gap of the semiconductor, photo generated electrons or holes are separated and the photo generated minority carriers arrive at the interface of the semiconductor-electrolyte. Meanwhile, the photogenerated majority carriers accumulate at the backside of the semiconductor. These photogenerated majority carries along with the help of a charge collecting substrate are then transported via a load to the counter electrode where these carriers electrochemically react with the redox electrolyte. Photosensitivity can be extended to longer wavelengths by the addition of dye to silver halid emulsions (Wei, 2010). Interest in photochemistry of semiconductors has led to the discovery of wet-type photoelectrochemical solar cells which was then extended by Gratzel to the Dye sensitized solar cells (DSSC) by adsorption of dye molecules on the nanocrystalline TiO_2 electrodes.

2. Dye Sensitized Solar Cells

Dye Sensitized Solar Cells (DSSCs) are third generation solar cells developed by O'Regan and Gratzell in 1991. They are amongst the newest and promising technologies that have been developed in the 21^{st} century and hold a high potential to be an alternative for the development of a new generation of photovoltaic devices. DSSC converts inexpensive photon from solar energy to electrical energy based on sensitization of wide band gap semiconductor dyes and electrolytes (Kimpa et al., 2012).

DSSCs are a successful combination of materials, consisting of a transparent electrode coated with a dye-sensitized mesoporous film of nanocrystalline particles of TiO_2, an electrolyte containing a suitable redox-couple and a Pt coated counter-electrode (Fig.1). The performance of DSSC is highly dependent on the sensitizer dye and wide band gap material such as TiO_2, SnO_2, and ZnO. However, TiO_2 is highly preferable due to its ability to resist the continuous transfer of electron under illumination (Kimpa et al., 2012). What makes DSSCs different form the conventional semiconductor devices used is the fact that they can separate the function of light absorption from charge carrier transport. According to Wei (2010), dye sensitizer absorbs the incident sunlight and exploits the light energy to induce vectorial electron transfer reaction. When compared to Silicon based photovoltaics, various advantages of DSSC include tolerance to the defects in semiconductors such as defects in Si, easy formation and cost effective production of the semiconductor-electrolyte interface (SEI) and the direct energy transfer from photons to chemical energy.

The use of a high surface area nanoporous TiO_2 was a major breakthrough in DSSC. A single monolayer of dye on the semiconductor surface was sufficient to absorb essentially all the incident light in a reasonable thickness of the semiconductor TiO_2 film (Wei, 2010). Due to this discovery TiO_2 became the semiconductor of choice with advantage properties of cheap, abundant and non-toxic. The application of nanoporous TiO_2 in DSSC has improved the light harvesting process by enhancing the effective surface area in 1000-fold when compared to dye sensitization of semiconductors focused on flat electrodes. According to Wei (2010), the charge transport of the photo-generated electrons passing through all the particles and grain boundaries is highly efficient in nanocrystalline TiO_2 film. DSSCs efficiencies of up to 10.4% have been reported for devices employing nanocrystalline TiO_2 films (Kimpa et al., 2012). The efficiency of a DSSC in the process

for energy conversion depends on the relative energy levels and the kinetics of electron transfer processes at the liquid junction of the sensitized semiconductor- electrolyte interface (Longo & De Paoli, 2003). As indicated by Longo and De Paoli (2003), for efficient operation of DSSC, the rate of electron injection must be faster than the decay of the dye excited state. Also, the rate or re-reduction of the oxidized sensitizer (or dye cation) by the electron donor in the electrolyte must be higher than the rate of back reaction of the injected electrons with the dye cation, as well as the rate of reaction of injected electrons with the electron acceptor in the electrolyte . Lastly, the kinetics of the reaction at the counter- electrode must also gurantee the fast regeneration of charge mediator or this reaction could also become rate limiting in the overall cell performance.

As described in Wei (2010), the initial photo excitation occurs in the light absorbing dye. The nanoporous semiconductors which are used (TiO_2) act as support for the sensitizer and function as electron acceptor and conductor at the same time. The subsequent injection of electrons from the photo-excited dye into the conduction band of semiconductors results in the flow of current travelling across the nanocrystalline TiO_2 film to the charge collecting electrode and then to the external circuit. Sustained conversion of light energy is facilitated by regeneration of the reduced dye sensitizer either by a reversible redox couple, which is usually I_3^-/ I^- or by the electron donation from a p-type semiconductor.

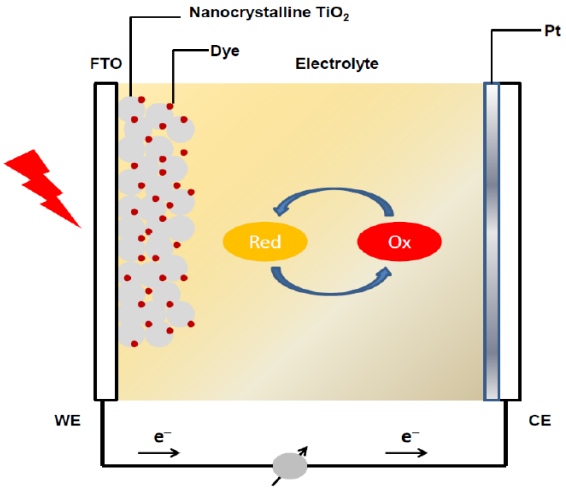

Figure 1: Structure of DSSC (Ze, 2012)

3. Pigments

All the color we see around us in the environment is produced by chemical compounds called pigments. Pigments are present almost everywhere from plants, animals, microorganisms to the food we eat and the juices we drink. However plants are considered to be the principal producers of pigments. Pigments are defined as chemical compounds that absorb light in the wavelength range of the visible region (Vargas et al., 2000). After light absorption takes place, color is produced due to a molecule specific structure called chromophore. The chromophore captures the energy from the incident light and the excitation of an electron from an external orbital to a higher orbital is produced. The non-absorbed energy is reflected to be captured by the eye, and generated neural impulses are transmitted to the brain where they could be interpreted as a color (Vargas et al., 2000).

There are various types of pigments that currently exist. Pigments are broadly classified according to their origin as natural, synthetic or inorganic. Natural Pigments are produced by living organism like plants, animals, fungi and other microorganisms. Synthetic pigments are obtained from chemicals.

4. Bacterial Pigments

Naturally produced pigments were extensively used as colorant before the introduction of synthetic dyes. Synthetic dyes are currently dominant colorant sources despite of their hazardous effect to humans, animals and environment. Bacterial pigments due to their better biodegradability and higher compatibility with the environment offer promising avenues for various applications such as in food, pharmaceuticals, cosmetics and textiles (Venil et al., 2013). Bacteria produce pigments for various reasons that play an important role in their survival. According to Ahmad et al. (2012), bacteria such as *Cyanobacteria* sp. produce phycobilin pigment where as other bacterial strains such as *Serratia marcescens* and *Chromobacterium violaceum* produce prodigiosin and violaceum respectively. Functions of these pigments include photosynthesis, UV protection, defense mechanism, secondary metabolites for storage of energy and others depending on the environmental conditions. The isolation of these bacterial strains can be conducted from various environmental sources such as water bodies, soil, plants, insects and animals. Pigment produced by bacteria can be easily isolated using solvents and can be further purified and characterized for physical and chemical characteristics using analytical techniques such as TLC, UV-vis Spectroscopy, FTIR, HPLC, NMR, ESI-MS and Gel Permeation Chromatography (Ahmad et al., 2012).

5. *Bacillus cibi*

Bacillus cibi are gram variable rod shaped bacteria that are 0.6 – 0.8 ×1.5 - 3.5 µm in size and motile by uniformly distributed flagella (Yoon et al., 2005). The bacteria colonies are smooth, circular, glistening about 3-4 mm in size and produce yellow pigment making them yellow in color. As stated in Yoon et al. (2005), the optimum temperature for *B. cibi* is 37 ºC with inhibited growth at 4 and 50 ºC and optimal pH for growth in the range of 6.5- 7.5 with inhibited growth at pH 5.0 and below. Many *Bacillus* sp. produce pigments

and their pigments have been identified as carotenoids. Carotenoids produced by the bacteria are said to play an important role in providing resistance to UV irradiation and reactive oxygen species being strong antioxidants (Manzo et al., 2011). They inhabit diverse environments such as soil, aquatic environments and the guts of various insects and animals.

6. *Chromobacterium Violaceum*

Chromobacterium violaceum is a heterotrophic, rod-shaped gram-negative organism found as a saprophyte in a wide variety of tropical and subtropical ecosystems mainly water and soil (Scholz et al., 2006). They are 0.6-0.9 µm × 1.5-3.0 µm in size using a single polar flagellum and up to four antigenically and structurally distinct lateral flagellae for locomotion (Likiriko, 2000). *C. violaceum* grow in the temperature of 20-40 ºC with optimum temperature achieved at 35-40 ºC. It is a violet pigmented bacterium due to the production of a non diffusible violacein pigment which possesses antibiotic, antihumoral and anti- *Trypanosoma cruzi* activity (Scholz et al., 2006). *C. violaceum* shows optimum growth at pH 7 and studies have shown that tryptophan is a precursor in the biosynthesis of violacein and its production is very essential for the production of pigment in *C. violaceum.*

7. *Nocardioides fonticola*

Nocardioides fonticola bacteria are yellow pigmented, gram positive, strictly aerobic and road shaped actinomycete that are widely distributed in soil and aquatic habitats including marine sediments (Chou et al., 2008). Cells are non motile having average size of 0.8 × 2.0-9.0 µm and with optimum growth temperature of 30 ºC and 7-8 pH (Chou et al., 2008). Studies have also showed that *N. fonticola* are resistant to nalidixix acid, ceftizoxime and sulfamethoxazole antibiotics but sensitive to ampicillin, chloramphenicol, gentamicin, kanamycin and novobiocin.

8. *Serratia maercescens*

Serratia maercescens is a gram negative, road shaped, facultative anaerobe and motile bacterium classified as opportunistic pathogen (Microbewiki, 2011). It grows at the temperature range of 5-40 ºC with optimum temperature of 37 ºC and at a pH range of 5-9. It is saprophyte in nature and widely distributed in the environment in places such as water, soil air, plants, and animals while it produces a distinct red pigment called prodigiosin (Hejazi & Falkiner, 1997). The movement of the bacterium is facilitated with the help of flagellum and the cells have an average size of 0.5-1 × 1.5-2.0 µm. Studies have shown that *S. maercescens* is resistant to many traditional antibiotics such as penicillin and ampicillin where as it succumbs to the group of antipseudomonal beta-lactam antibiotics that inhibit cell wall synthesis (Microbewiki, 2011).

9. Prodigiosin

Prodigiosin is a secondary metabolite family of tripyrrole red pigments that contains a common 4-methoxy, 2-2 bipyrrole ring systems, produced by *S. maercescens, Pseudomonas magneslorubra, Vibrio psychroerythrous* and other bacteria (Giri et al., 2004). As stated in Casullo de Araujo et al. (2010), prodigiosin is a red pigment that has three rings forming pyrrolylpyrromethane skeleton with a C-4 methoxy group, a molecular formula of $C_{20}H_{25}N_3O$ and molecular weight of 323.44 Da. It was also found to be light sensitive, insoluble in water, moderately soluble in alcohol and ether, and soluble in methanol, acetonitrile, and chloroform. Pigment production by *S. maercescens* depends on many factors that include incubation time, pH, carbon and nitrogen sources and other inorganic salts available for consumption (Casullo de Araujo et al., 2010). Studies have shown that prodigiosin exhibits antifungal, antibacterial and antiprotozoal activities. Prodigiosin has a chemical formula of 5[(3-methoxy-5-pyrrol-2-ylidene-pyrrol-2-ylidene)-methyl]-2-methyl-3-pentyl-1*H*pyrrole (Fig.2)

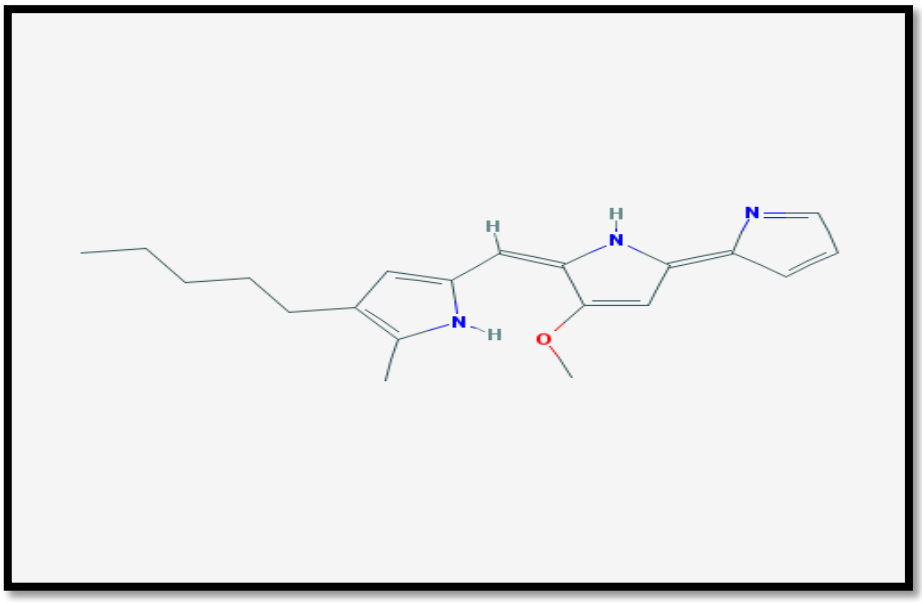

Figure 2: Chemical structure of prodigiosin (PubChem, n.d.)

10. Violacein

Violacein is an indole derivative violet pigment that is naturally produced by bacteria of the genus *Chromobacterium* (Duran et al., 2007) Violacein is bactericidal, trypanocidal, tumoricidal and posses anti-viral activity (Rettori & Duran, 1998). Furthermore as stated in Ahmad et al. (2012), it possesses anti leishimanial and anti- *Mycobacterium tuberculosis* activities. It has a molecular mass of 343.34 Da [3-(1, 2-dihydro-5-(5-hydroxy-1*H*-indol-3yl)-2-oxo-3*H*-pyrrol-3-ilydene)-1, 3-dihydro-2*H*-indol-2-one] having chemical structure as shown in Fig.3. Violacein is soluble in methanol, acetone, ethyl acetate and DMSO, whereas it is in soluble in water and slightly soluble in ethanol (Durian et al., 2007).

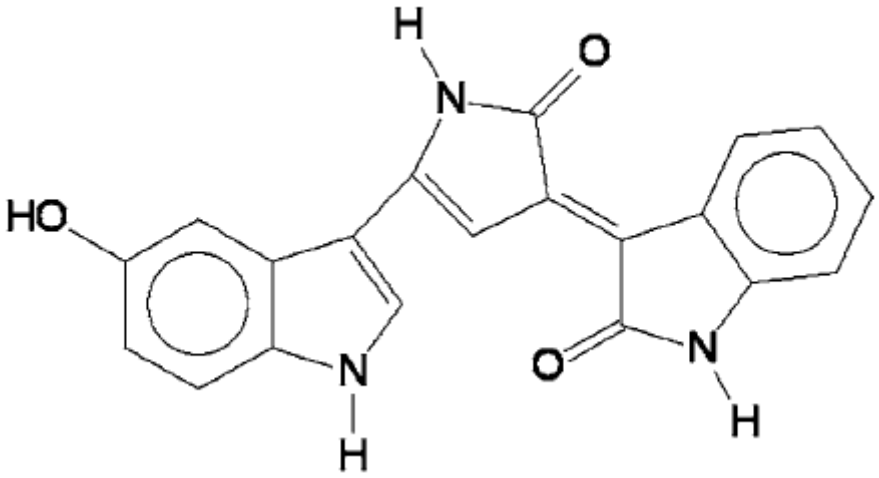

Figure 3: Chemical structure of violacein (Duran et al., 2007)

11. Carotenoids

Carotenoids are pigments that are yellow, orange or red in color formed by the condensation of isoprenyl units and are naturally produced by a wide variety of organisms such as prokaryotes and higher plants (Asker et al., 2007). They have diverse functions in various organisms and posses interesting properities. As stated in Bhosale (2004), Acetyl-CoA is the key precursor of carotenoid biosynthesis in microorganisms. Crotenoids serve as accessory pigments in light capturing complexes present in plants and protect cells from oxygen radicals in nonphototrophic organisms (Asker et al., 2007). Studies have associated the formation of carotenoids with illumination in microorganisms living in the natural environment.

III. MATERIALS & METHODS

1. Sampling

Water samples were taken from two separate locations. First sampling location was Gunung Gading National Park water fall and the second sample was collected from University Malaysia Sarawak's Lake. Water samples were collected from subsurface (10 - 15 cm) of the water bodies.

2. Isolation of Pigmented Bacteria Colonies

For the isolation of pigmented bacteria first the water samples were filtered using a 0.22 µm vacuum driven bottle top filter (GP Millipore Express) and the filter paper was aseptically cut out and re suspended in 50 ml centrifuge tubes containing distilled water. Complex undefined mediums such as Nutrient agar CM003 (NA: Oxoid), Luria –Bertani agar (LB: Difco) and modified Bennett's agar (yeast extract + dextrose+ peptone) were prepared. 100 µl aliquot was then spread plated in different agar medias and petri dishes were left in room temperature for 1-2 days to allow the growth of micro organisms. All colored colonies developing on the plates were then isolated and sub cultured to give a pure culture of the respective bacteria species.

3. Morphological Identification

3.1 Gram Staining

In Gram staining method, first the pigmented bacteria colonies were grown in a dark place and the non pigmented colonies were used for gram staining. A smear was prepared by first placing two loopfulls of distilled water in a target circle of a slide. Following, a very small amount of bacteria from the petri dishes was dispersed with inoculating loop/needle in the distilled water over the entire area of the target circle. The smear was left to dry and then passed through flame to heat kill and fix organisms to the slide. Once the smear was

prepared, it was completely covered with crystal violet and left for 20 seconds. Then the crystal violet was briefly washed with distilled water and the smear was covered with Gram's Iodine solution and left standing for 45 seconds before being poured off. The smear was then flooded with 95% ethyl alcohol for 10 to 20 seconds until a complete decolorization occurred. Slide was rinsed with water and the smear covered by safranin for 20 seconds. Following this step it was washed with distilled water for 2 seconds and blot dried before being examined under oil immersion. This method was adopted from Benson (2006).

3.2 Scanning Electron Microscope

For a higher resolution and magnification of the bacteria, scanning electron microscope (SEM) was used. The first two pigmented bacteria isolates were viewed under SEM. The SEM specimen preparation protocol stated in David & Robert (2006) was followed with slight modifications. Culture broth was first centrifuged in a 2 ml centrifuge tube and supernatant was discarded. About 1 ml of 2.0% gluteraldehyde (prepared in 0.1 M PBS) was poured into the tube for fixation and left overnight at room temperature. The gluteraldehyde was then removed and a series of dehydration with enthanol (10% to 100%) was performed. Cell pellets were coated with gold and examined by SEM (JEOL: JSM-6390LA).

4. Molecular Identification

4.1 DNA Extraction

DNA was extracted from the cell pellets using the CTAB DNA extraction method. 1.5 ml of an overnight culture was transferred into eppy tubes and centrifuged (WiseSpin: CF-10) for 2 minutes. After removing the supernatant, the cell pellet was suspended in 567 µL TE buffer and mixed well by vortexing. 30 µL of 10% SDS and 3 µL of 20 mg/ml Proteinase

K were added and the mixture was left in a water bath at 37 °C for one hour. Following the water bath 100 µL of 5M Nacl and 80 µL of CTAB/Nacl were then added and the mixture was incubated at 65 °C for 15-20 minutes.

Equal volume of PCI (25:24:1) was then added to the eppy tubes and centrifuged for 5 minutes. Viscous supernatant was transferred into a new eppy tube and mixed with equal volume of CI (24:1) and centrifuged for 5 minutes. The clear supernatant was transferred into a new eppy tube and 0.6 volume of isopropanol was added and the tube inverted until a white precipitate appeared. It was then centrifuged for 2 minutes and the supernatant removed. The pellet was washed with 200 µL of 70% ethanol and centrifuged for 2 minutes before being air dried. The Dried DNA was dissolved in 100 µL of TE buffer and stored at -20 °C before proceeding with PCR. This method was obtained from Moore et al. (2004).

4.2 Polymerase Chain Reaction

Polymerase chain reaction was performed to amplify and sequence the 16S rRNA from the bacteria genome. The protocol stated in Barghouthi (2011) was adopted with slight modifications. For this process universal primers and master mix (Vivantis) were used. 50 µL reactions were prepared by adding 36.5 µl distilled water, 5 µl PCR buffer (10×), 2 µl of $Mgcl_2$ (50 mM), 1 µl dNTPs (10 mM), 1 µl 27F primer (20 pmol), 1 µl 1525R primer (20 pmol), 3 µl DNA and 0.5 µl Taq polymerase (5µ/µl). The amplification reaction was hot started at 95 °C for 5 minutes. The PCR protocol followed was, 94 °C for 90 s, 48 °C for 35 s, 72 °C for 105, a final extension step at 72 °C for 3 minutes and a total of 33 cycles.