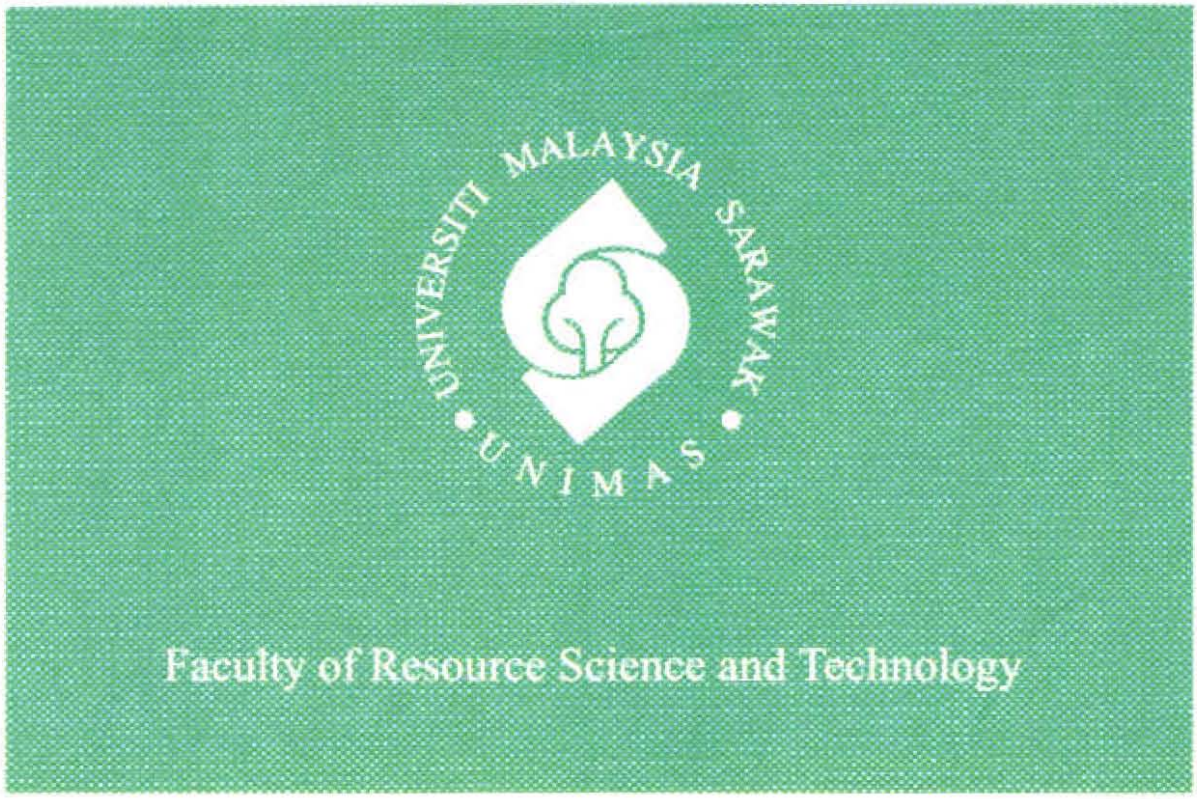

ISOLATION AND CHARACTERIZATION OF PIGMENTED MARINE BACTERIA FOR DYE SENSITIZED SOLAR CELL (DSSC) APPLICATION

Cheong Wei Shien

(33714)

Bachelor of Science with Honours
(Resource Biotechnology)
2014

Isolation and Characterization of Pigmented Marine Bacteria for Dye Sensitized Solar Cell (DSSC) Application

Cheong Wei Shien (33714)

This project is submitted in fulfilment of the requirement for the Degree of Bachelor of Science with Honours
(Resource Biotechnology)

Supervisor: Dr. Azham Zulkharnain

Resource Biotechnology
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I, Cheong Wei Shien, 33714, Faculty of Resource Science and Technology hereby declare that the work entitled "Isolation and Characterization of Pigmented Marine Bacteria for Dye Sensitized Solar Cell (DSSC) Application" is my original work. I have not copied from any other students' work or from any other sources except where due reference or acknowledgement is made explicitly in the text, nor has any part been written for me by another person.

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Date submitted

Cheong Wei Shien (33714)
Name of the student (Matric No.)

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I, Azham Zulkharnain hereby certifies that the work entitled "Isolation and Characterization of Pigmented Marine Bacteria for Dye Sensitized Solar Cell (DSSC) Application" was prepared by the above named student, and was submitted to the "FACULTY" as a partial/full fulfilment for the conferment of Bachelor of Science with Honours, and the aforementioned work, to the best of my knowledge, is the said student's work.

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LIST OF ABBREVIATIONS

CTAB	Cetyl Trimethyl Ammonium Bromide
$CDCl_3$	Chloroform
DCM	Dichloromethane
ddH_2O	Deionized water
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
DSSC	Dye Sensitized Solar Cell
EtBr	Ethidium bromide
FTIR	Fourier-Transform Infrared Spectroscopy
FTO	Fluorine doped tin oxide
MA	Marine Agar
MB	Marine Broth
$MgCl_2$	Magnesium chloride
NaCl	Sodium chloride
NMR	Nuclear Magnetic Resonance
TiO_2	Titanium dioxide
KBr	Potassium bromide
PCR	Polymerase Chain Reaction
Rf	Retention factor
rRNA	Ribosomal ribonucleic acid
TAE	Tris-acetae-EDTA
TiO_2	Titanium dioxide

TLC	Thin layer chromatography
SDS	Sodium Dodecyl Sulphate
SnO_2	Tin dioxide
TE	Tris-EDTA
UV/Vis	Ultraviolet/Visible
λ_{max}	Maximum wavelength

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Isolation and Characterization of Pigmented Marine Bacteria for Dye Sensitized Solar Cell (DSSC) Application

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ABSTRACT

Solar energy could be used in most regions on the surface of the Earth, and is renewable. Nowadays, dye sensitized solar cell (DSSC) which could use natural pigments as dye sensitizer has attracted considerable attention. Since there are some drawbacks of pigments extracted from plants and fruits, bacterial pigments could be an alternative source for the dye sensitizer. Thus, the aim of this project was to isolate and characterize the marine bacteria which can produce pigment to be used as dye sensitizer for DSSC. In this study, seawater samples were collected from Jeti Muara Tebas and Pantai Pasir Pandak. The bacterium that produced red pigment was selected. The selected bacterium was characterized as *Pseudoalteromonas* sp. by using Polymerase Chain Reaction (PCR) and 16S rRNA gene sequencing. Then, the bacterial pigment was extracted and characterized as prodigiosin by Thin Layer Chromatography (TLC), UV/Vis Spectroscopy, Fourier-Transform Infrared Spectroscopy (FTIR) and Nuclear Magnetic Resonance (NMR). The result of the performance of the extracted bacterial pigment as dye sensitizer in DSSC showed that bacterial pigment can be used as an alternative dye sensitizer in DSSC.

Key words: Pigmented marine bacteria, Bacterial pigments, Dye sensitizer, Dye sensitized solar cell (DSSC)

ABSTRAK

Tenaga solar boleh digunakan di kebanyakan rantau di permukaan Bumi, dan boleh diperbaharui. Pada masa kini, pewarna sensitif sel solar (DSSC) yang boleh menggunakan pigmen semula jadi sebagai pewarna pemeka telah menarik perhatian yang besar. Oleh sebab terdapat beberapa kelemahan pigmen yang diekstrak daripada tumbuhan dan buah-buahan, pigmen bakteria boleh dijadikan sumber alternatif bagi pemeka pewarna Oleh itu, tujuan projek ini adalah untuk mengasingkan dan mencirikan bakteria marin yang boleh menghasilkan pigmen untuk digunakan sebagai pemeka pewarna kepada DSSC Dalam kajian ini, sampel air laut dikumpulkan dari Jeti Muara Tebas and Pantai Pasir Pandak. Bakteria yang menghasilkan pigmen merah telah dipilih. Bakteria yang dipilih dicirikan sebagai Pseudoalteromonas sp. dengan menggunakan Polymerase Chain Reaction (PCR) dan gen penjujukan 16S rRNA. Kemudian, pigmen bakteria diekstrak dan dicirikan sebagai prodigiosin oleh Kromatografi Lapisan Nipis (TLC), Spektroskopi UV/Vis, Spektroskopi-Fourier inframerah transformasi (FTIR) dan Magnetic Resonance Nuklear (NMR) Hasil prestasi pigmen bakteria yang diekstrak sebagai pewarna pemeka dalam DSSC menunjukkan bahawa pigmen bakteria boleh digunakan sebagai pemeka pewarna alternatif dalam DSSC.

Kata kunci: Bakteria laut berpigmen, Pigmen bakteria, Pewarna pemeka, Pewarna sel solar sensitif (DSSC)

1.0 INTRODUCTION

Renewable energies are important because of the depletion of fossil fuel. Hence, solar energy as one of the renewable energies could be used in most regions on the surface of the Earth. DSSC which use the organic pigments as dye sensitizer has gained much attention nowadays because this solar cell is considerably cheaper to manufacture. It will most likely replace silicon based solar cell on the market in the future. There have been reports on the use of natural pigments extracted from plants and fruits as dye sensitizer of these sunlight harvesting cells. However, the use of plants and fruits are impractical because of the high cost and low availability. Therefore, pigments extracted from bacteria could be an alternative dye sensitizer.

A large number of different species of bacteria are used to produce pigments. Bacterial pigments are advantageous, in terms of production, when compared to pigments extracted from the plants because they can be exploited easily by using culturing techniques (Hendry & Houghton, 1997). Besides, the pigment production by bioprocesses involving bacteria has relatively high growth rate (Hendry & Houghton, 1997). This will cut the production time, and thus brought them industrially competitive. Moreover, the production is flexible and can easily be controlled compared to plant or animal sources (Hendry & Houghton, 1997).

The pigments of higher organisms, such as plant, animal and fungal, are less accessible to be exploited because of the structural complexity of the pigment-bearing tissue and the pigment is formed only at critical points of development within a complex life cycle (Hendry & Houghton, 1997). In addition, pigments extracted from plant are expensive and uncompetitive

due to their high production costs. Such disadvantages could be solved with the development of bacterial pigments.

It is important to note that bacteria are abundant in nature which can be isolated from the environment, especially from marine environment. So, the collection of bacteria is sustainable and has no negative impact on environment. For the propagation of bacteria, only minimal medium is needed, as bacteria have the ability to utilize cheap sources of carbon and nitrogen to produce valuable low and high molecular-weight metabolites (Demain, 1980). In order to minimize the operating cost, extraction of bacterial pigment can be performed by using simple solvent extraction technique (Demain, 1980).

Thus, the aim of this study is to screen for the marine bacteria which can produce pigment to be used as dye sensitizer in DSSC. Therefore, the main objectives of this project were to:

1. isolate and characterize marine bacterial strain which has potential to produce coloured pigment.
2. characterize the pigment extracted from selected pigmented marine bacteria.
3. assess the performance of the bacterial pigments as dye sensitizer using DSSC.

2.0 LITERATURE REVIEW

2.1 Marine Bacteria

Bacteria are found on all possible regions on the Earth, based on their habitat, diversity, ecological function, and biotechnological application. Marine and terrestrial microfloras differ from each other due to the influence of their respective environmental conditions (Kim, 2013). Bacteria which live in marine environment have unique properties because they have to be able to survive in extreme condition of marine environment, such as low nutrition, high salinity, high or low temperature, and high pressure (Kim, 2013). Hence, marine bacteria can be divided base on their habitat into psychrophiles (living at low temperatures), halophiles (living at high salinity), and barophiles (living under high pressure) (Kim, 2013). Although these characteristics show the differences between marine and terrestrial microorganisms, it remains difficult to separate bacterial genera on the basis of habitat due to the ubiquitous presence of similar species in both environments. As such, most bioactive compounds have been isolated from bacteria in both environments.

There is resurgence in the search for good sources of natural pigments which are eco-friendly. Accordingly several investigators have started screening natural pigments from various sources. The marine environment, which covers three quarters of the surface of the Earth, remains largely unexplored. Marine bacteria are an untapped source for pigments that can have wide range of applications in industries.

2.2 Bacterial Pigment

Among the natural sources of pigments, bacteria offer the great scope and hope. Some factors have made their choice more feasible, such as the ease of cultivation, extraction, the genetic diversity in microbes and sophistication of technology (Juailova *et al.*, 1997). Thus, the work on bacterial pigments should be intensified, especially in finding cheap and suitable growth medium which can cut the cost of production and increase its applicability for industrial production (Venil *et al.*, 2013).

Several intensely coloured compounds have been isolated from certain bacteria which have little resemblance to pigments in other biological systems. An example of the bacterial pigment is violacein (Figure 2.1), a purple pigment which produced by *Chromobacterium violaceum* It is an indole derivative characterized as 3-(1, 2-dihydro-5-(5-hydroxy-1H-indol-3-yl)-2-oxo-3H-pyrrol-3-ilydene)-1, 3-dihydro-2H-indol-2-one (Duran & Menck, 2001). The pigment appears to be similar to that of other known species of *Chromobacterium* and helped in identification of the genus of the causative organisms (Eugene *et al.*, 1961).

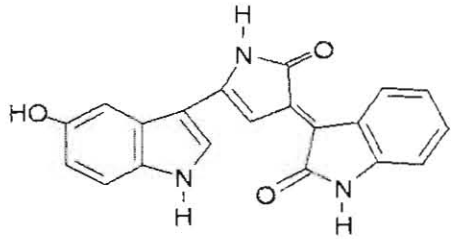

Figure 2.1: Violacein.

Carotenoids are the most widely distributed pigments, and they play an important role in bacteria, especially in photosynthetic processes to prevent photo-damage, and conferring resistance to oxidative damage due to production of activated forms of oxygen (Cardona-Cardona *et al.*, 2010). They are yellow, orange, or red in colour, and the biosynthesis of the carbon skeleton is based on condensation of isoprenyl units. Until now, over 700 different structures of carotenoids are identified, which obtained through various modifications of the carbon backbone (Stafsnes *et al.*, 2010). Carotenoids are mostly extracted from plant tissues or chemical synthesis. However, microbial production has the potential in terms of both the efficiency of production and the diversity of carotenoid structures due to the increasing demand on these compounds (Stafsnes *et al.*, 2010). Currently, carotenoids are used as food colourants, animal feed supplements, and more recently, also used for cosmetic, nutraceuticals, and pharmaceutical purposes (Sasidharan *et al.*, 2013). Astaxanthin (Figure 2.2) is one of the carotenoids that have commercial value as a food supplement for humans and as food additives for animals. A carotenoid biosynthesis gene cluster for astaxanthin production has been isolated from *Agrobacterium aurantiacum* (Misawa *et al.*, 1995). Another astaxanthin-producing marine bacterium, *Paracoccus haeundaensis*, was also isolated recently (Lee *et al.*, 2004).

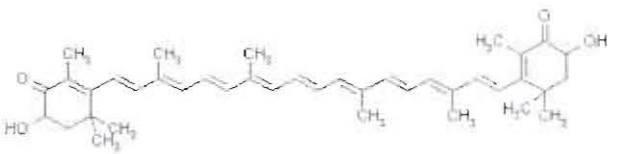

Figure 2.2: Astaxanthin.

Figure 2.3 show that prodigiosin is a tripyrrole. The prodigiosin tripyrrole was shown to be localized in extracellular and cell-associated vesicles and in intracellular granules (Kobayashi & Ichikawa, 1991). Its name is derived from the word "prodigious", meaning something marvellous. It is a red pigment produced by a wide variety of bacteria, including *Serratia marcescens, Serratia rubidaea, Vibrio gazogenes, Vibrio psychroerythrous, Pseudomonas magneslorubra, Alteromonas rubra, Rugamonas rubra, Streptomyces longisporus, Streptomyces spectabilis* and *Streptoverticillium rubrireticuli* (Variyar *et al.*, 2002). It has an unusual structure with three pyrrole rings and is a pyrryldipyrryhnethene; two of the rings are directly linked to each other, while the third is attached by way of a methene bridge (Gerber, 1975; Qadri & Williams, 1972). The highly conjugated system of seven double bonds presumably accounts for the intense pigmentation.

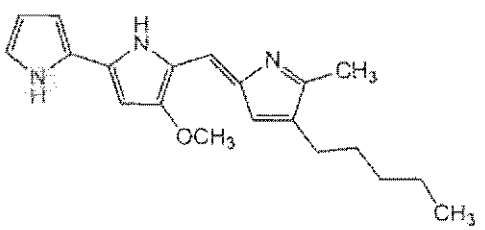

Figure 2.3: Prodigiosin.

2.3 Dye Sensitized Solar Cells (DSSC)

DSSC, also known as "Grätzel cell", has been invented by Michael Grätzel and Brian O'Regan in 1988 (Soutter, 2013). The technology of DSSC arose from the concept of "artificial photosynthesis" that mimics the process of photosynthesis, which converts sunlight into energy (Soutter, 2013). In DSSC, dye sensitizer plays the crucial role in light-harvesting and energy conversion (Wang & Tamiaki, 2009). Although this solar cell is still in relatively early

stages of development, it shows great promise as an inexpensive alternative to costly silicon solar cell because it offers moderate conversion efficiency with other advantages, such as low production cost, easy fabrication, good performance under low light, and compatibility with building window glass and flexible substrates (Lee & Yang, 2011). Therefore, DSSC has been placed as one of the promising alternatives to silicon based solar cell which is currently dominant in the market (Lee & Yang, 2011).

The dye sensitizers used in DSSC can be classified into two, which are inorganic dyes and organic dye (Karmakar & Ruparelia, 2011). Among these two categories of dye, inorganic dyes have given better results since the stability towards photo degradation is less for the organic dyes (Karmakar & Ruparelia, 2011). The inorganic dyes that are used for DSSC are mainly metal complex dyes, such as ruthenium, iridium, osmium, and so on (Karmakar & Ruparelia, 2011). On the other hand, the organic dyes used in this purpose are mainly fruit dyes and other natural extract dyes (Karmakar & Ruparelia, 2011). Many researches have been done to find transition-metal complexes as well as organic dyes (Karmakar & Ruparelia, 2011). However, none of it has been able to match the performance of the ruthenium complex based on conversion yield and long term stability (Karmakar & Ruparelia, 2011).

As shown in Figure 2.4, A DSSC consists of two conducting glass electrodes in a sandwich arrangement, and each layer has a specific function. The glass electrodes are transparent which allows the light to pass through the cell. The tin dioxide (SnO_2) coating is a transparent, conductive layer. The titanium dioxide (TiO_2) serves as a holding place for the dye. The dye molecules act as light harvester produce excited electrons which cause a current in the cell

(Narayan, 2012). The iodide solution acts as electron donor and oxidation product. The bottom conductive layer is coated with graphite.

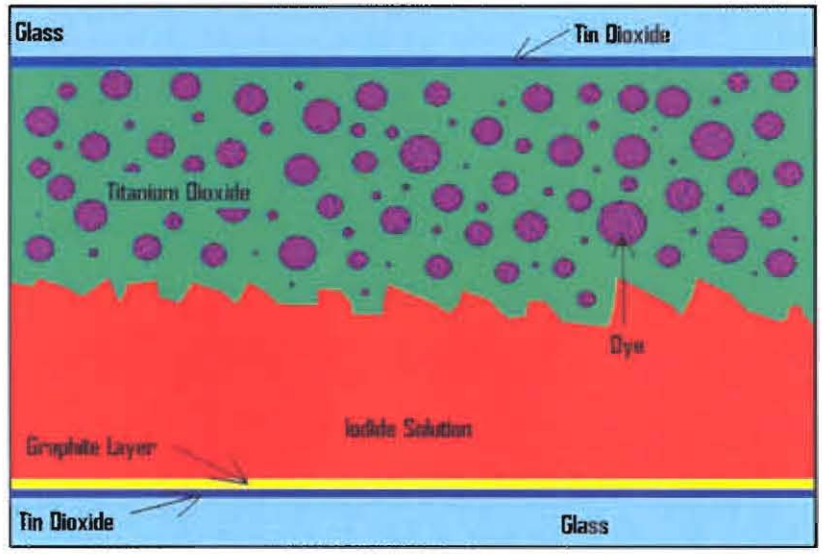

Figure 2.4: Structure of DSSC.

Figure 2.5 shows that DSSC produce electricity through electron transfer. Light passes through the conductive glass electrode (Grätzel, 2003). The dye absorbs the photons of light and one of the electrons in the dye goes from a ground state to an excited state, and so called photo excitation (Grätzel, 2003). The excited electron jumps to the TiO_2 layer and diffuses across the film. The electron then reaches the conductive electrode, travels through the wire, and reaches the counter electrode (Nagata & Murakami, 2009). The dye molecule, having lost an electron to the TiO_2, is now oxidized, which means it has one less electron than before. The dye wants to recover its initial state so it obtains electron from the iodine electrolyte and the dye goes back to ground state (Grätzel, 2003). This causes the iodine to become oxidized. When the original lost electron reaches the counter electrode, it gives the electron back to the electrolyte.

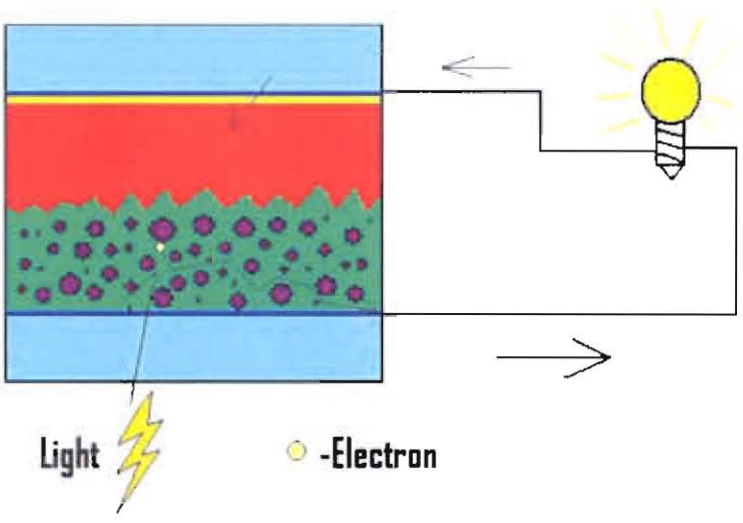

Figure 2.5: Electron transfer process in DSSC.

3.0 MATERIALS AND METHODS

3.1 Seawater Sample Collection

Seawater samples were collected from Jeti Muara Tebas and Pantai Pasir Pandak. The samples were taken up from the surface of the seawater. Sterile plastic bottles were used to collect the seawater samples, and closed tightly in order to avoid external contamination. Then, the samples were labelled, and transported to the laboratory and stored under the bench for further used.

3.2 Preparation of Media

3.2.1 Marine Agar (MA)

MA was prepared by suspending 4.5 g of agar powder and 11.22 g of MB in 300 ml of distilled water. The medium was mixed well by using magnetic stirrer. The medium was then sterilized by autoclaving at 121°C, 121 kPa for 20 minutes. After that, the medium was cooled to 60°C, and then mixed well and dispensed into sterile Petri dishes. The prepared MA plates were stored at 4°C.

3.2.2 Marine Broth (MB)

MB was prepared by suspending 1.87 g of the medium in 50 ml of distilled water. The medium was mixed well by using magnetic stirrer, and then sterilized by autoclaving at 121°C, 121 kPa for 20 minutes. The prepared MB was stored at 4°C.

3.3 Isolation of Pigmented Marine Bacteria

Pour plate technique was used for plating the samples. The prepared MA plates were inoculated with 50 µl of seawater sample, and then quickly spread evenly on the MA plates with a spreader. The plates were shield with plastic cover to avoid contamination by air-borne bacteria. A strip of parafilm was wrapped around the plates to prevent drying. The plates were inverted and incubated at room temperature for 24 hours.

After the incubation period, a single, well separated pigment producing single cell colonies that appeared on the plates were selected, purified by sub-culturing onto new MA plates. The process above was repeated until pure pigmented marine bacteria were obtained. Each plate was incubated at room temperature for 24-48 hours.

3.4 Selection of Potential Strain

Potential strains that produced pigment were selected based on the colour of the pigment. During the first phase, all chromogenic cultures that showed bright pigmentation were short listed. During the second phase, those cultures that produced red pigment were selected and subjected to further studies towards selection of potential strain. The criteria employed for selection of potential strain included production of intense red pigmentation in the broth. After screening, the isolated strain which recorded considerable amount of red pigmentation on the agar as well as in the broth was selected.