ISOLATION AND CHARACTERIZATION OF HYDROGEN PRODUCING MARINE PHOTOSYNTHETIC BACTERIA

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A dissertation submitted in partial fulfillment of the
requirements for the award of the degree of
Master of Science (Biotechnology)

Faculty of Biosciences and Medical Engineering
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JULY 2014

To my beloved father and mother for their moral support

To my wife and children

And

My brothers and sisters

ACKNOWLEDGEMENT

All praises and all thanks are to Allah, the lord of the mankind who bestow knowledge and guidance to His servants. I thank Allah for making it possible for me to write down this Dissertation and complete my study.

I wish to acknowledge my supervisor, Dr Mohd Firdaus Abdul Wahab for his hard work, patience, support and encouragement from the beginning to the end of this work.

I also wish to acknowledge the support of my beloved parents for their wonderful training, upbringing and support throughout my life.

I wish to appreciate my lovely family: my magnificent and caring wife, Ummu Nu'aym and my two Adorable kids: Nu'aym and Ummul Fadhl for their patience, perseverance and tolerance during the long period of this study.

I also wish to thank all those who assisted me towards the success of this work from my lab colleagues and technicians, class mates and all those who had contributed in any way towards the completion of this work. It is not possible to list everyone due to limited space.

Finally, appreciation goes to my brothers and sisters, friends and colleagues at home and abroad as well as well-wishers who always pray for the success of my study. I appreciate you all.

May Allah (*Subhanahu wa taala*) reward all of you abundantly.

ABSTRACT

Photosynthetic bacteria are widely distributed in nature and are a good source of sustainable products. The aim of this work is to investigate the potential of photosynthetic bacteria from marine environments to produce hydrogen, a renewable, clean and efficient fuel. Marine environments have been identified as major source of new natural products. Samples of water were collected from marine environments around Kuala Terengganu. Using aseptic techniques, the samples were inoculated in succinate medium and incubated under light at a temperature of 28-30^{o}C. Two suspected photosynthetic bacteria were isolated from the sample. Both are Gram negative rods and produce *carotenoid* and *bacteriochlorophyl* photosynthetic pigments. The isolated bacteria were then investigated for their potential to produce hydrogen using acetate and synthetic waste water media containing starch as carbon sources incubated under light. The gas produced were collected and analyzed using a Residual Gas Analyzer (RGA). Both isolates show potential to produce hydrogen gas. The bacteria were then subjected to molecular characterization using 16s rRNA method to identify the isolated organisms. The two isolates were identified using NCBI BLAST tool *Pseudomonas species ID1* with accession number KJ950496 and *Aeromonas species ID2* with accession number KJ950497.

ABSTRAK

Bakteria fotosintetik terdapat secara meluas di alam semula jadi dan merupakan sumber yang baik sebagai produk lestari. Tujuan kajian ini adalah untuk menyelidik potensi bakteria fotosintesis dari persekitaran marin untuk menghasilkan hidrogen, bahan api yang boleh diperbaharui, bersih dan cekap. Persekitaran marin telah dikenal pasti sebagai sumber utama produk semula jadi baru. Sampel telah dikumpulkan dari persekitaran marin di sekitar Kuala Terengganu. Dengan menggunakan teknik aseptik, sampel telah dibiakkan dalam medium succinate dan diinkubasikan di bawah cahaya pada suhu 28-30°C. Dua bakteria fotosintetik telah dikenal pasti daripada sampel. Kedua-duanya adalah rod Gram negatif dan menghasilkan karotenoid dan bakterioklorofil pigmen fotosintesis. Bakteria diasingkan kemudiannya diselidik potensi mereka untuk penghasilan hidrogen dengan menggunakan asetat dan media sintetik air sisa yang mengandungi kanji sebagai sumber karbon diinkubasi di bawah cahaya. Gas yang dihasilkan telah dikumpulkan dan dianalisis menggunakan Residual Gas Analyzer (RGA). Kedua-dua bakteria menunjukkan potensi untuk menghasilkan gas hidrogen. Bakteria kemudiannya diberikan pencirian molekul dengan menggunakan kaedah 16S rRNA untuk mengenal pasti bakteria berkenaan. Dua bakteria tersebut telah dikenal pasti sebagai *Pseudomonas* sp ID1 (KJ950496) dan *Aeromonas sp ID2* (KJ950497).

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LIST OF ABBREVIATION AND SYMBOLS

ATP	Adenosine Triphosphate
Bchl a	Bacteriochlorophyl a
BHP	Biological Hydrogen Production
BLAST	Basic Local Alignment Search Tool
bp	Base pairs
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleoside triphosphate
EDTA	Ethylenediamine Tetraacetic Acid)
LH-1	Light Harvesting complex 1
LH-2	Light Harvesting complex 2
NCBI	National center for biotechnology information
OD	Optical Density
PCR	Polymerase Chain Reaction
PNS	Purple Non Sulphur bacteria
PS	Purple Sulphur Bacteria
PSB	Photosynthetic Bacteria
rDNA	Ribosomal DNA
RGA	Residual Gas Analyser
RNA	Ribonucleic Acid
Rpm	Revolution Per Minute
rRNA	Ribosomal RNA
TAE	Tris Acetate EDTA
Taq	*Thermus aquaticus*
USA	United State of America
VFA	Volatile Fatty Acids
μ	Specific Growth Rate

γ	Gamma
α	Alpha
β	Beta
g/L	Gram Per Liter
mM	Milli Molar
nm	Nano Meter
µL	Micro Liter
mL	Milli Liter
v/v	Volume By Volume
^{o}C	Degrees Celcius
e^{-}	Electron

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CHAPTER 1

INTRODUCTION

1.1 Background

The search for renewable sources of energy is a driving tool towards sustainable development. The idea of a sustainable development is to solve future challenges affecting humanity in general (Hopwood et al., 2005). Solving the future challenge pose by environmental problems can be considered as a sustainable development that requires a long term action plan. The use of fossil fuels as transportation fuel comes with several environmental challenges. It is not renewable, its extinction has been predicted and one of the major causes of greenhouse effect following its combustion as a consequence of human activities. These activities are responsible for releasing organic carbon in the past decades at an amount that is equivalent to that of organic carbon accumulated over millions of years ago (Mohan and Pandey, 2013). Transportation fuel used by motor vehicles contributed only about 19% of carbon dioxide (CO_2) emissions and accounted for at least 70% of the bulk of carbon monoxide emission globally (Goldemberg, 2008). Sustainable sources for energy production need to be obtained from renewable sources, independent of fossil fuel and cost effective. Despite the potential negative effect of fossil fuel such as the production of gaseous products upon combustion (the major products are CO_2, Sulphur dioxide SO_2, as well as carbon monoxide, CO) that adversely have impact on the environment by effecting global warming, it is not easily possible to stop the use of

fossil fuel without providing an alternative and reliable replacement; fossil fuels are major economic driven tool today and contributed close to 80% of total energy demands worldwide (Ngô and Natowitz, 2009). Hydrogen gas is regarded as one of the valuable substitute for fossil fuels in the future as transportation fuel. It has some unique features that made it a promising alternative to fossil fuels. It is environmentally friendly, clean, renewable and was reported to have high energy content and free emissions of CO_2, SO_2 and nitrogen oxide (NO_2) (Argun and Kargi, 2011). Upon combustion, hydrogen gas does not release carbon dioxide and it is possible to convert hydrogen to electricity using fuel cells (Kim et al., 2011). Depending on its source, hydrogen use in fuel cell vehicles can lead to an ecologically friendly transport system (Granovskii et al., 2006).

Hydrogen is regarded as the most abundant element on earth, but it does not exist in natural molecular form but rather has to be produced from various feedstock sources. Hydrogen does not emit carbon; therefore it does not contribute to greenhouse effect. Burning of hydrogen does not have negative environmental effects such as greenhouse effect, acid rain or contribute to ozone layer depletion instead it emit water as it end product (Nath and Das, 2004; Allakhverdiev et al., 2010). It has many added advantages to its credit such as social, economic and environmental (Meher Kotay and Das, 2008). Hydrogen production is performed domestically; hence it has the future potentiality of effectively reducing import of crude oil in many oil importing countries.

The production of hydrogen is possible from various energy sources. Hydrogen is produced using variety of methods which can be either biological or non-biological. In today's conventional (non-biological) method, industrial hydrogen production starts from methane gas, which is the major constituent of natural gas (Sorensen, 2011). More than 50% of commercial hydrogen production today comes from fossil fuel (Liu et al., 2005). Three techniques are basically used to produce hydrogen conventionally: steam reforming, partial oxidation, and auto thermal reforming (Sorensen, 2011). These techniques according to Kim and Kim (2011) and lee et al (2010), indicates that hydrogen is not produced renewably but rather from non-renewable sources. The sources are dependent on fossil while the product, hydrogen is a clean and an efficient

energy carrier. Hydrogen that is produced from renewable sources is qualified to be termed as clean energy production process. To produce clean hydrogen and to alleviate the effect of greenhouse gases as a result of reliance on fossil fuel, an alternative method that is free from fossil is required that is renewable, environmentally friendly and acceptable to the public. Hydrogen produced by biological processes of hydrogen production stands a better chance of being the alternative method of hydrogen production for sustainable development. Renewable sources of hydrogen also include wind and solar energy. Electricity production from these sources *via* water electrolysis produces hydrogen but hydrogen produced from wind is more favourable for reducing greenhouse gas emissions and therefore is much in agreement with sustainable development (Granovskii et al., 2006).

Biological methods are regarded as the most efficient and most promising tool for hydrogen production. Research in recent years has proven that biological hydrogen production to be an absolute method for hydrogen production for its ability to produce hydrogen renewably in large quantities (Cai et al., 2012). It is a cost effective method of hydrogen production, renewable and carbon neutral. Biological hydrogen production (BHP) will ensure hydrogen production using a technology that is both environmentally friendly and suppress the problem of limiting resources because it is renewable (Kim et al., 2011). Biological production of hydrogen can be achieved by several biological methods. The methods are carried out at ambient temperatures and pressure which confer an added advantage over chemical or electrochemical ways of hydrogen productions that require more energy (Meher Kotay and Das, 2008). Microbial metabolism can be used to produce hydrogen (Hallenbeck and Benemann, 2002). Metabolism of hydrogen is carried out by bacteria and microalgae and there is possibility of hydrogen production from many microorganisms including photosynthetic bacteria (Nandi and Sengupta, 1998). With the discovery of the ability of microorganisms to produce hydrogen, an effective renewable source of hydrogen has been established. Microbial sources will provide a sustainable production route of renewable energy and will cause a reduction in price since they are much in abundance. The energy generated by microorganisms are advanced fuels with properties like that of petroleum based fuels and can be used in transportation (Peralta-Yahya et al., 2012). Several microorganisms including photosynthetic bacteria had been reported to have

biohydrogen production (Zürrer and Bachofen, 1979; Barbosa et al.; 2001, Das and Veziroğlu, 2001; Suwansaard et al., 2009; Suwansaard et al., 2010). These organisms will rely on several sources of carbon which could be from waste materials when used as feed stocks. The use of waste materials as carbon source will contribute to waste recycling (Manish and Banerjee, 2008). Photosynthetic microorganisms have the capacity to absorb light energy (photons). They store it as chemical energy *via* the formation of chemical bonds. Biohydrogen produced by photosynthesis is the ultimate source of renewable hydrogen, it relies on resources that cannot be exhausted (Lee et al., 2010). Biological hydrogen production using photosynthesis was predicted to be a reliable alternative to hydrogen productions instead of chemical and electrochemical technologies (Allakhverdiev et al., 2010).

In the world today, water covers more than 70 percent of earth's surface and serves as a vital resource to all organisms on the planet. The largest bodies of water on earth are the oceans or the marine environment. As marine environments occupied two third of the planet surface, it is possible that marine organisms outnumber land organisms. Microorganisms are present widely in many environments and new species are constantly discovered (Shin, 2013). Marine environments offer a special living environment for many organisms. These organisms serve as sources of natural products. Due to the special living condition of marine environments, microorganisms found in marine habitat possess unique potential for the production of novel and biologically active substances with unique structures and new functions (Fenical, 1993, Zhao et al., 2009). The involvement of marine microbes in the search for new compound is due to its nature as a renewable resource and a complex life form (Bernan et al., 1997).

Marine photosynthetic microbes comprise of aerobic and anaerobic anoxygenic phototrophic bacteria. These bacteria either use organic compounds or reduced sulphur as electron acceptor. They are respectively called non sulphur and sulphur bacteria. They are further subdivided into purple non sulphur, purple sulphur, green non sulphur and green sulphur bacteria. The purple non sulphur bacteria usually inhabit the planktonic part of marine water and account for at least 11% of total marine

microbes (Kolber et al., 2001). They are photoheterotrophs with bacterial pigment called *bacteriochlorophyl a* (*BChl a*) that is used to harvest light (Ritchie and Johnson, 2012). They are efficient hydrogen producers with their ability to utilize organic acids (Asada and Miyake, 1999). The bacteria are suitable for large hydrogen productions (Cai and Wang, 2012). This is attributed to their potential high conversion of different substrates for hydrogen production and growth (Koku et al., 2002).

1.2 Problem Statement

The world today relied heavily on fossil fuel as its source of energy. This fuel is used as transportation fuel in vehicles and contributes to global warming by releasing carbon dioxide in to the atmosphere that adds to greenhouse effect. The source is not renewable and a shortfall in fossil fuel has long been predicted. Despite this, it is not possible to terminate the use of fossil fuel easily because of its global energy and economic benefits. However, an alternative to fossil fuel is needed that can effectively serve as replacement. As the search for renewable energy intensifies, hydrogen has been identified as a clean and promising fuel for the future with ability to reduce most of the problems arisen from the use of fossil fuels. At present, sources of hydrogen production methods rely heavily on fossil with methane contributing to over 50% of commercial hydrogen production today. The methods are not carbon neutral which necessitates the need for an alternative source that is cost effective, renewable and carbon neutral. Biological hydrogen production with photosynthetic microorganisms has been identified as a potential source of hydrogen generation. Marine environment is a rich ecosystem, which serves as a habitat for many photosynthetic bacteria which makes it a reliable source of biohydrogen producers.

1.3 Objectives

2. To isolate photosynthetic bacteria from marine environment
3. To screen the isolated photosynthetic bacteria for hydrogen production.
4. To identify and characterise the photosynthetic hydrogen producers using 16S rRNA characterisation.

1.4 Scope of Study

Photosynthetic bacteria (PSB) were isolated from marine environments in this study. The bacteria isolated were then be evaluated for hydrogen production using a residual gas analyser (RGA), available at the Faculty of Chemical Engineering, Universiti Teknologi, Malaysia (UTM). The PSB with hydrogen producing ability were identified further using physical, biochemical and 16S rRNA characterizations.

1.5 Significance of Study

Marine environments have been identified as major source of new natural products. It harbours many photosynthetic bacteria that survive under harsh conditions. This study is hoped to increase our understanding of the role of photosynthetic bacteria isolated from marine environments in hydrogen production. It will serve as a preliminary to further research on commercial hydrogen production from marine environments.

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