ISOLATION AND CHARACTERIZATION OF PHOTOSYNTHETIC BACTERIA FROM AQUACULTURE WASTEWATER FOR HYDROGEN PRODUCTION

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Dedicated to my beloved parents, siblings and beloved Haifa

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ABSTRACT

Hydrogen is a clean energy substitute to fossil fuels, also environmentally friendly and does not lead to global warming. Photosynthetic bacteria produce hydrogen from organic compounds by an anaerobic light-dependent electron transfer process. Two hydrogen-producing photosynthetic bacteria have been isolated from aquaculture wastewater enriched with succinate (electron donor) under facultative condition. The isolated bacteria were identified as Gram negative and rod shaped bacteria. During the photohydrogen production process, hydrogen gas is produced at 30ºC, initial pH of 7, and light intensity provided using white fluorescent lamp. The two isolated bacteria were then screened for hydrogen production using acetate and starch as substrate. Hydrogen production was measured using residual gas analyzer. These strains have the ability to utilize starch better than acetate as carbon sources for hydrogen production. The two strains, designated as AQ1 and AQ4, were identified to be Pseudomonas sp. via 16S rRNA characterization. The sequences have been deposited to GenBank with the accession number KJ854409 for Pseudomonas sp. AQ1 and KJ854410 for Pseudomonas sp. AQ4. In conclusion, the two isolates (AQ1 and AQ4) have been characterized as hydrogen producer in photohydrogen production process using starch and acetate as substrate. They have the potential to be used in simultaneous wastewater treatment and clean energy generation.

ABSTRAK

Hidrogen adalah pengganti tenaga bersih untuk bahan api fosil, mesra alam sekitar serta tidak menyumbang kepada pemanasan global. Bakteria fotosintetik menghasilkan hidrogen daripada sebatian organik oleh proses pemindahan elektron bergantung kepada cahaya dalam keadaan anaerobik. Dua bakteria penghasil hidrogen telah diacingkan daripada airsisa akuakultur yang diperkaya dengan suksinat (penderma elektron) di bawah keadaan fakultatif. Bakteria yang terpencil telah dikenal pasti sebagai Gram negatif dan berbentuk rod. Semasa proses foto penghasilan hidrogen, gas hidrogen telah dihasilkan pada 30ºC, pH 7, dibawah lampu pendarfluor putih. Bakteria terpencil itu kemudiannya telah digunakan untuk penghasilan hidrogen menggunakan asetat dan kanji sebagai substrat. penghasilan hidrogen ini diukur dengan menggunakan *Penganalisis Gas Sisa*. Strain ini mempunyai keupayaan untuk menggunakan asetat dan kanji sebagai sumber karbon bagi pengeluaran hidrogen. Strain AQ1 dan AQ4, telah dikenal pasti sebagai *Pseudomonas* sp. melalui pencirian 16S rRNA, dan telah didepositkan ke GenBank dengan *accession number* KJ854409 and KJ854410. Kesimpulannya, strain ini (AQ1 dan AQ4) telah dikenalpasti sebagai penghasil hidrogen di dalam proses penghasilan foto-hidrogen menggunakan kanji dan asetat sebagai substrat. Strain-strain ini mempunyai potensi untuk digunakan di dalam proses rawatan air sisa dan penghasilan tenaga bersih secara serentak.

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CHAPTER 1

INTRODUCTION

1.1 Background

Hydrogen is a cleaner fuel that can potentially substitute fossil fuels (Barbosa *et al*., 2001; and Koku *et al*., 2003). Hydrogen is known as an energy carrier that does not cause pollution, environmentally friendly and does not lead to global warming (Melis, 2006). Furthermore, hydrogen can be used for electricity generation (Yu; and Takahashi, 2007). The sun energy can be used by photosynthetic bacteria (PSB) for producing hydrogen. At the same time, a small amount of organic acids will be consumed (Melis, 2005; Barbosa *et al*., 2001).

There are several parameters such as light intensity, substrate concentration, and initial pH affecting hydrogen production (Koku *et al*., 2003). Photo hydrogen production is a well-developed technology, thus PSB uses recycled organic materials and does not need fossil fuels as input (Kondo *et al*., 2002; Kondo *et al*., 2006). Now the technology is rapidly developing to improve hydrogen production and efficiency of conversion (Kondo *et al*., 2002).

There are varieties of biological ways to produce hydrogen from either water, organic acids or from biomass (Melis, 2006). Many prokaryotes with different groups have the ability to produce hydrogen. This is especially true for bacteria that belong to photosynthetic bacteria family. They are characterized by the ability to produce hydrogen from organic substrates with light dependency (Sunita, and Mitra, 1993). PSB has two different pathways of energy metabolism and the ability to live in both dark-anaerobic and light-anaerobic conditions (Lu *et al*., 2011).

Both PSB and anaerobic bacteria have the ability to provide a photohydrogen production system by using residual carbohydrates as substrates (Barbosa *et al*., 2001). Hyvolution is a hybrid system of dark and photofermentation bacteria, which lead to higher yield of hydrogen. In this integrated project, biomass is used by dark fermentation (thermophilic bacteria) for hydrogen production, then the products of dark fermentation is utilized for photofermentation (by photosynthetic purple non-sulfur bacteria) (Boran *et al.*, 2010; Gebicki *et al.*, 2010; and Wu *et al*., 2010). Ordinarily obligate and facultative anaerobic bacteria such as *Clostridium*, *Escherichia* and *Enterobacter* have the ability to produce high rate of hydrogen from glucose, but the produced hydrogen will be limited due to the production of organic materials. These bacteria can also be co-cultured with photosynthetic bacteria to gain a higher yield of hydrogen from glucose (Asada *et al.*, 2006; and Asada, 2008).

1.2 Problem Statement

The production and utilization of fossil fuels causing are negative impacts on the environment. Combustion of fossil fuels leads to the emission of greenhouse gases into the atmosphere. In response, hydrogen has emerged as an attractive substitute to the conventional fuels, as the combustion product is only water. Various sources for feasible hydrogen production are being researched, and production from biological sources is of high potential. Hydrogen production by using photosynthetic bacteria is being actively studied. They are known as the most powerful hydrogen producers, as the use of the photobiological hydrogen evolution could provide a very simple and efficient system of solar energy conversion. So, PSB with efficient hydrogen production need to be identified, from various sources.

1.3 The Objectives of the Study

1. To isolate and characterize photosynthetic bacteria (PSB) from aquaculture wastewater.

2. To screen the isolated PSB for hydrogen production.

3. To identify the photosynthetic hydrogen producer by using 16S rRNA characterization.

1.4 Scope of Study

In this study PSB were isolated from aquaculture wastewater. Isolation was performed in several steps, such as dilution, shake, and plate cultivation. Furthermore, the colonies and cells of isolated PSB were characterized and the pigment was screened. PSB were then screened for the ability to produce hydrogen. Identification of hydrogen producer was done using 16S rRNA technique.

1.5 Significance of Study

This study contributed to the knowledge on the production hydrogen from biological sources, especially via photosynthetic method. Hydrogen is a clean energy source and can be used as feedstock for some industries. Therefore, demand on hydrogen production has increased considerably in recent years. This PSB isolated in this study has the potential to be applied in photosynthetic bioreactors, such as for waste treatment, with usable energy generation.

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