STARCH DEGRADING BACTERIA FOR BIOHYDROGEN PRODUCTION

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

Faculty of Biosciences and Medical Engineering
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JANUARY 2016

In loving memory of my late grandparents Sheikh Muhammad Bello Gusau and Hajiya Fatima (Inno). May Allah forgive their shortcomings and make Jannatul Firdaus to be their final abode.

ACKNOWLEDGEMENT

All thanks are to Allah most Beneficent the most Merciful, for His unquantifiable favour and specifically for keeping me healthy and alive to witness this memorable time of my academic pursuit. May His peace and Blessing be upon the pathfinder, the incomparable General, Prophet Muhammad (S.AW), members of his family, his companions and all those who responded to this call up to the Day of Judgement.

First and foremost, I would like to express my deepest gratitude to my able intellectual supervisor, Dr. Mohd Firdaus Abdul Wahab, I remain forever grateful for his support, guidance, patience and encouragement throughout the period of the research. Thank you for disciplining my professional life. Special thanks and appreciation to my parents, Khalifa Attahiru Bello and Hajiya Fatima, whom their support both financially and morally can never be estimated, and for cultivating in me the habit of early rising. May Allah reward them abundantly AMEEN.

Next, I would like to thank all the staffs in FBME and postgraduate students who had guided me throughout the project. I am grateful for their advice and motivation without which this thesis would not have been the same as presented here.

Last but not least, I would like to extend my deepest and sincere appreciation to my family members. Thank you for their unconditional love and support at all time. Besides, I would like to express my gratitude to all my friends and colleagues for their help and encouragement. May almighty Allah bless and enrich each and every one for his or her contributions.

ABSTRACT

Global research is moving forward in developing biological production of hydrogen as a renewable energy source in order to reduce the use of non-renewable energy. Biohydrogen has the potential of replacing the hydrogen production by fossil fuels which is one of the major factors that cause the global warming. The amylolytic activity of several microorganisms capable of utilizing starch in the dark fermentation process has provided a great potential in biohydrogen production by the starch degrading bacteria. In this study, starch utilizing bacteria have been successfully isolated from cassava chips processing wastewater and sludge. Based on the morphological characteristics, the isolate was found to be Gram positive bacteria with spherical cell shape. 16S rRNA analysis identified the strain *Lactococcus* sp. SDB4 with 92% similarity to *Lactococcus lactis.* The partial 16S rRNA sequence has been deposited to GenBank with Accession Number KU160544. Furthermore, the isolate was subjected to anaerobic dark fermentation process using synthetic media with tapioca starch as the only source of carbon. The findings in this study indicate that the *Lactococcus* sp. SDB4 possesses an amylolytic activity and capable of utilizing starch efficiently (75%). This strain has the potential to be used in biohydrogen production using starch containing wastewater as substrate.

ABSTRAK

Penyelidikan global dalam pembangunan pengeluaran biohydrogen mengurangkan sumber tenaga yang boleh diperbaharui semakin berkembang untuk mengurangkan penggunaan sumber tenaga yang tidak boleh diperbaharui. Biohidrogen berpotensi untuk menggantikan pengeluaran hydrogen daripada bahan api fosil yang merupakan salah satu faktor utama pemanasan global. Pembangunan mikroorganisma yang boleh menggunakan kanji berdasarkan aktiviti amylolytic dalam proses fermentasi gelap menyumbang kepada potensi pengeluaran biohydrogen. Dalam kajian ini, bacteria yang boleh menggunakan kanji telah berjaya diperoleh daripada sisa pemprosesan kerepek ubi kayu. Daripada segi morfologi, sel bacteria tersebut merupakan bakteria Gram positif yang berbentuk sfera. Berdasarkan analisis 16S rRNA, bacteria tersebut merupakan *Lactococcus* sp. SDB4 dan mempunyai 92% persamaan dengan *Lactococcus lactis.* Julukan 16S rRNA bacteria tersebut telah didaftarkan ke dalam GenBank dengan nombor pendaftaran KU160544. Bakteria teersebut diperoleh melalui proses fermentasi gelap yang menggunakan media sintetik yang ditambah kanji ubi kayu sebagai sumber karbon. Kajian ini juga menunjukkan bahawa bakteria *Lactococcus* sp. SDB4 mempunyai aktiviti amylolytic dan berupaya untuk menggunakan (75%) kanji. Bakteria ini berpotensi untuk diaplikasi dalam proses pengeluaran biohidrogen bersumberkan sisa air mengandungi kanji sebagai substrat.

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LIST OF ABBREVIATIONS AND SYMBOLS

ABI	-	Application Binary Interface
ATP	-	Adenosine Triphosphate
B.	-	Bacillus
BLAST	-	Basic Local Alignment Search Tool
bp	-	Base pairs
CaCl2	-	Calcium chloride
CH4	-	Methane
cm	-	Centimeter
CO2	-	Carbon dioxide
Da	-	Dalton
dNTPs	-	Deoxynucleotide triphosphates
DNA	-	Deoxyribunuclei Acid
E value	-	Expected value
EDTA	-	Ethylenediaminetetraacetic Acid
EtBr	-	Ethidium bromide
et al.	-	And others
ESWA	-	Enriched Starch Wastewater Agar
FeCl3.5H2O	-	Iron (III) chloride
g	-	Gram
g/L	-	Gram per Litre
GC-TCD	-	Gas Chromatography Thermal Conductivity
H2	-	Hydrogen gas
HCl	-	Hydrochloric acid
H_2O	-	Water
H_2S	-	Hydrogen Sulfide
kDa	-	Kilo Dalton

kb	-	Kilo base
kbp	-	Kilo base pair
$KJ\ g^{-1}$	-	Kilo Jole per gram
K_2HPO_4	-	Dipotassium hydrogen phosphate
KH_2PO_4	-	Potassium dihydrogen phosphate
L	-	Liter
LAB	-	Lactic Acid Bacteria
MEGA	-	Molecular Evolutionary Genetics Analysis
MgCl2	-	Magnesium chloride
MgSO4.7H2O	-	Magnesium sulphate
µ	-	Specific growth rate
µL	-	Microliter
µg	-	Microgram
µm	-	Micrometer
µmax	-	Maximum growth rate
mL	-	Mililiter
mg/L	-	Milligram per liter
mM	-	Milimolar
M	-	Molar
NaCI	-	Sodium chloride
NaOH	-	Sodium hydroxide
NCBI	-	National Center of Biotechnology Information
NH_4NO_3	-	Ammonium nitrate
ng/ µL	-	Nano gram per microliter
nm	-	Nano meter
NO_2	-	Nitrogen
OD	-	Optical Density
PCR	-	Polymerase Chain Reaction
POME	-	Palm Oil Mill Effluent
RNA	-	Ribonucleic acid
rpm	-	Revolutions per minute
rRNA	-	Ribosomal ribonucleic acid
RNase	-	Ribonuclease
SDB	-	Starch Degrading Bacteria

sp.	-	Species
TAE	-	Tris-Acetate electrophoresis buffer
td	-	Doubling time
UV	-	Ultraviolet
V	-	Voltage
VFA	-	Volatile fatty acids
v/v	-	Volume per volume
w/v	-	Weight per volume
α	-	Alpha
β	-	Beta
°C	-	Degree Celsius
%	-	Percent
× g	-	Times gravity

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CHAPTER 1

INTRODUCTION

1.1 Background of the Research

The current global economy and energy needs are being covered by fossil fuels. This has led to hyper consumption of these non-renewable resources which in turn has resulted in both the increase in CO_2 concentration in the atmosphere as well as rapid depletion of fossil fuels. The combustion of fossil fuels and its by-product contribute to the increase emission of greenhouse gas (CO_2, NO_2, CH_4 and other chemicals) which is responsible for the current global warming and climate change (Show *et al.*, 2012). For these reasons, a novel and safe energy carriers are introduced that substitute fossil fuels. Among the various alternative sources developed, hydrogen is considered the cleanest of all renewable energy source and the most promising fuel with high energy content (142KJ g^{-1}) (Rosales-Colunga and Rodríguez, 2014; Thakur *et al.*, 2014)

Hydrogen is considered to be the inspiring fuels for the environment and economy because it produces a non-toxic substance (water) upon combustion and provide all the requirements for a clean and renewable fuel (Ramprakash and Muthukumar, 2014). In addition, hydrogen is safer to handle than domestic natural gas and it can be used directly in internal combustion engines or in fuel cell to generate electricity (Boboescu *et al.*, 2014). Despite the fact that hydrogen has many social, economic and environmental benefits, but (Kotay and Das, 2008) revealed that the major problem encounter in the utilization of hydrogen gas as a fuel is its non-availability in nature and its production is very expensive.

1.2 Production of Hydrogen

Conventionally, hydrogen is produced in different forms of processes which includes electrolysis, photolysis, thermochemical and thermolysis process of water (Audu and Wahab, 2014). (Keskin and Hallenbeck, 2012) revealed that hydrogen can also be produced from fossil fuels by steam reforming or thermal cracking of natural gas, partial oxidation of hydrocarbons and coal gasification or pyrolysis. However, these processes are very expensive, mostly requires an external energy source for the process to function and also not constantly eco-friendly (Kanso *et al.*, 2011).

In the last few years, attention has shifted towards the novel and less energy intensive technologies for the hydrogen production. Biological production of hydrogen arises as an ideal way for hydrogen production due to its flexibility, low energy demand, low cost as its requires organic wastes for the process and also very environmental friendly (Kim *et al.*, 2011).

The biohydrogen production not only provide a clean fuel, but also provide the reduction of environmental pollution from the uncontrolled degradable organic waste (Keskin and Hallenbeck, 2012). As reported by (Rosales-Colunga and Rodríguez, 2014) 40% of hydrogen is produced from natural gases, 30% from heavy oil and naphtha, 18% from coal, 4% from electrolysis and only 1% from biomass.

1.3 Applications of Hydrogen

Hydrogen is a colourless, odourless and non-toxic gas, and also a promising fuel with high energy content which is said to be 2.75 times higher energy yield than hydrocarbon fuels(Ramprakash and Muthukumar, 2014). It has a high calorific value mass 122 KJ g^{-1} which is significantly higher than gasoline (Patel *et al.*, 2015).

Hydrogen is widely used in many industries; it is used for the production of ammonia and methanol as well as hydrogenation of fats and oils in the food industry and production of electronic devices (Rosales-Colunga and Rodríguez, 2014). Refineries used it in steel processing, reformulation of gasoline and production of compounds with low molecular weight (Audu and Wahab, 2014; Kapdan and Kargi, 2006).

In the transportation sector, hydrogen also plays a vital role where it can be used as a fuel for vehicles due to its non-polluting nature thereby reducing significantly green house gas emission (Audu and Wahab, 2014; Show *et al.*, 2011). Among the electrical generators, hydrogen serves as an oxygen scavengers and a coolant which can be used in the generation of electricity by converting chemical energy into electrical energy. In addition, hydrogen have a high “low heating value” (LHV) which can be used in fuels cells as well as internal combustion engines (Patel, *et al.*, 2015).

1.4 Problem Statement

Over the years fossil fuels have been the main source of energy and global economy. The massive use of these fossil fuels has not only brought environmental threats and health problems, it has also resulted in energy shortage globally and emission of green house gases like carbon dioxide (CO_2), nitrogen (NO_2) and methane. The emission of these gases has contributed to the current global warming and climate change.

To overcome the global warming and energy shortage crisis, there is a need to search for alternative renewable energy sources. Hydrogen is considered as one of the promising fuel in the future for its renewable energy sources, low cost and inexhaustible. Microbial hydrogen production through fermentation using waste materials is an attractive option as the waste materials are readily available and the fermentation process is technically feasible. Several studies have been done on the biohydrogen production using different substrate but the relatively low yield of hydrogen, cost of production and unstable hydrogen production are the major challenges of biohydrogen production (Show, *et al.*, 2012). The capability of starch utilizing bacteria to hydrolyze starch in wastewater and sludge and produce hydrogen via anaerobic fermentation provides an advantage for cost competitive and sustainable hydrogen production. Hence, this underscores the need to identify and characterize the bacterial strains that can utilize starch efficiently with a hydrogen production potential.

1.5 Research Objectives

This study was designed to address the following objectives:

(1) To isolate starch degrading bacteria from cassava chips industrial waste

(2) To screen for starch utilizing ability qualitatively

(3) To identify phylogenetic characteristics of the selected isolate based on molecular techniques.

1.6 Significance of the Research

The increasing public concerns on the environmental problems and energy crisis has urged the development of an alternative clean fuel to substitute the conventional fossil fuels, in which biohydrogen is among the promising ones. Therefore, starch utilizing bacteria isolated from cassava chips processing wastewater and sludge is identified and characterized using 16S rRNA sequencing. The preliminary study conducted here will provide an insight for possibility of hydrogen gas production via dark fermentation by using starch-based waste materials as substrate. From the isolation and identification of the bacteria isolated, further studies on the hydrogen-producing ability of the bacteria can be carried out, allowing the hydrogen gas to be commercially available as clean fuel in the future.

1.7 Scope of the Research

This study focused on the isolation of starch degrading bacteria from cassava chips processing industrial waste sample (wastewater and sludge) in enriched starch wastewater media. The isolates obtained were screened for starch utilization and 16S rRNA sequence analysis. The isolates will then be evaluated for hydrogen production using gas chromatography (GC) equipped with thermal conductivity detector (TCD).

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