ISOLATION, IDENTIFICATION AND CHARACTERISATION OF LIGNOCELLULOLYTIC BACTERIA FROM MANGROVE ROOTS

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

Faculty of Biosciences and Medical Engineering
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JULY 2018

To my beloved family

ACKNOWLEDGEMENT

First and foremost, I would like to express my highest gratitude towards my supervisor, Dr. Chong Chun Shiong for his continuos supports, patience, encouragement, immense knowledge and time spent for me throughout my master journey. . His guidance helped me to get through all the difficulties and problems faced along conducting the research and completing the thesis writing. I could not have imagined having a better supervisor for my research project.

Deepest appreciation for my all my lab members especially my seniors, Lam, Jinn and Suganthi for their knowledge sharing, guidance, advices, and assistances given in order for me to complete this project. My sincere thanks also goes to my co-supervisor, Puan Huszalina bt. Hussin for her guidance and help in completing the project. Not to forget, million thanks to all FBME lab assistants and staffs for their limitless guidance and assistances in term of knowledge and facilities along the project conducted. I also thank my fellow classmates especially Adila, Aimi, Azian, Najwa and Christine for the companions, opinions, motivations and entertainment along my journey here.

Most importantly, my token of appreciation goes to my beloved family, who has been the constant resources of strength, motivations, and supports in term of time and financial for all the time.

I also place on record, my sense of gratitude to everyone, who directly or indirectly lend their hands for me in the journey of the study.

ABSTRACT

Globally, 998 million tonnes of agricultural waste is disposed into landfills per year and Malaysia contributes 1.2 million tonnes of the amount. The waste consists of lignocellulosic biomass, a chainlike sugars called cellulose and hemicellulose embedded in a woody material called lignin, which could be converted into biofuel. Enzymes secreted by microorganisms are needed for the conversion of these materials into biofuels. One of good sources to find the microorganisms that are able to degrade the lignocellulosic materials is mangrove. It is a rich environment containing plenty of decayed plant materials, thus it could be a potential resource of highly productive and diverse microbial community. In this study, five bacterial strains have been isolated from mangrove roots collected in Tanjung Piai, Johor. From the lignocellulolytic screening, CN4, CN7 and CN10 showed positive results for cellulose degradation, CN10 and CN12 for xylan degradation, and CN20 for lignin degradation. Strain CN10 was selected for further investigation due to its abilities to degrade both cellulose and xylan. Gram-staining performed showed that CN10 is a Gram-positive rod-shaped bacterium. Based on 16S rRNAgene sequence analysis, the selected strain was identified as *Exiguobacterium* sp. CN10. Growth profile was carried out at 35°C, pH 7 and 5% (w/v) salt using tryptic soy broth as medium. The effect of temperature, pH and salinity on cellulose and xylan degradation using crude enzyme of CN10 were investigated. For cellulose degradation, the optimal temperature, pH and salinity for strain CN10 were 50°C, pH 8.0 and 12% (w/v) respectively. For xylanase activity, the optimal temperature and pH for the strain were also at 50°C and pH 8.0, while the optimal salinity was at 10% (w/v) salt. Collectively, the findings suggested that the strain CN10 may have a great potential in the lignocellulosic biomass degradation which could be of great commercial value.

ABSTRAK

Secara global, sebanyak 998 juta tan sisa pertanian dibuang ke tapak pelupusan sampah setiap tahun dan Malaysia menyumbang sebanyak 1.2 juta tan daripada jumlah tersebut. Sisa buangan tersebut terdiri daripada biojisim lignoselulosik, satu rantaian gula dipanggil selulosa dan hemiselulosa yang tersirat dalam bahan kayuan dipanggil lignin, yang boleh ditukar menjadi biofuel. Enzim yang dirembes oleh mikroorganisma diperlukan untuk pertukaran bahan ini menjadi bio-fuel. Salah satu sumber terbaik mendapatkan mikroorganisma yang boleh mendegrad bahan lignoselulosik ialah bakau. Bakau ialah persekitaran yang kaya dengan tumbuhan yang sedang mereput, justeru ia adalah satu sumber potensi untuk komuniti mikroorganisma yang sangat produktif dan pelbagai. Dalam kajian ini, lima strain bakteria telah diasingkan dari akar bakau yang diambil dari Tanjung Piai, Johor. Daripada saringan lignoselulosik, CN4, CN7 dan CN10 menunjukkan hasil positif untuk degradasi selulosa, CN10 dan CN12 untuk degradasi xilan, dan CN20 untuk degradasi lignin. Strain CN10 telah dipilih untuk kajian seterusnya disebabkan kebolehannya untuk mendegrad selulosa dan xilan. Pewarnaan Gram yang dijalankan menunjukkan bahawa CN10 adalah bakterium Gram-positif yang berbentuk rod. Berdasarkan analisis jujukan gen 16S rRNA, strain terpilih dikenalpasti sebagai *Exiguobacterium* sp. CN10. Profil pertumbuhan dijalankan pada suhu 35°C, pH 7.0 dan 5% (w/v) garam menggunakan kaldu tripsin soya sebagai medium. Kesan suhu, pH dan saliniti terhadap degradasi selulosa dan xilan menggunakan enzim mentah CN10 telah dikaji. Bagi degradasi selulosa, suhu, pH dan saliniti optimal untuk strain CN10 masing-masing adalah 50°C, pH 8.0 dan 12% (w/v). Bagi aktiviti xilan, suhu dan pH optimal untuk strain ini adalah 50°C dan pH 8.0, manakala saliniti optimal adalah pada 10% (w/v) garam. Secara keseluruhan, hasil carian ini memberi cadangan tentang potensi besar strain CN10 dalam degradasi bahan lignoselulosik yang boleh memberi hasil komersial yang besar.

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LIST OF ABBREVIATIONS

$NH_4H_2PO_4$	-	Ammonium dihydrogen phosphate
BLASTn	-	Basic Local Alignment Search Tool of Nucleotide
CMC	-	Carboxymethylcellulose
dNTPs	-	Deoxynucleotide triphosphates
DNA	-	Deoxyribonucleic acid
DNS	-	dinitrosalicylic acid
et al.	-	et alia
EDTA	-	Ethylenediaminetetraacetic acid
HCl	-	Hydrochloric acid
$FeCl_3$	-	Iron(III) chloride
$MgCl_2$	-	Magnesium chloride
MEGA 7		Molecular Evolutionary Genetic Analysis version 7.0
NCBI	-	National Center for Biotechnology Information
ND		Not determined
OD	-	Optical density
PCR	-	Polymerase chain reaction
pH	-	Potential of hydrogen
RNase	-	Ribonuclease
NaCl	-	Sodium chloride
NaOH	-	Sodium hydroxide
TAE	-	Tris-Acetic-EDTA
TSA		Tryotic soy agar
TSB		Tryptic soy broth
UV	-	Ultraviolet
16S rRNA	-	16 Subunit Ribsomal Ribonucleic Acid

LIST OF SYMBOLS

α	-	Alpha
β	-	Beta
bp	-	Base pair
℃	-	Celsius
CFU	-	Colony forming unit
°	-	Degree
g	-	Gram
g/L	-	Gram per litre
h	-	Hour
∞	-	Infinity
kb	-	kilo basepair
µL	-	microlitre
mA	-	milliampere
mg	-	milligramme
mL	-	millilitre
mm	-	millimetre
mV	-	millivolt
M	-	Molarity
ng/µL		nanogram per microlitre
nm	-	nanometer
-	-	Negative

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

The world is gradually marching towards a severe energy crisis, in which the demand of energy is overstepping its supply. Fossil fuel is an example of energy source that is still becoming the top global demand despite its dwindling resources (Mohapatra, 2017). This scenario leads to development of diverse research to find the alternative sources that can replace the dependence towards fossil fuels as the source of energy in order to meet future demands. One of the alternative sources of energy that is being widely studied is the biofuel produced from lignocellulosic materials (Dhiman, 2009). Other than becoming sources for biofuel, lignocelluloses can also be a resource for structural materials such as paper and fiber.

Lignocellulosic materials are one of promising alternative resources due to its continuous availability as they are the main components found in plant materials. Many studies have reported that lignocellulosic biomass holds enormous potential for sustainable production of fuels. Baêta*et al.*(2016) reported the optimisation of net energy recovery from production of hydrogen and methane through anaerobic digestion of the hemicelluloses hydrolysate obtained from pretreatment of sugarcane bagasse. Another study by Faraco and Hadar (2011) focused on the production of bioethanol from degradation of lignocellulosic wastes such from cereal crops, tomatoes and olive trees in Mediterranean Basin. To achieve the production of biofuels, the lignocellulosic materials need to be degraded by certain

enzymesfollowed by fermentation process, in which the source of the enzymes can be originated from microorganisms.

Vast numbers of studies have been done for the detection of lignocelluloses degrading microorganisms using agrowastessources like palm residues, sugarcanes wastes, rice straws and others. Microorganisms in the environment like the mangrove would have necessary enzymes such as the lignolytic enzymes, hemicellulases and cellulase to breakdown the biopolymer into simple sugars for glycolysis and respiration. These microorganisms are typically distributed in the intertidal zones with the fluctuating temperature, pH, salinity and tidal (Kathiresan and Bingham, 2001). The sediments and plant materials of the mangrove area is one of the suitable environment to explore lignocelluloses degrading microorganisms because of continuous input of lignocellulosic carbon in the form of litter which can function as a substrate for decomposition by microbes (Behera*et al*., 2017). Due to the richness of nutrients and its unique environment, this location could potentially be the source to obtain novel microorganisms for lignocelluloses degradation.This study focused on the identification and characterization of bacteria from mangrove partss to be the source of enzymes for degradation of lignocellulosic materials such as from agricultural wastes.

1.2 Problem Statement

The increasing demand of the energy derived from fossil fuels has been one of the global problems as its supply cannot fulfil the demand due to the depletion of this particular resource. Hence, alternative source of energy must be developed in order to prevent the energy shortage as well as to meet the future demands.

One of the alternatives for sustainable and renewable energy that can be a promising source due to their abundance on the earth is lignocellulosic materials. The degradation of these materials can lead to the production of biofuels such as ethanol, methane and hydrogen. For the lignocellulosic materials to be degraded into sugars

before transforming into biofuels, presence of respective enzymes are needed and microorganisms can be the resources for these enzymes. In natural environments, microorganisms can effectively degrade lignocellulose materials and the degraded compounds could be used as their carbon and energy source for growth. Hence, these microorganisms could be the potential sources of biocatalysts for plant biomass into biofuel.

Most of the researches concentrated on fungi as the lignocellulose degrader. However recently, the role of bacteria as the lignocelluloses degrader begin to receive more attention as they are more cost-efficient because bacteria can grow more rapidly, produce multi-enzyme complexes with increased functionality and higher specificity (Maki *et al*., 2009).

So far, the microbial degradation of lignocelluloses biomass from mangrove is not well characterized as it is from other sources such as agroindustrial biomass. Hence, this study was conducted using the sample from mangrove area to identify the roles and abilities of the microorganisms from mangrove area in the degradation of lignocellulose materials.

1.3 Objectives of the Study

1) To isolate and screen of the lignocellulolytic bacteria from mangrove roots
2) To identify the bacteria using 16S rRNA gene analysis
3) To characterize the bacteria from the aspects of physiology and its cellulolytic enzyme activity

1.4 Significance of Study

As the supply for the fossil-derived fuels has become limited, the development of studies involving the degradation of lignocellulolytic materials as a

source for energy production is significantly increase because this approach can provide renewable and sustainable resources. The degradation of lignocelluloses must be aided with presence of effective enzymes, which can naturally be produced by microorganisms. Nigam (2013) stated that bacteria is one of the good sources of enzyme, the one with the ability to degrade lignocellulosic materials can become interesting research area. Lignocellulolytic bacteria from mangrove roots can potentially be one of the microbial sources that can be used to degrade lignocellulosic biomass, which then could be converted to produce valuable end product such as biofuel. The isolation of lignocellulolytic bacteria from mangrove roots is a potential source for the discovery of novel lignocellulolytic bacteria as the environment provides unique conditions for the microbial growth.

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