UNIVERSITI PUTRA MALAYSIA

CHARACTERISATION OF CELLULOLYTIC ACTIVITY AND BIOCONVERSION OF PALM KERNEL CAKE BY LACTIC ACID BACTERIA

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CHARACTERISATION OF CELLULOLYTIC ACTIVITY AND BIOCONVERSION OF PALM KERNEL CAKE BY LACTIC ACID BACTERIA

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Thesis submitted to the Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia in fulfilment of the requirement for the Bachelor of Science (Hons.) Biotechnology

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FACULTY OF BIOTECHNOLOGY AND BIOMOLECULAR SCIENCES
UNIVERSITI PUTRA MALAYSIA

Date : 2 June 2015

LETTER OF PERMISSION

It is hereby to approve that I, **Wan Suet Ying (Matric No: 161407)** have done my final year project entitled **"Characterisation of cellulolytic activities and bioconversion of palm kernel cake by lactic acid bacteria"** under supervision of Assoc. Professor. Dr. Foo Hooi Ling from Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia.
I hereby give the permission to my supervisor to write and prepare manuscript from the results of my research to be published in any form if I did not do it in six (6) months from the above date. The addition of my name in the manuscript depends on the supervisor herself.

Yours sincerely,

……
(Wan Suet Ying)

FACULTY OF BIOTECHNOLOGY AND BIOMOLECULAR SCIENCES
UNIVERSITI PUTRA MALAYSIA

Date : 2 June 2015

APPROVAL SHEET

This attached project report entitled **"Characterisation of cellulolytic activities and bioconversion of palm kernel cake by lactic acid bacteria"** was prepared by **Wan Suet Ying (Matric No: 161407)** in fulfilment of the requirement for Degree of Bachelor of Science (Honour) Biotechnology in the Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia.

Approved by,

……..
Assoc. Prof. Dr. Foo Hooi Ling
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ABSTRACT

Abstract of thesis presented to the Faculty of Biotechnology and Biomolecular Sciences in fulfilment of the requirement for the Degree of Bachelor of Science (Hons.) Biotechnology

CHARACTERISATION OF CELLULOLYTIC ACITIVITIES AND BIOCONVERSION OF PALM KERNEL CAKE BY LACTIC ACID BACTERIA

By:

Wan Suet Ying
June 2015

Supervisor : Assoc. Professor Dr. Foo Hooi Ling
Faculty : Faculty of Biotechnology and Biomolecular Sciences

Cellulolytic enzyme is becoming more important due to its application in agro-wastes, which appear to be a potential substitution for production of bioenergy and biofuel. Lactic acid bacteria (LAB) isolated from Malaysian fermented food (*Lactobacillus plantarum* RG14, RG11, RI11, RS5, TL1, I-UL4 and B4) were characterised for their cellulolytic activities (CMCase, FPase, β-glucosidase, xylanase and mannanase assays) under different pH conditions (pH 5, 6.5 and 8). Two quantification methods (Bradford and modified Lowry methods) of protein concentration were conducted for the determination of specific celluloytic activity. Specific cellulolytic activities were 45 to 52 times higher when the protein concentration of cell-free supernatant was determined by Bradford method as compared to modified Lowry method. *L. plantarum* RG14, RG11 and RI11 were observed with higher scoring in overall cellulolytic activity and hence they were selected for biotransformation of palm kernel cake (PKC) via solid state fermentation (SSF) for 14 days with 2-day sampling intervals. The PKC extract was collected from fermented PKC to determine the cellulolytic activities and LAB population. The highest LAB cell population was observed at day 2 PKC extract treated by *L. plantarum* RG14 and RG11 with 8.15 log CFU/mL and 8.11 log CFU/mL, respectively. As for *L. plantarum* RI11, the highest cell population was noted at day 4 with 8.13 log CFU/mL. The cell population of LAB was maintained throughout the SSF. The solubilised protein concentration of PKC extract determined using modified Lowry method increased

according to the SSF period, whereas a decrement in solubilised protein concentration was observed when Bradford method was employed. This might due to lower sensitivity and linearity in Bradford method in comparison to modified Lowry method. Hence, the specific cellulolytic activity of PKC extract was determined using modified Lowry method under pH condition at 5. The CMCase activity was enhanced at day 6, followed by a drastic decline. The β-glucosidase activity was increased during the SSF with the highest activity observed at day 12 when PKC was treated by *L. plantarum* RG14 and day 14 by *L. plantarum* RG11 and RI11respectively. A significant enhancement in specific mannanase activity was exhibited at the 8^{th} day of incubation. Unfortunately, no FPase activity was detected throughout the fermentation period. Interestingly, the specific cellulolytic activity obtained through submerged fermentation in MRS broth was higher as compared to PKC extract obtained through SSF. This was due to MRS broth provided sufficient moisture and nutrient to support the growth and enzyme production in LAB isolates. In conclusion, the LAB isolates were able to produce versatile cellulolytic enzymes to degrade various forms of polysaccharides and PKC. Thus, the cellulolytic enzymes of LAB possessed vast potential for biotransformation of biomass.

ABSTRAK

Abstrak tesis dihasilkan bagi Fakulti Bioteknologi and Sains Biomolekul untuk memenuhi keperluan Ijazah Sarjana Muda Sains (Hons) Bioteknologi

PENCIRIAN AKTIVITI CELLULOLYTIC DAN PENUKARAN BIO UNTUK HAMPAS ISIRONG KELAPA SAWIT OLEH BAKTERIA ASID LAKTIK

Oleh:

Wan Suet Ying
Jun 2015

Penyelia : Profesor Madya Dr. Foo Hooi Ling
Fakulti : Fakulti Biotekologi and Sains Biomolekul

Enzim cellulolytic menjadi lebih penting kerana penggunaannya dalam bahan buangan pertanian, yang berpotensi menjadi pennggganti dalam pengeluran biotenaga dan biofuel. Aktivity cellulolytic bakteria asid laktik (LAB) yang diperolehi daripada makanan peraman Malaysia (*Lactobacillus plantarum* RG14, RG11, RI11, RS5, TL1, I-UL4 dan B4) ditentukan melalui ujian CMCase, FPase, β-glucosidase, xylanase dan mannanase pada pH 5, 6,5 dan 8. Dua kaedah kuantifikasi bagi kepekatan protein larut (kaedah Bradford dan Lowry yang diubahsuai) digunakan sebagai penentuan aktiviti cellulolytic khusus. Aktiviti cellulolytic khusus adalah 45 hingga 52 kali lebih tinggi apabila kepekatan protein larut bagi supernatan tiada sel ditentukan dengan kaedah Bradford berbanding kaedah Lowry yang diubahsuai. *Lactobacillus plantarum* RG14, RG11 dan RI11 dengan pemarkahan yang lebih tinggi dalam aktiviti cellulolytic keseluruhan dipilih untuk menjalani biotransformasi selama 14 hari dengan teknologi fermentasi pepejal (SSF) menggunakan hampas isirong kelapa sawit (PKC) sebagai substrat dengan sampel ujian diambil setiap dua hari. Ekstrak PKC yang difermentasi telah dikumpulkan untuk menentukan prestasi aktiviti cellulolytic dan pertumbuhan sel LAB. Pertumbuhan sel tertinggi diperhatikan pada hari ke-2 oleh *L. plantarum* RG14 and RG11 masing-masing dengan 8.15 log CFU/mL dan 8.11 log CFU/mL. Pada hari ke-4, *L. plantarum* RI11 menunjukkan pertumbuhan sel tertinggi iaitu 8.13 log CFU/mL. Pertumbuhan sel dalam PKC adalah stabil sepanjang proses teknologi fermentasi pepejal. Kepekatan protein larut daripada ekstrak PKC yang telah difermentasi ditentukan dengan

menggunakan kaedah Lowry yang diubahsuai meningkat manakala kaedah Bradford menunjukkan penurunan kepekatan protein larut. Hal ini mungkin disebabkan oleh tahap sensitiviti dan kelinearan yang rendah dalam kaedah Bradford berbanding kaedah Lowry yang diubahsuai. Oleh itu, aktiviti cellulolytic khusus bagi ekstrak PKC ditentukan dengan menggunakan kaedah Lowry yang diubahsuai pada pH 5. Aktiviti CMCase telah menunjukkan peningkatan pada hari ke-6 diikuti dengan penurunan drastik. Aktiviti β-glucodidase bagi ekstrak PKC meningkat semasa SSF dengan aktiviti tertinggi pada hari ke-12 oleh PKC yang dirawat oleh *L. plantarum* RG14 dan hari ke-14 oleh *L. plantarum* RG11 and RI11. Aktiviti mannanase khusus menunjukkan peningkatan pada hari ke-8 fermentasi. Aktiviti FPase tidak menunjukkan sebarang aktiviti sepanjang tempoh SSF. Aktiviti enzim khusus bagi ekstrak PKC yang diperolehi melalui fermentasi tenggelam menggunakan media MRS adalah lebih tinggi berbanding proses teknologi fermentasi pepejal. Hal ini disebabkan media MRS menyediakan kelembapan dan nutrien yang mencukupi untuk menyokong pertumbuhan sel dan penghasilan enzim dalam LAB. Kesimpulannya, LAB mampu menghasilkan enzim yang mempunyai aktiviti cellulolytic dan dapat meleraikan pelbagai bentuk polisakarida dan PKC. Oleh itu, enzim cellulolytic LAB berpotensi untuk biotransformasi bagi biomass.

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LIST OF ABBREVIATIONS

Abbreviation used in this text:

g	gram
log	logarithm with base 10
M	Molarity
h	hour
min	minute
mL	millilitre
nmol	nanomole
pH	exponent of hydrogen ion
rpm	rotation per minute
µg	microgram
µL	microlitre
µm	micrometer
°C	degree celsius
%	percentage
CMC	carboxymethylcellulose
FPase	Filter paperase
LAB	Lactic acid bacteria
NSP	Non starch polysaccharides
PKC	Palm kernel cake
SSF	Solid state fermentation

CHAPTER 1

1.0 INTRODUCTION

The explosive increment of global population has been a popular topic for recent decades due to the consequences it brings along. Problems caused by exponentially increment of human population are given great attention and well publicized. Environmental pollution and food demand are the problems we are facing today. With limited resources available such as non renewable fossil fuel and inconvenience living condition, it could be a challenge for the living condition of human beings.

Scientists are putting a lot of efforts in exploring potential solutions for the problems we are facing nowadays. It was found that cellulosic materials, the most abundant renewable resource on earth (Robson & Chambliss, 1984), could be a possible solution for the state of difficulties we are facing. These biological resources have a great potential to serve as an alternative for diminishing fossil energy resources (Yin *et al.*, 2010). The complete utilization of cellulosic materials has become increasingly important due to the global demand of resources.

According to Malaysia Palm Oil Board (MPOB) (2014), Malaysia produced around 1.7 million tonnes of crude palm oil and was known to be the second largest export in the year of 2013. Production of agro-wastes from oil palm industry is a common problem for environmental pollution issue. Agro-wastes or agricultural wastes refer to the waste produces from various agricultural processes, usually from farms, poultry houses and plantation sites. Hence, the production of biofuel and bioenergy from agro-wastes has gained vast attention from the public, instead of consumable crops.

Palm oil industry is one of the major economy engines of Malaysia with the production of around 19 million tonnes of crude palm oil in 2014, as reported by MPOB (2014). The wealth generated by palm oil industry comes along with the bulk amount of residual wastes or biomass which produces as the by-product that contributes to environmental pollution. There are 4.7 million arches of oil palm plantation in Malaysia, with the potential to produce 77.2 million tonnes of palm biomass, which is around 94% of the biomass produce in Malaysia, as reported by Sin Chew Daily News (2014).

Palm kernel cake (PKC) is one of the by-products results from palm kernel extraction process (Tuan Lah *et al.*, 2012). Raw PKC with low economic value is usually disposed as waste and creates environmental issue. Therefore, recent research suggests that PKC can be used as substrate for solid state fermentation (SSF) in order to produce value-added products such as enzymes. In addition, SSF of PKC with microorganism can be used to enhance the nutritive quality of agro-wastes that can be applied as animal feed. The low moisture content of SSF is favourable because a lower chance of contamination by other microorganism and higher porosity to provide aeration (Sindhu *et al.*, 2006).

Waste transformation is a trend to overcome the environmental issue as well as sustaining the food security worldwide. The presence of cellulolytic and hemicellulolytic enzymes is essential for the degradation of PKC due to the characteristic of PKC which mainly consists of non starch polysaccharides (NSPs) and crude fibre, with the composition of 78% mannan, 3% arabinoxylan, 3% glucuronoxylan and 12% cellulose (Alimon, 2004).

Synergetic reaction of cellulase complex: endo-β-1,4-glucanase (EC 3.2.1.4), exo-β-1,4-glucanase (EC 3.2.1.91) and β-D-glucosidase (EC 3.2.1.21) provide effective biological hydrolysis β-1,4 linked of cellulose into glucose (Yin *et al.*, 2010).Apart from cellulose, hemicellulose is another component found mainly in primary and secondary layer of plant cell wall that closely associated with cellulose and lignin. The presence of hemicellulolytic enzyme such as xylanase and mannanase are required to degrade the xylan and mannan in PKC to ensure effective biodegradation of PKC.

Fungus is a common producer for cellulase, yet the cost of production is high due to the substrates use and its slow growth (Ariffin *et al.*, 2006). Hence, the focus has directed to bacteria with ability to produce cellulase as a potential substitution for fungus in the production of cellulase enzyme due to its faster growth rate compared to fungus. In this study, the effort was put forward to investigate the potential of lactic acid bacteria (LAB) to produce cellulolytic enzyme.

Simple fermentative nature of LAB which involves one or a few fermentation end products is considerable academic interest and provides an excellent model system for the study of energy transduction, solute transport and membrane biology (Axelsson, 1998). On the other hand, the diverse metabolic capacity that enables them to adapt to a variety of conditions has made LAB more applicable in wide range of industrial fields. Varieties of food products incorporated with LAB produce in commercial scale due to their positive health aspects reported in the recent decades (Melgar *et al.*, 2012).

The general purpose of this study was directed toward the cellulolytic activity of LAB isolated from Malaysian fermented food. Cellulolytic activity of isolated LAB was characterised through different cellulolytic enzyme assays. The selected cellulolytic activity of LAB isolates were then employed for SSF of PKC for 14 days of incubation was determined.

1.1 Objectives

The specific objectives of this project are:

1. To characterise cellulolytic activities of LAB isolated from Malaysian fermented food.
2. To biotransform palm kernel cake by cellulolytic LAB isolates via SSF.

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