ISOLATION AND CHARACTERISATION OF FIBROLYTIC AND LIPOLYTIC ENZYME-PRODUCING BACTERIA FROM OIL PALM EMPTY FRUIT BUNCH COMPOST

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I herby declare that the material in this thesis is my own except for quotations, excerpts, equations, summaries and references, which have been duly acknowledged.

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Elaine Remi Anak Douglas Telajan
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ABSTRAK

Bungkil inti kelapa sawit (PKC) adalah salah satu bahan buangan kelapa sawit yang penting. PKC telah digunakan sebagai makanan bagi haiwan ruminan dan ternakan itik dan ayam. Kegunaan PKC dalam makanan ternakan ayam dan itik adalah terhad kerana sifat anti- nutrisi seperti galaktomanan dan xylan yang terdapat dalam PKC. Sifat anti-nutrisi ini meningkatkan keviskusan diet akibat daripada penyerapan air yang tinggi dan ini akan menghadkan penyerapan nutrisi oleh haiwan ternakan. Satu cara untuk mengatasi masalah ini ialah dengan merawat PKC dengan enzim atau mikroorganisma yang menghasilkan enzim yang boleh mendegredasikan komponen tegar kepada nilai pemakanan. Oleh itu, projek ini dilaksanakan untuk mengasingkan bakteria mesofilik dan termofilik serta menyaring bakteria tersebut dalam keupayaan mereka menghasilkan enzim galactomannanase, cellulase, xylanase dan lipase. Enzim-enzim tersebut dapat meningkatkan kualiti nutrisi PKC sebagai "single cell protein (SCP)". Pengasingan dilakukan dengan menggunakan kaedah 'dilution plate' diatas Nutrient agar untuk bacteria, Starch Casein Nitrate agar (SCA) untuk actinomycete mesofilik, Czapex-dox Yeast Extract Casamino Acid agar (CYC) untuk actinomycete termofilik, yeast extract peptone glucose agar (YEPD) untuk yeast dan potato dextrose agar (PDA) untuk fungi. Substrat komersil; Azo-carob-galactomannan, Azo-xylan (oat) dan Azo-CM-cellulose digunakan untuk menyaring galactomannanase, xylanase dan cellulase manakala sobitan monolaurate (Tween 20) digunakan sebagai substrat untuk aktiviti lipase. Sebanyak 1146 mikroorganisma telah diasingkan daripada beberapa sumber kompos "empty fruit bunch" (EFB), larutan mikrob efektif dan EFB mentah. Daripada itu, 627 bakteria, 219 actinomycete, 101 yis dan 199 fungi telah diasingkan dan disaring. Enam belas asingan dengan keupayaan menghasilkan enzim mannanase, cellulase, xylanase telah dikira aktiviti mannanase, cellulase dan xylanase secara quantitatif. Didapati tiga asingan Bacillus sp. yang berlainan memberikan aktiviti mannanase (Asingan 7DY7, 7DU3 and 4DB3 masing-masing dengan aktiviti maximum sebanyak 1.30 U/µg protein, 0.95 U/µg protein and 0.92 U/µg protein), cellulase (Asingan 7DY7, 7DU3 and 4DB13 masing-masing dengan aktiviti maksimum sebanyak 0.08 U/µg protein, 0.35 U/µg protein and 0.11 U/µg protein) dan xylanase (Asingan 7DY7, 7DU3 and 4DB8 masing-masing dengan aktiviti maksimum sebanyak 0.15 U/µg protein, 0.08 U/µg protein and 0.21 U/µg protein) yang agak tinggi jika dibandingkan dengan 13 asingan bakteria yang lain. Enam belas asingan bakteria tersebut dicari kenalpasti menggunakan jujukan 16S rDNA masing-masing. Sebelas Bacillus sp. dan asingan tunggal bagi Micromonospora sp., Streptomyces sp. dan Thermoactinomyces sp. masing-masing dikenalpasti dengan membandingkan jujukan separa 16S rDNA asingan-asingan tersebut dengan jujukan 16S rDNA yang terdapat dalam GenBank dengan menggunakan Basic Alignment Search Tool (BLAST). Mikroorganisma yang diasingkan dapat digunakan untuk merawat PKC untuk meningkatkan nilai nutrisi makanan dalam PKC.

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%	Percent
:	Ratio
/	per
=	Equal
>	More than
<	Less than
^{o}C	Degree of Celsius
α	Alpha
AIA	Actinomycete Isolation Agar
ARDRA	Amplified Ribosomal DNA Restriction Analysis
AFLP	Amplified Fragment Length Polymorphism
β	Beta
BLAST	Basic local alignment search tool
bp	Base pair
BSA	Bovine Serum Albumin
Ca	Calcium
CaCl2	Calcium chloride
CYC	Czapex-dox Yeast extract Casamino acid
DNA	Deoxyribonucleic acid
DN*ase*	Deoxyribonuclease
CBDs	Cellulose-binding domains
CMC	Carboxymethyl Cellulose
CTAB	Cetyltrimethly ammonium bromide
CPO	Crude Palm Oil
DP	Degree of polymerization
dNTP	deoxynucleoside-5'-triphosphate
DNS	Dinitrosalicylic Acid
dH_2O	Distilled water
EDTA	Ethylenediaminetetra- acetate

EFB	Empty Fruit Bunch
EM	Effective microbe
EtBr	Ethidium Bromide
E-value	Expected value
FFB	Fresh Fruit Bunch
g	Gram
h	hour
kb	Kilo base
kg	kilogram
L	Litre
LBG	Locust Bean Gum
LMW	Low Molecular Weight
m	meter
MJ	Mega joule
mm	Millimetre
mM	Millimolar
mg	Milligram
ml	Millilitre
MgCl2	Magnesium chloride
µ	Micro
M	Molar
Min	Minute
MF	Mescarp Fibre
MOP	Molded Oil Palm
µL	Microlitre
µg	Microgram
µmol	Micromole
NaOH	Sodium hydroxide
NA	Nutrient agar
NB	Nutrient Broth

nm	Nanometre
PCI	Phenol-Chloroform-Isoamyl
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PFAD	Palm Fatty Acid Distillate
PKC	Palm Kernel Cake
PKE	Palm Kernel Expeller
PKM	Palm Kernel Meal
POME	Palm Oil Mill Effluent
POS	Palm Oil Sludge
Psi	Pounds per square inch
RFLP	Restriction Fragment Length Polymorphism
RAPD	Randomly Amplified Polymorphic DNA
RNA	Ribonucleic acid
RBB	Remazol Brilliant Blue
RN*ase*	Ribonuclease
RPM	Revolutions per minute
S	Svedberg unit
sdH_2O	Sterile distilled water
SDS	Sodium dodecile sulphate
SCN	Starch Casein Nitrate
TBE	Tris- Boric acid-EDTA
T-RFLP	Terminal Restriction Fragment Length Polymorphism
U	Unit
V	Volt
x	Times
YEPD	Yeast Extract Peptone Dextrose

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CHAPTER 1

INTRODUCTION

The oil palm sector is one of the major industries in Malaysia. The growth of the palm oil industry in Malaysia has been phenomenal over the last 30 years. From merely 400 hectares planted in 1920, the total planted oil palm area increased progressively to 54,000 hectares by 1960 and by 1998, the oil palm planted area had increased to more than 3.0 million hectares (Industrial Processes and the Environment. Handbook No.3. Crude Palm Oil Industry, 1999). In 2007, the total oil palm planted area increased by 3.4% to 4.3 million hectares where Sabah remained as the largest oil palm planted State with 1.27 million hectares or 30% of the total planted area (Malaysian Palm Oil Board, 2007).

Today, Malaysia is the world's largest producer and exporter of palm oil accounting for nearly 49.5% of world production and 64.5% of world exports (Industrial Processes and the Environment. Handbook No.3. Crude Palm Oil Industry, 1999). In the year 2007, Malaysia produced 15.8 million tonnes of crude palm oil and 1.91 million tonnes of crude palm kernel oil which showed a decline of 0.4% and 2.5% compared to the year 2006 for crude palm oil and crude palm kernel oil respectively. The decline was mainly attributed to the effects of flood damage during the early part of the year and biological stress, which affected the palm trees especially during the first half of 2007. However, the crude palm oil production is predicted to rise to 16.2 million tonnes in 2008 because of improvement in yields and an expansion in matured area (Malaysian Palm Oil Board, 2007). Malaysia exported a variety of oil palm products which include palm oil, palm kernel oil, palm kernel cake, oleochemicals and finished products. The total export volume of oil palm products declined by 3.0% or 0.60 million tonnes to 19.56 million tonnes in 2007 from 20.16 million tonnes in 2006 (Malaysian Palm Oil Board, 2007).

The extensively rapid expansion of the palm oil sector had generated abundant of by-products. Palm kernel cake (PKC), empty fruit bunches (EFB), fibre, shell and potato ash are among the major by-products generated in the palm oil extraction process. This has subsequently given rise to their disposable problem. The government has opted for a "zero waste" concept which is environment friendly and is centered on complete recycling or utilization of all perceived waste components and by-products generated by the oil palm sector (Industrial Processes and the Environment. Handbook No.3. Crude Palm Oil Industry, 1999).

Palm kernel cake (PKC) is one of the many major oil palm by-products and is obtained from the kernel after the oil has been extracted. Nutritionally, PKC contain a moderate amount of protein and carbohydrate making it a useful source of protein and energy for livestock and it is commonly used in animal feed (Hutagalung, 1981). PKC has been widely used as ruminant feed (Broderick *et al.*, 1988; Moss and Givens, 1994; Umunna *et al.*, 1994; Chandrasekariah *et al.*, 2001), pig diets (Thorne *et al.*, 1989; Agunbiande *et al.*, 1999; Kim *et al.*, 2001) and rabbit diets (Aduku *et al.*, 1988; Aganga *et al.*, 1991). Due to the presence of fibrous materials in PKC such as mannan, galactomannan, xylan and arabinoxylan coupled with high fibre content, low palatability and lack of several essential amino acids, their inclusions in poultry diet are very limited. Much research has been carried out to determine the quality of PKC and its maximum level in poultry diets (Wignjosoesastro *et al.*, 1972; Onwundike, 1986; Paniraghi, 1992; Perez *et al.*, 2000) but few studies have been done to overcome the physical and nutritional barriers. Methods that had been used to improve the quality of PKC are through supplementation with biotin (Oloyo, 1991), sodium hydroxide (Nwokolo *et al.*, 1977) and enzymes (Pluske *et al.*, 1997).

Treatment of PKC with enzymes to improve the availability of nutrients and proteins of PKC is done either by adding fibrolytic enzyme-producing microorganisms to the PKC-based poultry feed or by adding purified fibrolytic enzymes to the PKC based feed. The latter is commonly practice in Malaysia but it is very costly as commercially available enzymes are expensive. Malaysia has to purchase the enzymes abroad either from Denmark, Netherlands, Belgium and

other country, making the cost of using PKC feed produced by Malaysia very expensive (Ibrahim, 2008).

One way to overcome the high cost of production of treated PKC is to treat PKC using locally isolated fibrolytic microorganisms. Malaysia has a very diverse genetic resources and microorganisms (Krishnapillay *et al.*, 2003). These microorganisms produce various useful enzymes such as galactomannanases, endoglucanases and xylanases that can be used to treat PKC (Arcand *et al.*, 1993; Stoll *et al.*, 1999). Studies on the use of enzymes to improve the nutritive value of PKC were mainly carried out by applying a single enzyme, particularly the mannanase. At the moment, no data is available on the supplementation of PKC with combinations of enzymes in feeding trials with poultry. As PKC contain a number of non-starch polysaccharides which are mostly indigestible, the inclusion of several non-starch polysaccharide-degrading enzymes can support and accelerate their digestion in the alimentary tract of poultry.

Hence, this study was carried out to identify the potential microorganisms with fibrolytic activities capable of digesting mannan, xylan, cellulose and other fibrous materials, and at the same time be able to produce lipase to enable them to grow on PKC. The focus of this study was on the fibrolytic enzymes which include galactomannanase, cellulase, and xylanase. Empty fruit bunch (EFB) compost was chosen as the source of microorganisms because EFB is another major by-product of oil palm and is highly fibrous in nature. During its composting process, three general categories of microorganisms: bacteria, actinomycetes and fungi were present (Thambirajah *et al.*, 1995). In general, composting is a process managed by humans involving the cultivation of microorganisms that degrade organic matter in the presence of oxygen. Additionally, microorganisms from the environment and the EFB itself will contribute to the composting process. At certain stage of composting, the compost becomes so heavily populated with thermophilic microorganisms that it generates massive heat in the composting process that formed an ideal environment for thermophilic microorganisms to grow (Cooperband, 2000). Thus, the diversity of microorganisms in the composting process coupled with the high incubation temperature of the compost makes EFB