CHARACTERIZATION AND BIOACTIVITIES OF CARBOXYMETHYL CELLULASE PRODUCED BY *ALCALIGENES FAECALIS* USING DISPOSED X-RAY FILM AS THE SUBSTRATE

NOOR AFIFAH BINTI FAUZI

MASTER OF SCIENCE (BIOTECHNOLOGY)

UNIVERSITI MALAYSIA PAHANG

SUPERVISOR'S DECLARATION

We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Master of Science (Biotechnology).

_____

(Supervisor's Signature)

Full Name : DR. ESSAM A. MAKKY

Position : ASSOCIATE PROFESSOR

Date :

_____

(Co-supervisor's Signature)

Full Name : DR. MOHD HASBI AB RAHIM

Position : ASSOCIATE PROFESSOR

Date :

STUDENT'S DECLARATION

I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

_____

(Student's Signature)

Full Name : NOOR AFIFAH BINTI BIINTI FAUZI

ID Number : MKT14003

Date :

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DEDICATION

Dedicated to my beloved family

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ABSTRAK

Selulase adalah enzim yang bermanfaat yang telah lama digunakan dalam pengeluaran makanan haiwan, dalam perumusan bahan pencuci, penjernihan jus, pembuatan kertas dan pengeluaran wain. Pada masa kini, pengeluaran enzim selulase dari biopertukaran sisa selulosa telah diamalkan secara meluas. Enzim biasanya boleh diperolehi daripada mikroorganisma terutamanya bakteria dan kulat. Keupayaan potensi mikroorganisma yang tidak diketahui untuk mencerna bahan selulosa dapat dieksploitasi menggunakan substrat baru, iaitu sisa filem sinar-x. Filem sinar-x adalah sejenis sisa berbahaya. Ia mengandungi sisa perak di samping selulase yang boleh dihasilkan oleh mana-mana mikrob yang memakan selulosa yang terdapat dalam sisa filem sinar-x. Objektif projek ini adalah (i) untuk memencil, menyaring, dan mengenalpasti mikroorganisma dari sumber air, tanah dan makanan, dengan itu memilih pencilan yang paling kuat (ii) untuk mengoptimumkan kesan pencilan bakteria menggunakan filem sinar-x yang dilupuskan sebagai substrat pada pengeluaran selulase (iii) untuk menulenkan dan mencirikan bioaktiviti selulase. Sampel Tanah Panching (PS1) dan sampel Hati Ayam (CL8A) dipilih sebagai pencilan yang paling kuat selepas pemeriksaan daripada 27 isolat awal. Selepas pengoptimuman penghasilan CMCase dan avicelase, hanya CMCase dipilih untuk proses selanjutnya kerana ia lebih gemar filem x-ray sebagai substrat. Keadaan yang optimum yang meningkatkan penghasilan CMCase disiasat dengan menggunakan kaedah “Satu Faktor Pada Satu Masa” (OFAT), yang melibatkan 7 faktor yang berbeza; keadaan inkubasi, sumber karbon, sumber nitrogen, pH awal, jumlah substrat, saiz inokulum dan vitamin. PS1 mencapai aktiviti CMCase tertinggi sebanyak 0.934 U / ml dalam keadaan bergetar, dengan 0.4 % w/v kanji, 0.1 % w/v ekstrak malt, 2.5 g jumlah substrat, 2 ml saiz inokulum dan 2 % w/v tiamin dalam pH 8 media penghasilan. Sedangkan CL8A mencapai aktiviti tertinggi CMCase sebanyak 4.559 U/ml dalam keadaan bergetar, dengan 0.4 % w/v laktosa, 1.5 g jumlah substrat, pH 9 media penghasilan dan faktor-faktor lain menunjukkan keputusan serupa dengan PS1. Walau bagaimanapun hanya CL8A dipilih untuk penulenan separa enzim kerana ia menunjukkan produktiviti enzim yang lebih tinggi. CL8A dihasilkan secara besar-besaran untuk melaksanakan pemendakan ammonium sulphate selepas proses pengoptimuman. Hasil CMCase sebanyak 6.49% telah diperolehi dan berat molekul enzim telah dianalisis menggunakan kaedah elektroforesis dengan hasilnya 60 kDa. Mikroorganisma PS1 dikenalpasti sebagai bakteria Gram negatif *Providencia rettgeri,* sementara CL8A dikenalpasti sebagai bakteria Gram negatif *Alcaligenes faecalis.* Pecahan enzim CMCase telah dimangkinkan menggunakan 2 faktor yang berbeza dan didapati stabil pada 25 ° C dan pH 5. Kesimpulannya, pencilan yang berasal dari sisa makanan (*A. faecalis*) dan tanah (*P. rettgeri*)) memperlihatkan potensi yang baik sebagai mikroorganisma selulosa dan dapat degradasikan filem sinar-x dan menghasilkan CMCase.

ABSTRACT

Cellulase is a beneficial enzyme that has been long used in production of animal feed, in the formulation of detergents, juice clarification, paper manufacturing and wine production. Nowadays, production of cellulases enzyme from the bioconversion of cellulosic waste has been extensively practiced. The enzymes typically can be acquired from microorganisms especially bacteria and fungi. Furthermore, potential capability of unknown microorganisms to digest cellulosic material can be exploited using a new substrate, which is x-ray film waste. X-ray film is a form of hazardous waste which contains silver residue, alongside cellulase which can be produced by any microorganisms that feed on cellulose found in x-ray film waste. The objectives of this project are (i) to isolate, screen and identify microorganisms from water, soil and food sources, thus select on most potent isolates, (ii) to optimize the effect of bacterial isolates using disposed x-ray film as the substrate on cellulase production, and (iii) to purify and characterize the bioactivity of cellulose. The samples of Panching Soil (PS1) and Chicken Liver (CL8A) were selected as the most potent isolates after screening from 27 initial isolates. After optimization of CMCase and avicelase productions, only CMCase was selected for further process as it preferred the x-ray film as substrate. The optimum condition that enhanced the CMCase production was investigated using "One Factor at a Time" (OFAT) method, involving 7 different factors; incubation condition, carbon source, nitrogen source, initial pH, amount of substrate, inoculum size and vitamin. PS1 achieved highest CMCase activity of 0.934 U/ml in shaking condition, with 0.4 % w/v starch, 0.1 % w/v malt extract, 2.5 g amount of substrate, 2 ml inoculum size and 2 % w/v thiamine with pH 8 of production media. Whereas, CL8A achieved highest CMCase activity of 4.559 U/ml in shaking condition, with 0.4 % w/v lactose, 1.5 g amount of substrate, pH 9 of production media and the rest of the factors were similar to PS1. However only CL8A was selected for partial purification of enzyme as it displayed higher enzyme productivity. CL8A was produced on a large scale to carry out ammonium sulphate precipitation after the optimization process. CMCase yield of 6.49 % was obtained and the molecular weight of the enzyme has been analysed using electrophoresis method resulting in 60 kDa. Microorganism of PS1 was identified as Gram negative bacteria *Providencia rettgeri*, while CL8A was identified as Gram negative bacteria *Alcaligenes faecalis*. The CMCase enzyme fraction was catalysed using 2 different factors and was found to be stable at 25 °C and pH 5. In conclusion, the isolates derived from food waste (*A. faecalis*) and soil (*P. rettgeri*) displayed a good potential as cellulolytic microorganisms and were able to degrade x-ray film and produce CMCase.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

%	Percent
°C	Degree Celsius
#	Highest value
/	Per
:	Ration
=	Double-bonded
β	Beta
×	Multiplication

LIST OF ABBREVIATIONS

APS	Ammonium persulfate
AZCL	Azurine-Crosslinked
A. faecalis	*Alcaligenes faecalis*
A. niger	*Aspergillus niger*
A. sulphate	Ammonium sulphate
BGL	Beta-glucosidases
BSA	Bovine serum albumin
B. subtilis	*Bacillus subtilis*
CBH	Cellobiohydrolase
CC	Corn cob
CFF	Cell free filtrate
CMC	Carboxymethyl cellulose
CMCase	Carboxymethyl cellulase
DNA	Deoxyribonucleic acid
DNS	Dinitrosalicylic
DEAE	Diethylaminoethyl cellulose
EDTA	Ethylenediaminetraacetic acid
EG	Endoglucanases
EH	Epoxide hydrolase
E.coli	*Escheria coli*
E. faecalis	*Enterococcus faecalis*
FDA	Food and drug administration
FPase	Filter paperase
g	Gram
GRAS	Generally recognised as safe
h	Hour
hrs	Hours
JDC	*Jatropha curcas* deoiled cake
km	kilometre
K. pneumonia	*Klebsiella pneumonia*
L	Litre

M	Molar
mg	Milligram
min	Minute
ml	Millilitre
MUC	Methyl-umbelliferyl-cellobioside
MWCO	Molecular weight cut-off
NA	Nutrient agar
NB	Nutrient broth
OBR-HEC	Ostazin brilliant red-hydroxyethyl cellulose
OD	Optical density
PASC	Phosphoric acid swollen cellulose
PET	Polyethylene terephthalate
pH	Potential hydrogen
ppm	Parts per million
P. aerogenosa	*Pseudomonas aerogenosa*
P. variotii	*Paecilomyces variotii*
rpm	Revolutions per minute
RS	Rice straw
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SD	Standard deviation
SEM	Scanning electron microscope
SmF	Submerged fermentation
sp.	Species
SSF	Solid state fermentation
S. typhii	*Salmonella typhii*
S. aureus	*Staphylococcus aureus*
TAE	Tris-acetate-EDTA
TEMED	Tetramethylethylenediamine
T. reesei	*Trichoderma reesei*
T. viride	*Trichoderma viride*
UMP	Universiti Malaysia Pahang
U/ml	Units per millilitre
w/v	Weight per volume

LIST OF CHEMICAL FORMULAS

$CaCl_2$	Calcium chloride
$CuSO_4.5(H_2O)$	Copper (II) sulphate pentahydrate
KH_2PO_4	Potassium dihydrogen phosphate
$KNaC_4H_4O_6 \cdot 4H_2O$	Potassium sodium tartrate
KNO_3	Potassium nitrate
$MgSO_4$	Magnesium sulphate
NaOH	Sodium hydroxide
$Na_2HPO_4.7H_2O$	Disodium phosphate heptahydrate
Na_2HPO_4	Disodium phosphate
$NaNO_3$	Sodium nitrate
NH_4Cl	Ammonium chloride
$(NH_4)_2SO_4$	Ammonium sulphate
NaCl	Sodium chloride
Na_2CO_3	Sodium sulphite
Na_2(tartrate)$2H_2O$	Sodium tartrate dehydrate

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