PRODUCTION OF MANNAN-DEGRADING ENZYMES BY *ASPERGILLUS NIGER* IN SHAKE FLASKS AND STIRRED TANK FERMENTER

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in the Fulfilment of the Requirements for the Degree of Master of Science

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Chairman: Professor Arbakariya Ariff, Ph.D.

Institute: Bioscience

Optimization of medium composition and culture conditions for mannan-degrading enzymes production by *Aspergillus niger* was carried out in shake flasks and 2 L stirred tank fermenter. Preliminary, three potential strains (*Sclerotium rolfsii, Rhizopus oryzae and A. niger*) were screened, and *A. niger* was used for subsequent study. The mannan-degrading enzymes were purified partially and characterized with regard to pH optima and stability, temperature optima and stability, and K_m and V_{max} values. The influence of agitation speed, aeration rate and incubation temperature on the production of mannan-degrading enzyme in batch fermentation using 2 L stirred tank fermenter were also investigated.

Highest level of β-mannanase activity was obtained when guar gum (1495 nkat mL^{-1}) and bacteriological peptone (1744 nkat mL^{-1}) were compared to other carbon (locust bean gum, cellulose, carboxymethylcellulose and glucose) and nitrogen (peptone from meat,

yeast extract, ammonium sulphate, nitrate and citrate) sources used. The conditions predicted for the maximum production of β-mannanase through the use of response surface methodology were at pH 5.47, 57 g L^{-1} bacteriological peptone and 21.3 g L^{-1} guar gum. The maximal β-mannanase, endoglucanase, β-mannosidase and galactosidase activity obtained from the predicted equation was of 2010.8, 34.8, 1.6 and 39.0 nkat mL^{-1}, respectively.

The optimal temperatures for β-mannanase activity were 50°C and 60°C, with half-life ($t_{1/2}$) of 6 h at 60°C and 4 h at 70°C. The optimal temperature for endoglucanase activity was 60°C, with $t_{1/2}$ of 6 h at 60°C and 45 min at 70°C. The optimal temperature for β-mannosidase was 70°C with $t_{1/2}$ of 1.5 h at 70°C. While the optimal temperature for α-galactosidase activity was 50 to 60°C with $t_{1/2}$ of 2.5 h at 60°C. The β-mannanase, endoglucanase and α-galactosidase had a pH optima at 3.5 while β-mannosidase at pH 3.0. The enzymes characterized in this study were defined as acidic proteins. The β-mannanase, β-mannosidase, α-galactosidase and endoglucanase showed good stability at pH values of pH 3.5 – 7.0, pH 3.5 – 6.5, pH 3.5 – 5.0 and pH 4 – 7, respectively after a prolonged incubation (24 h at 50°C). High substrate specificity of crude culture filtrate, with low K_m value of β-mannanase (0.04 mg mL^{-1}), endoglucanase (0.54 mg mL^{-1}), β-mannosidase (1.67 mM) and α-galactosidase (1.34 mM) indicates the synergistic effect of the enzyme mixture had occurred. The value of V_{max} for β-mannanase, endoglucanase, β-mannosidase and α-galactosidase were 0.52, 0.12, 1.72 x 10^{-3} and 4.68 x 10^{-3} nmol mL^{-1} min^{-1}, respectively.

A fermentation in 2 L stirred tank fermenter using optimized medium yielded 678 nkat mL^{-1} β-mannanase, associated with 1.25 nkat mL^{-1} β-mannosidase, 18.46 nkat mL^{-1} α-galactosidase and 40.15 nkat mL^{-1} endoglucanase at impeller tip speed of 0.82 m s^{-1}, aeration rate of 0.1 vvm and incubation temperature of 35^{o}C. Higher degree of agitation speed and aeration rate had an inhibitory effect on the production of mannan-degrading enzymes.

Abstrak tesis dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan Ijazah Master Sains

PENGHASILAN ENZIM BAGI MENDEGRADASIKAN MANNAN OLEH *ASPERGILLUS NIGER* MENGGUNAKAN KELALANG PENGGONCANG DAN TANGKI FERMENTER PENGADUK

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Februari 2005

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Institut: **Biosains**

Pengoptimaan komposisi media dan keadaan kultur untuk penghasilan enzim bagi mendegradasikan mannan oleh *A. niger* dijalankan di dalam kelalang penggoncang dan tangki fermenter pengaduk 2 L. Sebagai permulaan, tiga strain (*Sclerotium rolfsii, Aspergillus niger* and *Rhizopus oryzae*) yang mempunyai potensi telah dipilih dan *A. niger* telah digunakan untuk kajian yang seterusnya. Enzim utk mendegradasikan mannan telah ditulinkan separa dan dicirikan untuk penentuan pH optima dan kestabilan, suhu optima dan kestabilan, dan nilai K_m and V_{max}. Kesan kelajuan putaran, kadar pengudaraan dan suhu inkubasi kepada penghasilan enzim bagi mendegradasikan mannan dalam fermentasi suapan sesekelompok menggunakan tangki fermenter pengaduk, 2 L juga dikaji.

Tahap tertinggi bagi aktiviti β-mannanase didapati apabila gum guar (1495 nkat mL^{-1}) dan pepton bakteriologikal (1744 nkat mL^{-1}) dibanding dengan sumber karbon (gum

kekacang locust, selulos, karboksimetilselulos and glukosa) and nitrogen (pepton daripada daging, yis ekstrak, ammonium sulfat, nitrat and sitrat) lain yang telah digunakan. Anggaran keadaan bagi penghasilan maksimum β-mannanase melalui penggunaan kaedah tindakbalas permukaan (response surface methodology) adalah pada pH 5.47, 57 g L^{-1} pepton bakteriologikal dan 21.3 g L^{-1} gum guar. Anggaran aktiviti maksimum bagi β-mannanase, endoglukanase, β-mannosidase and galaktosidase yang didapati dari persaman, masing-masing adalah 2010.8, 34.8, 1.6 and 39.0 nkat mL^{-1}.

Suhu optima bagi aktiviti β-mannanase ialah 50^{o}C dan 60^{o}C, dengan jangka separa hayat ($t_{1/2}$) selama 6 jam pada 60^{o}C dan 4 jam pada 70^{o}C. Suhu optima bagi aktiviti endoglukanase ialah 60^{o}C, dengan $t_{1/2}$ selama 6 jam pada 60^{o}C dan 45 minit pada 70^{o}C. Suhu optima bagi β-mannosidase adalah pada 70^{o}C dengan $t_{1/2}$ selama 1.5 jam pada 70^{o}C. Sementara itu, suhu optima bagi aktiviti α-galaktosidase ialah pada 50 ke 60^{o}C dengan $t_{1/2}$ selama 2.5 jam pada 60^{o}C. β-mannanase, endoglukanase dan α-galaktosidase mempunyai pH optima yang sama iaitu pada 3.5 sementara β-mannosidase pada pH 3.0. Enzim yang dicirikan di dalam kajian ini didefinasikan sebagai protein berasid. Kestabilan yang baik pada pH ditunjukkan oleh β-mannanase, β-mannosidase, α-galaktosidase and endoglukanase, masing-masing pada pH 3.5 – 7.0, pH 3.5 – 6.5, pH 3.5 – 5.0 dan pH 4 – 7 selepas inkubasi (24 jam pada 50^{o}C). Kespesifikan substrat yang tinggi bagi filtrasi kultur kasar, dengan nilai K_m yang rendah bagi β-mannanase (0.04 mg mL^{-1}), endoglukanase (0.54 mg mL^{-1}), β-mannosidase (1.67 mM) and α-galaktosidase (1.34 mM) menggambarkan yang kesan sinergistik telah berlaku pada campuran enzim.

Nilai V_{max} untuk β-mannanase, endoglukanase, β-mannosidase and α-galaktosidase masing-masing adalah 0.52, 0.12, 1.72×10^{-3} and 4.68×10^{-3} nmol mL^{-1} min^{-1}.

Fermentasi dalam tangki fermenter pengaduk 2 L menggunakan medium yang telah dioptimakan menghasilkan 678 nkat mL^{-1} β-mannanase, berserta dengan 1.25 nkat mL^{-1} β-mannosidase, 18.46 nkat mL^{-1} α-galaktosidase dan 40.15 nkat mL^{-1} endoglukanase pada kelajuan hujung pemutar 0.82 m s^{-1}, kadar pengudaran 0.1 vvm dan suhu inkubasi pada $35^{o}C$. Peningkatan darjah kelajuan putaran dan kadar pengudaraan mempunyai kesan perencatan pada penghasilan enzim untuk mendegradasikan mannan.

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TABLE OF CONTENTS

DECLARATION

I hereby declare that the thesis is based on my original work expect for quotation and citation which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other at UPM or other institutions.

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SITI NORITA BINTI MOHAMAD

Date:

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

nkat mL^{-1}	:	nanokatal/ millilitre
U mL^{-1}	:	Unit/millilitre
mg mL^{-1}	:	milligram/millilitre
PDA	:	Potato Dextrose Agar
*p*NP-αGal	:	*p*-nitrophenyl-α-D-galactoside
*p*NP-αMan	:	*p*-nitrophenyl-β-D-mannoside
h	:	hour
min	:	Minute
L	:	litre
mM	:	millimolar
µL	:	microlitre
GG	:	Guar gum
LBG	:	Locust bean gum
CMC	:	Carboxymethylcellulose
^{o}C	:	Degree Celsius
DNS	:	Dinitrosalicylic acid
DOT	:	Dissolved oxygen tension
rpm	:	Rotation per minute
Re	:	Reynolds number based on diameter of rotation
vvm	:	Volume of air per min/ volume of fermentation media