PRODUCTION, OPTIMIZATION AND CHARACTERIZATION OF L-GLUTAMINASE FROM MARINE BACTERIA

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MASTER OF SCIENCE

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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.

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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.

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Dedicated to both my husband and parents

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ABSTRACT

L-glutaminase present in living organisms. L-glutaminase has been identified having potential applications for several industries including pharmaceutical, food, and health care. However, industrial sectors nowadays demand natural producing enzyme compared to artificial enzymes. L-glutaminase extracted from microorganisms has more advantages to fulfil demands from industries. Up until now, less research about bacterial production of L-glutaminase from local marine environments had been carried out. Hence, the study aims to investigate the L-glutaminase production, optimization and characterization of partially-purified L-glutaminase from bacterial isolates of local marine environment. In this study, the screening of bacteria-producing L-glutaminase had been implemented from three different Pahang Beaches known as Pantai Teluk Cempedak, Pantai Batu Hitam, and Pantai Balok. A total of 17 isolates showed positive response on production of L-glutaminase. However, only 12 isolates showed positive response with strong colour change which then proceeded for biochemical characterization and 16S rRNA sequence analysis. The biochemical characterizations analysis showed negative results for all tests that had been carried out. Then, 16S rRNA gene sequencing were further proceeded to identify the identity of bacteria that showed positive results on L-glutaminase production. Additionally, production, optimization, enzyme purification and characterization were carried out from the best L-glutaminase producer. The two highest enzyme activity recorded was from *Kosakonia radicincitans* with enzyme activity of 0.103 U/ml and *Shigella flexneri* which has the enzyme activity of 0.100 U/ml. Furthermore, two isolates that had the highest enzyme activity were selected for optimization of process parameters through One Factor at A Time method. The parameters involved are temperature, pH, additional of organic and inorganic nitrogen sources, and additional of carbon sources. It was found out that *K. radicincitans* and *S. flexneri* have the highest enzyme activity at temperature 37°C, pH 7, additional of ammonium chloride as inorganic nitrogen source, and additional of glucose for carbon source. However, *K. radicincitans* has the highest enzyme activity when beef extract was added to its culture media while *S. flexneri* prefer yeast extract. An experiment with all parameters at its optimal level was conducted and *K. radicincitans* had the highest enzyme activity of 0.3542 U/ml at temperature of 37°C, pH 7, additional of beef extract for organic nitrogen source, additional of ammonium chloride for inorganic nitrogen source, and additional of glucose as the carbon source. L-glutaminase extracted from *K. radicincitans* was further partially purified and characterized. It had a molecular weight of 70 kDA. L-glutaminase was most reactive and stable at temperature of 37ºC and pH 7. This knowledge on production, optimization, and characterization of L-glutaminase are very important in order to understand its characteristics and suitability for industrial usage.

ABSTRAK

L-glutaminase boleh didapati di dalam organisma. L-glutaminase telah dikenalpasti mempunyai peluang aplikasi di dalam sektor perindustrian seperti farmasuetikal, makanan, dan perubatan. Walaubagaimanapun, enzim yang dihasilkan secara semulajadi berbanding secara tiruan mendapat permintaan yang tinggi daripada sektor industri pada masa kini. Selain itu, L-glutaminase yang diekstrak daripada mikroorganisma mempunyai lebih banyak kelebihan dan kebaikan untuk memenuhi permintaan daripada industri. Kekurangan kajian mengenai bakteria yang menghasilkan L-glutaminase daripada persekitaran marin Malaysia masih kurang sehingga sekarang. Oleh itu, objektif kajian ini adalah untuk membuat penyelidikan dengan lebih mendalam mengenai penghasilan L-glutaminase, pengoptimum dan pencirian separa penulenan L-glutaminase daripada bakteria marin Malaysia. Di dalam kajian ini, saringan bakteria yang menghasilkan L-glutaminase telah dijalankan daripada tiga pantai berbeza di Pahang iaitu Pantai Teluk Cempedak, Pantai Batu Hitam, dan Pantai Balok. Sejumlah 17 entiti menunjukkan respon positif mengenai penghasilan L-glutaminase. Walaubagaimanapun, hanya 12 entiti yang menunjukkan perubahan warna yang ketara dan seterusnya telah digunakan untuk proses karakter biokimia dan analisis 16S rRNA. Keputusan analisis karakter biokimia adalah negative untuk kesemua ujian yang telah dijalankan. Seterusnya, pencirian gen 16S rRNA telah dijalankan untuk mengenal pasti identiti bakteria yang telah menunjukkan keputusan positif dalam penghasilan L-glutaminase. Tambahan pula, penghasilkan, pengoptimum, penulenan enzim, dan pencirian telah dilakukan terhadap produser terbaik L-glutaminase. Dua enzim aktiviti tertinggi telah direkodkan iaitu daripada *Kosakonia radicincitans* dengan bacaan sebanyak 0.103 U/ml dan *Shigella flexneri* yang mempunyai bacaan sebanyak 0.100 U/ml. Selain itu, dua bakteria yang mempunyai bacaan aktiviti enzim tertinggi telah dipilih untuk menjalani proses pengoptimum parameter melalui kaedah Satu Faktor dalam Satu Masa. Parameter yang terlibat adalah suhu, pH, penambahan organic dan bukan organic sumber nitrogen, dan penambahan sumber karbon. *K. radicincitans* and *S. flexneri* telah didapati mempunyai enzim aktiviti tertinggi pada suhu 37°C, pH 7, penambahan ammonium klorida sebagai sumber nitrogen tidak organik dan penambahan glukosa sebagai sumber karbon. Walaubagaimanapun, *K. radicincitans* mempunyai bacaan enzim tertinggi apabila medium kulturnya telah ditambah dengan ekstrak daging lembu manakala *S. flexneri* lebih menyukai ekstrak ragi. Eksperimen dengan semua parameter pada tahap optimum telah dijalankan dan *K. radicincitans* mempunyai bacaan enzim tertinggi iaitu 0.3542 U/ml pada suhu 37°C, pH 7, penambahan ekstrak daging lembu sebagai sumber nitrogen organik, penambahan ammonium klorida sebagai sumber nitrogen bukan organik, dan penambahan glukosa sebagai sumber karbon. Kemudian, separa penulenan L-glutaminase yang diekstrak daripada *K. radicincitans* telah dijalankan dan dipercirikan. Berat molekularnya adalah 70 kDA. L-glutaminase yang paling aktif dan stabil adalah pada suhu 37°C dan pH 7. Ilmu mengenai penghasilan, pengoptimum, dan pencirian L-glutaminase adalah sangat penting untuk memahami ciri-ciri L-glutaminase dan kesesuaiannya untuk diaplikasikan dalam sektor industri.

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M_1	Concentration of the concentrated solution
M_2	Concentration of the diluted solution
^oC	Degree Celsius
µl	Microliter
µm	Micrometer
µM	Micromolar
µmol	Micromole
%	Percentage
V_1	Volume of the concentrated solution
V_2	Volume of the diluted solution

LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate
BLAST	Basic local alignment search tool
BSA	Bovine serum albumin
C	Cytosine
DNA	Deoxyribonucleic acid
DEAE	Diethylaminoethyl cellulose
E	East
F	Forward
G	Gram
g/l	Gram per litre
G	Guanine
GC	Guanine-cytosine
HIV	Human Immunodeficiency Virus
kDA	Kilo-Dalton
LB	Luria Bertani
T_m	Melting point
mg	Miligram
mg/ml	Milligram per millilitre
mL	Mililitre
ml/min	Millilitre per min
mM	Milimolar
M	Molarity
MEGA7	Molecular evolutionary genetic analysis 7 software
Nm	Nanometers
NCBI	National Center for Biotechnology Info
NJ	Neighbour-joining
N	North
OFAT	One factor at a time
PCR	Polymerase chain reaction
R	Reverse
rRNA	

rpm	Ribosomal ribonucleic acid
SDS-PAGE	Rotation per minute
sp.	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
UV	Species
US	Ultraviolet
U/gds	Undiluted sample
U/l	Unit per gram dry substrate
U/ml	Unit per litre
V	Unit per millilitre
v/v	Voltage
w/w	Volume per volume
	Weight per weight

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