UNIVERSITI PUTRA MALAYSIA

ISOLATION AND PURIFICATION OF PROTEOLYTIC ENZYME PRODUCED BY LACTIC ACID BACTERIA FROM BUDU AND BAMBANGAN

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ISOLATION AND PURIFICATION OF PROTEOLYTIC ENZYME PRODUCED BY LACTIC ACID BACTERIA FROM BUDU AND BAMBANGAN

By

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ISOLATION AND PURIFICATION OF PROTEOLYTIC ENZYME PRODUCED BY LACTIC ACID BACTERIA FROM BUDU AND BAMBANGAN

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September 2012

Chairman : Foo Hooi Ling, PhD

Faculty : Biotechnology and Biomolecular Sciences

Proteolytic enzymes have wide applications in various industries. Microorganisms represent an excellent source of proteolytic enzymes owing to their broad biochemical diversity, rapid growth, and low cost for their cultivation. However, the information of extracellular protease of Lactic Acid Bacteria (LAB) is limited. Therefore, an attempt was conducted to study the extracellular protease produced by LAB isolated from local fermented foods, *Budu* and *Bambangan*. The extracellular protease activity was determined by qualitative assay using skim milk agar plate and quantitative assay by spectrophotometric method. Out of 41 LAB isolates, 21 LAB exhibited extracellular protease activity over a broad pH range by quantitative spectrophotometric assay. Isolate B12m9 was the highest extracellular protease producer under acidic condition and hence it was selected for further study.

The isolate B12m9 was identified as *Pediococcus pentosaceus* B12m9 by phenotypic and genotypic analyses. Nucleotide sequence of 16S rDNA showed 97% homology to *P. pentosaceus*. Thus, this isolate was designated as *P. pentosaceus* B12m9 and its proteolytic enzyme was designated as extracellular protease B12m9. The experiments of cultivation conditions such as initial pH, temperature and incubation time showed that maximum protease production by *P. pentosaceus* B12m9 occurred at pH 7.0 and 30˚C over 20 h of incubation time.

The extracellular protease B12m9 was further purified by using Fast Protein Liquid Chromatograph. Four protease isozymes were produced by *P. pentosaceus* B12m9 as different protease activity of the ammonium precipitated proteases were detected. Peak G extracellular protease was purified to apparent homogeneity by ammonium sulphate precipitation, Resource Q anion-exchange chromatography and Superose-12 gel filtration chromatography with a recovery yield of 19% and purification fold of 62.53. The molecular mass of the purified protease was estimated to be 14.4 and 14.5 kDa by Glycine sodium dodecyl sulphate – polyacrylamide gel electrophoresis and Superose-12 gel filtration chromatography, respectively. The isoelectric point of the purified protease as revealed by isoelectric focusing electrophoresis was approximately 8.0. In conclusion, different LAB produced different types of extracellular protease and the purified extracellular protease B12m9 was an alkaline protease, where the potential of the purified extracellular protease has to be explored further.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PEMENCILAN DAN PENULENAN ENZIM PROTEOLITIK DIHASILKAN OLEH BAKTERIA ASID LAKTIK DARIPADA BUDU DAN BAMBANGAN

Oleh

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Enzim proteolitik mempunyai aplikasi yang luas dalam pelbagai industri. Mikroorganisma adalah sumber enzim proteolitik yang digemari disebabkan oleh ciri biokimia yang luas dan berbeza, pertumbuhan yang cepat dan kos rendah dalam pertumbuhan mereka. Walaubagaimanapun, maklumat mengenai protease ekstrasel Bakteria asid laktik (BAL) adalah terhad. Oleh itu, satu kajian dijalankan untuk mengkaji protease ekstrasel yang dihasilkan oleh BAL yang dipencilkan daripada makanan tertapai, Budu dan Bambangan. Aktiviti protease ekstrasel ditentukan menggunakan asai kualitatif dengan menggunakan piring agar susu dan asai kuantitatif dengan kaedah spektrofotometrik. Di antara 41 pencilan BAL, hanya 21 pencilan sahaja didapati mempamerkan aktiviti protease ekstrasel dalam julat pH yang besar dengan menggunakan kaedah kuantitatif spektrofotometer. Pencilan B12m9 mempamerkan aktiviti protease ekstrasel yang tertinggi, maka itu ia dipilih

untuk kajian selanjutnya.

Pencilan B12m9 telah dikenalpasti sebagai *Pediococcus pentosaceus* B12m9 berdasarkan kajian biokimia fenotipik dan genotipik. Jujukan 16S rDNA menunujukkan persamaan 97% dengan *P. pentosaceus.* Oleh itu, ia dinamakan sebagai *P. pentosaceus* B12m9 dan enzim proteolitiknya dikenali sebagai protease extrasel B12m9. Eksperimen untuk penentuan keadaan pengkulturan seperti pH awal, suhu dan masa pengeraman telah menunjukkan bahawa penghasilan maksimum protease *P. pentosaceus* B12m9 berlaku pada pH 7.0 dan 30˚C selepas pengeraman selama 20 jam.

Protease ekstrasel B12m9 ditulenkan selanjutnya dengan menggunakan *Fast Protein Liquid Chromatography*. Empat isozim protease telah dihasilkan oleh *P. pentosaceus* B12m9 kerana aktiviti protease yang berbeza daripada mendakan ammonium proteases dapat dikesan. Peak G protease ekstrasel ditulenkan ke tahap kehomogenan yang nyata melalui pemendakan ammonium sulfat, kromatografi penukaran anion Resource Q dan kromatografi penurasan gel Superose-12 dengan hasil perolehan 19% dan faktor penulenan 62.53. Berat molekul protease yang telah ditulenkan dianggar sebanyak 14.4 dan 14.5 kDa masing-masing dengan menggunakan gel elektroforesis glisina poliakrilamida natrium dodesil sulfat dan kromatografi penurasan gel Superose-12. Titik isoelektrik bagi protease yang ditulenkan seperti yang ditentukan melalui kaedah pemusatan isoelektrik adalah kira-kira 8.0.

Kesimpulannya, BAL yang berbeza menghasilkan protease ekstrasel yang berbeza dan protease ekstrasel B12m9 yang ditulenkan adalah protease jenis alkali, di mana potensi protease ekstrasel yang ditulenkan perlu diterokai selanjtnya.

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I certify that a Thesis Examination Committee has met on 14 September 2012 to conduct the final examination of Thung Tze Young on his thesis entitled "Isolation and Purification of Proteolytic Enzyme Produced by Lactic Acid Bacteria from *Budu* and *Bambangan*" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the Requirements for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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THUNG TZE YOUNG

Date: 14 September 2012

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