Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of requirement for the degree of Master of Science

CLONING, SEQUENCING AND EXPRESSION OF AN ORGANIC SOLVENT TOLERANT PROTEASE FROM *BACILLUS PUMILUS* 115B

By

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Five out of the nine isolated bacteria species screened earlier, demonstrated high protease production on Skim Milk Agar and were also to Benzene Toulene Xylene Ethylbenzene (BTEX). Among these, isolate 115b was found to produce the highest protease production. Besides being stable in 25% (v/v) benzene and toluene, protease from isolate 115b was found to be activated by n-dodecane and n-tetradecane by 1.7 and 2.5 folds respectively. Isolate 115b was identified as *Bacillus pumilus* 115b via biochemical tests and 16S rDNA sequencing analysis.

The gene encoding protease of *Bacillus pumilus* 115b was amplified via polymerase chain reaction (PCR) using consensus primers based on the sequences of alkaline serine protease genes from related species. The

complete nucleotide sequence of the protease from *Bacillus pumilus* 115b was determined. Sequence analysis showed an open reading frame (ORF) of 1149 that encoded a polypeptide of 383 amino acid residues and the calculated protein molecular mass of 39,448 Da. The ORF also encoded a single peptide consisting of 29 residues and a propeptide of 79 residues. The mature protein comprised 275 amino acids with a calculated molecular mass of 27,846 Da. Homology searches revealed that the amino acid residues from *B. pumilus* 115b protease shared a high homology (90%) with the alkaline serine protease from *B. pumilus* TYO-67 and *B. pumilus* UN-31-C-42.

The gene coding for an organic solvent tolerant 115b protease gene was cloned into pQE-30 UA expression vector. The recombinant plasmid was then transformed into *E. coli* M15[pREP4]. The organic solvent tolerant 115b protease gene was successfully expressed by induction with IPTG and was detected by SDS-PAGE analysis with a molecular weight of around 35 kDa. The expression of recombinant *E. coli* M15[pREP4] was optimized by inducing it with 1.0 mM of IPTG at 4 hour of induction time.

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

PENGKLONAN, PENGKODAN DAN PENGEKSPRESAN PROTEASE YANG TOLERAN TERHADAP PELARUT ORGANIK DARIPADA *BACILLUS PUMILUS* 115B

Oleh

SHALIHAH BINTI MAHAMAD

Februari 2006

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Lima daripada sembilan spesis bakteria yang dipencilkan telah menunjukkan penghasilan protease di atas agar susu dan merupakan bakteria yang stabil terhadap benzena, toluena, xilena dan etilbenzena (BTEX). Bakteria 115b telah dikesan menghasilkan protease yang tertinggi. Selain ia stabil terhadap 25% (v/v) benzena dan toluena, aktiviti daripada protease bakteria 115b didapati meningkat dalam n-dodecana dan n-tetradecana sebanyak 1.7 dan 2.5. Bakteria 115b ini kemudiannya dikenalpasti sebagai *Bacillus pumilus* 115b berdasarkan ujian biokimia dan analisa jujukan gen 16S rDNA.

Gen yang mengkodkan protease daripada *B. pumilus* 115b telah digandakan melalui tindakbalas polimerasi berantai (PCR) dengan

menggunakan primer yang berdasarkan jujukan gen protease alkali serina daripada spesis yang sama. Jujukan lengkap nukleotida protease daripada *Bacillus pumilus* 115b telah berjaya ditentukan. Analisa jujukan menunjukkan rangka bacaan terbuka (ORF) bersaiz 1149 bp yang mengkodkan polipeptida yang terdiri daripada 383 residu asid amino dan berat molekul protein dianggarkan 39,488 Da. Rangka bacaan terbuka ini mengandungi 29 residu peptida tunggal dan 79 residu propeptida. Protein matang pula terdiri daripada 275 asid amino residu dengan berat molekul yang dianggarkan 27,486 Da. Perbandingan residu asid amino protease daripada *B. pumilus* 115b menunjukkan homologi yang tinggi, iaitu 90 % dengan protease alkali serina daripada *B. pumilus* TYO-67 dan *B. pumilus* UN-31-C-42.

Gen yang mengkodkan protease 115b yang toleran terhadap pelarut organik telah diekpres ke dalam vektor pQE-30 UA. Plasmid rekombinan ini kemudiannya telah dimasukkan ke dalam bakteria *E. coli* M15[pREP4] sebagai perumah. Gen protease 115b yang toleran pelarut organik berjaya diekspres dengan induksi IPTG dan protein ekspres dapat dikesan melalui analisa SDS-PAGE dengan berat molekul sekitar 35 kDa. Pengekspresan rekombinan *E. coli* M15[pREP4] dioptimumkan dengan menggunakan 1.0 mM kepekatan IPTG selama 4 jam masa post-induksi.

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I certify that an Examination Committee has met on 14th February 2006 to conduct the final examination of Shalihah Binti Mahamad on her Master of Science thesis entitle "Cloning, Sequencing and Expression of an Organic Solvent Tolerant Protease from *Bacillus pumilus* 115b" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

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DECLARATION

I hereby declare that the 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 UPM or other institutions.

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