MOLECULAR ANALYSIS OF PUTATIVE *FIBROBACTER SUCCINOGENES* XYLANASE GENES SUBCLONED FROM RECOMBINANT XYLANOLYTIC PLASMID pBX6

By

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pBX6 is a recombinant plasmid containing a 3 kb fragment of insert DNA from *Fibrobacter succinogenes* S85 genomic DNA which encodes a xylanase gene. An *E.coli* HB101 colony cell, carrying pBX6 expresses xylanase activity which can be detected on Remazol Brilliant Blue-Xylan (RBB-Xylan) agar plates supplemented with Ampicillin as a clear halo zone against a dark blue background. This characteristic allows xylanase gene to be used as a selectable chromogenic marker on any vector system. The insert DNA from *Fibrobacter succinogenes* S85 in the plasmid pBX6 encodes two putative xylanase regions which are named ORF 1 and ORF 2. These two ORF were amplified by the polymerase chain reaction (PCR). A pair of PCR primers for the detection of two ORF from plasmid pBX6 has been synthesized and has revealed the putative xylanase gene of approximately 1,224 bp for ORF 1 and 1,450 bp for ORF 2. Both ORF was separately subcloned into *E.coli* JM109 by using pGEM T-Easy

vector. Recombinant plasmid DNA from a positive clone for both ORF was designated as pGEM-X1 and pGEM-X2. The insertion of the ORF 1 and ORF 2 was confirmed using restriction enzyme analysis and PCR amplification. The nucleotide sequence determined showed high similarity to the original gene and also high homology with other bacterial xylanases such as *Pseudomonas fluorescence* and *Butyrivibrio fibrisolvens*. The homology ranged from 57% to 94% for clone pGEM-X1 while for clone pGEM-X2, the homology ranged from 88% to 92% with xylanase from *F. succinogenes* S85 (xynC-xyl). Xylanase activity assay was done to further confirm its presence in the recombinant plasmid pGEM-X1 and pGEM-X2. However, the results showed there was no activity for both subclone using the Somogyi-Nelson assay for reducing sugar. Based on the results obtained, recombinant plasmids pGEM-X1 and pGEM-X2 were successfully inserted into pGEM-T Easy vector and introduced into *E.coli* JM109.

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

ANALISIS MOLEKULAR KE ATAS GEN XILANASE *FIBROBACTER SUCCINOGENES* YANG DISUBKLON DARI PLASMID REKOMBINAN pBX6

Oleh

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Februari 2006

Pengerusi: **Profesor Madya Abdullah Sipat, PhD**

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pBX6 ialah plasmid rekombinan yang mengandungi satu DNA selitan dari DNA genomik *Fibrobacter succinogenes* S85 yang mengkodkan gen xilanase. *E.coli* HB101 membawa plasmid rekombinan pBX6 akan mengekspresikan aktiviti xilanase yang boleh dikesan sebagai zon cerah terhadap latar belakang yang biru menggunakan petri agar yang ditambah dengan Ampisillin dan mengandungi substrat Remazol Brilliant Blue-xylan (RBB-xylan). Sifat ini menjadikan gen xilanase berpotensi sebagai penanda pilihan jenis kromogenik dalam sebarang sistem vektor. DNA selitan dari *Fibrobacter succinogenes* S85 di dalam plasmid pBX6 mengkodkan dua *Open Reading Frame* (ORF) iaitu ORF 1 dan ORF 2. Kedua-dua ORF ini telah diamplifikasi menggunakan tindak balas berantai polimeras. Kombinasi primer untuk pengesanan gen xilanase daripada plasmid pBX6 telah disintesis dan dianggarkan 1,224 bp bagi ORF 1 dan 1,450 bagi ORF 2. Kedua-dua ORF telah di subklon secara berasingan ke

dalam *E.coli* JM109 menggunakan pGEM-T-Easy sebagai vektor. Kedua-dua plasmid rekombinan telah di tandakan sebagai pGEM-X1 dan pGEM-X2. Pengesanan dan penentuan orientasi gen xilanase dibuat dengan penganalisasi enzim pembatas dan ampifikasi secara PCR. Jujukan DNA gen xilanase dalam klon pGEM-X1 dan pGEM-X2 telah dilaksanakan untuk memastikan kehadiran gen xilanase dan menunjukkan bahawa ia mempunyai persamaan dengan original gen xilanase. Jujukan DNA gen xilanase bagi kedua-dua klon ini juga menunjukkan ia mempunyai homologi dengan bakteria xilanase yang lain seperti *Pseudomonas fluorescence* dan *Butyrivibrio fibrisolvens* (57% sampai 94%) untuk klon pGEM-X1, manakala klon pGEM-X2 mempunyai nilai peratus dalam lingkungan antara 88% hingga 92% dengan xilanase dari *Fibrobacter succinogenes* S85 (xynC-xyl). Plasmid rekombinan pGEM-X1 dan pGEM-X2 tidak menunjukkan aktiviti apabila dilakukan kaedah 'Somogyi-Nelson assay'. Berdasarkan daripada keputusan yang diperolehi, kedua-dua plasmid rekombinan pGEM-X1 dan pGEM-X2 telah berjaya diperolehi dan dimasukkan ke dalam *E.coli* JM109.

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I certify that an Examination Committee has met on 9th February 2006 to conduct the final examination of Nurhasmimi Bt Hassan on her Master of Science thesis entitled "Molecular Analysis of Putative *Fibrobacter succinogenes* Xylanase Genes Subcloned from Recombinant Xylanolytic Plasmid pBX6" 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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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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NURHASMIMI BT HASSAN

Date: 27 September 2006

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