BACTERIAL CELLULASE FROM A LOCAL ISOLATE, *BACILLUS PUMILUS* EB3

By

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

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Chairman: Professor Mohd Ali Hassan, PhD

Faculty: Engineering

Cellulase production from bacteria can be an advantage as the enzyme production rate is normally higher due to bacterial high growth rate. Screening of bacteria, optimisation of fermentation conditions and selection of substrates are important for the successful production of cellulase. This study is conducted to produce cellulase from our local isolate *Bacillus pumilus* EB3, using oil palm empty fruit bunch (EFB) and carboxymethyl cellulose (CMC) as substrate. The effect of physical, chemical and thermal pretreatment on the EFB chemical composition and physical structure was studied, aimed at reducing lignin and hemicellulose, and making EFB structure more amorphous. The effect of pretreatment on reducing sugars production using commercial cellulase (Celluclast 1.5L) was also examined. Production of cellulase was conducted in shake flask and 2L stirred tank reactor (STR). The effect of initial pH, temperature, nitrogen source and carbon source on cellulase production in the shake flask was investigated. Following that, cellulase produced from *B. pumilus* EB3 was purified using ion exchange chromatography with anion exchanger (HiTrap QXL) for characterisation of the cellulase. The results of EFB pretreatment revealed that combination of pretreatments involving

physical, chemical and thermal treatment was most suitable to affect the chemical composition and physical structure of the EFB. Initial cellulose, hemicellulose and lignin content in untreated EFB were 51%, 28% and 15% respectively. After combination of pretreatments, the cellulose composition increased to 67% while hemicellulose and lignin content decreased to 17% and 10% respectively. The physical structure of the EFB was altered after pretreatments as based on the SEM micrograph. Alteration of EFB was due to removal of lignin and hemicellulose. Combination of pretreatments increased the hydrolysis of the EFB with the yield of 0.53 g reducing sugars / g EFB as compared to the untreated EFB where only 0.07 g reducing sugars being produced from 1 g of EFB. Study on cellulase production confirmed that fermentation parameters such as initial pH, temperature, carbon source and nitrogen source affected cellulase production. Cellulase from *B. pumilus* EB3 was found to be secreted the most at temperature 37°C, initial pH 7.0, 1% CMC as carbon source and 2 g/L of yeast extract as organic nitrogen source. The activity recorded during the fermentation was 0.006 U/mL, 0.076 U/mL and 0.032 U/mL respectively for FPase, CMCase and β-glucosidase. As production in the shake flask showed that EFB gave a competitive cellulase production as CMC, EFB was tested as carbon source in 2L STR. Due to hydrophobic characteristic of the treated EFB, the experiment was not so successful. Comparison of cellulase production using CMC as substrate in shake flask and 2L STR revealed that cellulase productivity was higher in the 2L STR than in the shake flask although overall, the maximum cellulase activity recorded was almost similar. Purification of cellulase from *B. pumilus* EB3 using ion exchange chromatography showed that 98.7% of total CMCase was recovered. Protein separation was however based on subtractive separation where the contaminants were bound to the column instead of

CMCase. Characterisation of the enzyme found that CMCase from *B. pumilus* EB3 has a molecular weight range from 30-65 kDa and was optimally active at pH 6.0 and temperature 60°C. The CMCase also retained its activity over a wide pH range (pH 5.0–9.0) and temperature range (30-70°C).

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

SELLULASE DARIPADA BAKTERIA TERPENCIL TEMPATAN, *BACILLUS PUMILUS* EB3

Oleh

HIDAYAH ARIFFIN

September 2006

Pengerusi: Profesor Mohd Ali Hassan, PhD

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Penghasilan enzim sellulase daripada bakteria mempunyai kelebihan oleh kerana kadar penghasilan enzim adalah lebih tinggi disebabkan kadar pertumbuhan bakteria yang lebih tinggi berbanding kulat. Proses penyaringan bakteria, pengoptimuman parameter dalam proses fermentasi dan pemilihan substrat yang sesuai adalah penting untuk menghasilkan sellulase dengan jayanya. Kajian ini dijalankan untuk menghasilkan sellulase daripada *B. pumilus EB3* dengan menggunakan tandan kosong sawit (TKS) dan karboksimetil sellulosa (CMC) sebagai substrat. Kesan pra-rawatan fizikal, kimia dan termal ke atas komposisi kimia dan struktur fizikal TKS dikaji, begitu juga dengan kesan pra-rawatan ke atas penghasilan guna penurun. Penghasilan gula penurun adalah menggunakan enzim komersial (Celluclast 1.5L). Penghasilan enzim seterusnya dijalankan di dalam kelalang dan reaktor 2L berpengaduk. Kesan pH awal, suhu, sumber nitrogen dan karbon ke atas penghasilan sellulase dikaji. Penulenan sellulase kemudiannya dijalankan menggunakan kromatografi penukaran ion dengan penukar anion (HiTrap QXL) digunakan. Penulenan dijalankan untuk pencirian sellulase. Keputusan daripada eksperimen pra-rawatan menunjukkan kombinasi pra-rawatan

fizikal, kimia dan termal paling memberi kesan ke atas komposisi kimia dan struktur fizikal TKS. Kandungan awal sellulosa, hemisellulosa dan lignin di dalam TKS yang tidak dirawat adalah 51%, 28% dan 15%. Selepas kombinasi pra-rawatan, komposisi sellulosa meningkat kepada 67% manakala komposisi hemisellulosa dan lignin menurun kepada 17% dan 10%. Selepas pra-rawatan juga, struktur fizikal TKS terubah seperti yang dilihat di dalam mikrograf SEM. Perubahan adalah disebabkan penyingkiran lignin dan hemisellulosa. Kombinasi pra-rawatan meningkatkan hidrolisis TKS di mana 0.53 g gula penurun dihasilkan daripada 1 g TKS. Perbandingan amat ketara di mana hanya 0.07 g gula penurun dihasilkan daripada 1 g TKS untuk TKS tanpa pra-rawatan. Kajian ke atas penghasilan sellulase pula menunjukkan parameter dalam proses fermentasi seperti pH awal, suhu, sumber karbon dan nitrogen memberi kesan ke atas penghasilan sellulase. Sellulase daripada *B. pumilus* EB3 dilihat dihasilkan pada tahap maksimum apabila suhu 37°C, pH awal 7.0, CMC sebagai sumber karbon dan 2 g/L ekstrak yis digunakan dalam fermentasi. Aktiviti sellulase yang direkodkan ketika fermentasi itu adalah 0.006 U/mL, 0.076 U/mL dan 0.032 U/mL untuk FPase, CMCase dan β-glucosidase. Disebabkan penghasilan sellulase di dalam kelalang menunjukkan TKS menyebabkan penghasilan aktiviti enzim yang hampir serupa dengan penggunaan CMC, maka TKS telah digunakan di dalam bioreaktor (2L). Bagaimanapun, eksperimen tersebut kurang berjaya disebabkan ciri hidrofobik TKS selepas rawatan. Perbandingan antara sellulase yang dihasilkan di dalam kelalang dan bioreaktor (2L) dijalankan dengan menggunakan CMC sebagai substrat. Penemuan menunjukkan produktiviti enzim yang dihasilkan di dalam bioreaktor lebih tinggi berbanding kelalang walaupun aktiviti maksimum adalah hampir serupa. Sellulase tersebut kemudiannya ditulenkan menggunakan kromatografi

penukaran ion dan keputusan menunjukkan 98.7% daripada keseluruhan CMCase berjaya diperolehi semula. Pemisahan protein yang berlaku walau bagaimanapun adalah berdasarkan pemisahan songsang di mana bahan cemar yang terikat di dalam turus, bukannya CMCase. Pencirian enzim menunjukkan CMCase daripada *B. pumilus* EB3 mempunyai julat berat molekul antara 30-65 kDa. Enzim ini juga aktif pada pH 6.0 dan suhu 60°C. CMCase juga mampu mengekalkan aktivitinya pada julat pH dan suhu yang besar, iaitu pada pH 5.0-9.0 dan suhu 30-70°C.

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I certify that an Examination Committee has met on 21 September 2006 to conduct the final examination of Hidayah Ariffin on her Master of Science thesis entitled "Bacterial Cellulase From a Local Isolate, *Bacillus pumilus* EB3" 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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Date: 9 November 2006

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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HIDAYAH ARIFFIN

Date: 7 July 2006

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