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INFLUENCE OF CULTIVATION CONDITIONS ON GROWTH, SPORULATION RATE AND □-ENDOTOXIN SYNTHESIS OF BACILLUS THURINGIENSIS MPK13

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INFLUENCE OF CULTIVATION CONDITIONS ON GROWTH OF *BACILLUS THURINGIENSIS* MPK13, SPORULATION RATE AND δ-ENDOTOXIN SYNTHESIS

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The influence of cultivation conditions on growth of Bt MPK13, sporulation rate, δ-endotoxin synthesis and its toxicity was the focus of this study. The Cry+ strain of Bt MPK13 with consistent ability in δ-endotoxin synthesis was selected by repeated isolation with polymerase chain reaction analysis and δ-endotoxin detection. The effect of monosaccharides (glucose, fructose and galactose) and disaccharides (sucrose, lactose and maltose) on growth of Bt MPK13, sporulation and δ-endotoxin synthesis was carried out using shake flask culture. The data generated was also used to find the relationship between intracellular and extracellular total carbon (TC), total nitrogen (TN) and C/N ratio on growth of Bt MPK13, sporulation and δ-endotoxin synthesis. The effect of dissolved oxygen tension (DOT) control strategies and also the influence of different

modes of bioreactor operation (batch, fed-batch and continuous) on the cultivation performance of Bt MPK 13 was investigated using 5 L stirred tank bioreactor. The existence of δ-endotoxin was detected using SDS-PAGE analysis and the toxicity of δ-endotoxin synthesized during different modes of bioreactor operation was carried out against early instars of bagworms, *Metisa plana*.

The selected Cry+ strain of Bt MPK13 was preserved as the stock culture in freeze dried form. The use of Cry+ strain of Bt MPK13 as the stock culture resulted to a consistent δ-endotoxin synthesis in repeated experiments. During cultivation, sucrose, fructose, galactose, lactose and maltose were able to support growth of Bt MPK13, but failed to enhance high percentage of sporulation and the existence of δ-endotoxin was not detected. The synthesis of δ-endotoxin was only detected in cultivation using glucose as the carbon source. Mixture of glucose with other sugars also recorded high cell growth (> 1.0×10^{12} cfu/mL) with high percentage of sporulation (> 35%) however, the synthesis of δ-endotoxin was only detected when glucose concentration used in a mixture was higher than 8 g/L, indicating that high glucose concentration (> 8 g/L) must be present in the culture to trigger δ-endotoxin formation.. The highest initial and final intracellular TC and TN were also recorded in cultivation using glucose as the sole carbon source which corresponded well to high cell growth, high percentage of sporulation and δ-endotoxin synthesis. High DOT level (80% saturation) during active growth phase of Bt MPK13 was compulsory for δ-endotoxin synthesis in batch cultivation using glucose (8 g/L) as a carbon source. Enhanced percentage of sporulation (up to 61%) with early δ-endotoxin synthesis (8 h of cultivation) was obtained in cultivation where the DOT was controlled

at 80% saturation during active growth and then switched to 60% or 40% saturation at mid-exponential growth phase (after 6 h). The application of this optimal DOT control strategy without affecting the cell growth could also be used to enhance the percentage of sporulation (> 45%) and δ-endotoxin synthesis in fed-batch cultivation. The highest viable cell count (1.5 x 10^{12} cfu/mL) and spore count (7.1 x 10^{11} spore/mL) with the existence δ-endotoxin was obtained in fed-batch cultivation with constant feeding of 2 L, 8 g/L glucose at 6 h of cultivation, and optimal DOT control strategy was applied. Compared to batch cultivation, fed-batch without DOT control strategy able to increase cell count (24% increment), however spore count was greatly decreased (50% decrement). Feeding of glucose at stationary phase and intermittent feeding resulted to non-existence of δ-endotoxin. In continuous cultivation, the highest cell (5.8 x 10^{14} cfu/L.h) and spore (1.6 x 10^{13} spore/L.h) productivities were obtained at the dilution rate (D) of 0.39 h^{-1} and 0.05 h^{-1}, respectively. Although the steady-state viable cell concentration (1.7 x 10^{12} cfu/mL) was substantially higher than those obtained in batch cultivation, more than 50% decrement in spore count was recorded. It is important to note that δ-endotoxin synthesis was not detected in all D tested in continuous cultivation, indicating that this cultivation technique is not suitable for Bt production.

The culture with the existence of δ-endotoxin recorded 100% mortality towards bagworms (*M. plana*) at 14 DAT which was related to oil palm defoliation, suggesting that Bt MPK13 has potential to be used as industrial biopesticide. The proposed optimal cultivation conditions may be used in the development of large scale cultivation of Bt MPK13 for subsequent use as biopesticides in oil palm plantation

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGARUH KEADAAN PENGKULTURAN TERHADAP PEMBIAKAN, KADAR PENSPORAAN DAN PENGHASILAN δ-ENDOTOKSIN *BACILLUS THURINGIENSIS* MPK13

Oleh

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Kesan keadaan pengkulturan terhadap pembiakan Bt MPK13, kadar pensporaan, penghasilan δ-endotoxin dan ketoksikannya merupakan fokus kajian ini. Strain Cry+ Bt MPK13 dengan keupayaan untuk hasilkan δ-endotoxin dengan konsisten telah dipilih melalui pemencilan berulang menggunakan analisis tindak balas rantaian polimerase dan pengecaman δ-endotoksin.

Kesan monosakarida (glukosa, fruktosa dan galaktosa) dan disakarida (sukrosa, laktosa dan maltosa) terhadap pembiakan Bt MPK13, kadar pensporaan dan penghasilan δ-endotoksin telah dijalankan menggunakan kelalang goncangan. Data yang diperolehi digunakan untuk mengkaji kaitan di antara jumlah karbon (TC), jumlah nitrogen (TN) dan nisbah C/N intraselular dan ekstraselular terhadap pembiakan Bt MPK13, pensporaan

dan juga penghasilan δ-endotoksin. Kesan strategi kawalan kepekatan oksigen terlarut (DOT) dan juga kesan operasi bioreaktor yang berbeza (kelompok, suap-kelompok dan selanjar) terhadap prestasi pengkulturan Bt MPK 13 juga telah dikaji menggunakan bioreaktor tangki adukan 5 L. Kehadiran δ-endotoksin dalam kultur dikesan menggunakan analisis SDS-PAGE dan ketoksikan δ-endotoksin yang terhasil semasa operasi bioreaktor yang berbeza diuji ke atas larva awal ulat bungkus, *Metisa plana*.

Strain Cry+ Bt MPK13 yang terpilih disimpan sebagai kultur stok di dalam bentuk sejuk-kering. Penggunaan strain Cry+ Bt MPK13 sebagai kultur stok menjanjikan penghasilan δ-endotoksin yang konsisten dalam experimen ulangan. Semasa pengkulturan, sukrosa, fruktosa, galaktosa, laktosa dan maltosa didapati mampu menyokong pembiakan Bt MPK13 yang baik namun tidak mampu untuk meningkatkan pensporaaan dan tidak menyokong penghasilan δ-endotoksin. Penghasilan δ-endotoksin hanya dikesan semasa pengkulturan menggunakan glukosa sebagai sumber karbon utama. Campuran glukosa dengan gula yang lain juga merekodkan pembiakan sel yang tinggi (> $1.0x10^{12}$ cfu/mL) dengan peratus pensporaan yang tinggi (> 35%), walaubagaimanapun penghasilan δ-endotoksin hanya dikesan sekiranya kepekatan glukosa yang digunakan dalam campuran adalah 8 g/L, yang mana ini menunjukkan kepekatan glukosa yang tinggi (> 8 g/L) adalah suatu kemestian dalam kultur untuk mencetuskan penghasilan δ-endotoksin. Nilai permulaan dan nilai akhir TC dan TN tertinggi semasa pengkulturan menggunakan glukosa sebagai sumber karbon utama menyokong pembiakan sel yang tinggi, peratus pensporaan dan tinggi dan juga penghasilan δ-endotoksin. Tahap DOT yang tinggi (80% ketepuan) semasa fasa pembiakan aktif Bt MPK13 adalah suatu kemestian untuk

menyokong penghasilan δ-endotoxin dalam kultur kelompok yang menggunakan glukosa (8 g/L) sebagai sumber karbon utama. Peningkatan pensporaan (sehingga 61%) dengan penghasilan δ-endotoxin yang cepat (seawal 8 j pengkulturan) diperolehi semasa pengkulturan dengan DOT dikawal pada 80% ketepuan semasa fasa pembiakan aktif dan ditukar ke 60% atau 40% ketepuan pada pertengahan fasa pembiakan eksponen (selepas 6 jam pengkulturan). Strategi kawalan DOT yang optimum yang diaplikasi tanpa mengganggu pembiakan sel juga boleh digunakan untuk meningkatkan peratus pensporaan sel (> 45%) dan penghasilan δ-endotoksin semasa pengkulturan suap-kelompok. Nilai sel hidup tertinggi (1.5×10^{12} cfu/mL) dan kiraan spora tertinggi (7.1×10^{11} spore/mL) dengan kehadiran δ-endotoksin diperolehi dalam kultur suap-kelompok suapan tetap dengan suapan 2 L, 8 g/L glukosa pada 6 jam pengkulturan dan aplikasi strategi kawalan DOT yang optima. Berbanding dengan kultur kelompok, kultur suap-kelompok tanpa strategi kawalan DOT mampu meningkatkan kiraan sel hidup (24% kenaikan), walaubagaimanapun kiraan spora didapati menurun dengan mendadak (50% pengurangan). Suapan glukosa pada fasa pembiakan pegun dan suapan berkala semasa pengkulturan didapati tidak menyokong penghasilan δ-endotoksin. Semasa pengkulturan selanjar, produktiviti sel hidup tertinggi (5.8×10^{14} cfu/L.h) dan produktiviti spora tertinggi (1.6×10^{13} spore/L.h) masing-masing diperolehi pada kadar pencairan (D) 0.39 h^{-1} dan 0.05 h^{-1}. Walaupun kepekatan sel hidup pada keadaan mantap (1.7×10^{12} cfu/mL) adalah lebih tinggi dari yang diperolehi semasa pengkulturan kelompok, lebih daripada 50% penurunan dalam kiraan spora telah direkodkan. Adalah penting diketahui bahawa δ-endotoksin tidak dikesan dalam semua D yang diuji dalam kultur selanjar, justeru menunjukkan teknik pengkulturan ini adalah tidak sesuai untuk penghasilan Bt.

Kultur yang mengandungi δ-endotoksin merekodkan kematian 100% terhadap ulat bungkus (*Metisa plana*) pada 14 hari selepas rawatan (HSR) yang mana ulat bungkus ini adalah penyebab utama kerosakan daun sawit dan ini menunjukkan bahawa Bt MPK13 mempunyai potensi yang tinggi untuk digunakan sebagai biopestisid industri. Keadaan pengkulturan yang optimal yang dicadangkan boleh digunakan dalam pembangunan pengkulturan Bt MPK13 skala besar untuk digunakan sebagai biopestisid di ladang-ladang sawit.

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I certify that a Thesis Examination Committee has met on (inset the data of vive voce) to conduct the final examination of (Mohamed Mazmira bin Mohd. Masri) on his thesis entitled "Production of Bacillus thuringiensis using different mode of fermentation" in accordance with the University 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 Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. 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 not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

MOHAMED MAZMIRA BIN MOHD MASRI

Date: **13 June 2013**

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