UNIVERSITI PUTRA MALAYSIA

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By

RAKSHA SUNHARE

**Thesis Submitted to the School of Graduate Studies,
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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

PRODUCTION AND PURIFICATION OF NIPAH VIRUS GLYCOPROTEIN IN *Spodoptera frugiperda* 9 (J.E. SMITH) INSECT CELL

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October 2013

Chairman: Associate Professor Tey Beng Ti, PhD

Institute: Institute of Bioscience

Nipah virus glycoprotein (NiVG) expressed in *Spodoptera frugiperda* 9 [*Sf*9 (J.E. Smith)] induces neutralizing antibodies and could be used as an early detection reagent against NiV infection to prevent. Several industrial problems during the development of recombinant truncated glycoprotein of NiV (tNiVG) of baculovirus expression vector system (BEVs) from *Sf*9 insect cells were investigated in this study that included production, cell disruption techniques and purification.

The production of recombinant tNiVG was enhanced by investigating the preferred medium and fermentation conditions of *Sf*9 cells. The maximum production of tNiVG was achieved by infecting *Sf*900 III serum free medium (SFM) developed middle exponential 5.0×10^6 cells/mL with quaternary amplified recombinant baculovirus at a multiplicity of infection (MOI) of 5. At the preferred condition, about 1.43 mg of tNiVG per 5.0×10^6 cells was obtained after 3 days of post–infection. The amount of tNiVG produced was equivalent to 40% of total cellular protein and thus maximum production was achieved from the insect cell–baculovirus

expression vector system. Subsequent replacement of fresh *Sf*900 III SFM (every 3 days) reduced the doubling time (22 h) and achieved the maximum density (15.7×10^6 cells/mL) of *Sf*9 cells after 4 days. Hence, fresh *Sf*900 III SFM was used to study the effect of amplification of recombinant baculovirus, MOI and time of infection (TOI) on tNiVG production.

The chemical lysis, freeze–thawing, high–pressure homogenisation and ultrasonication cell disruption methods were investigated to release the recombinant tNiVG from *Sf*9 cells. Among the investigated methods, high–pressure homogenisation with a single–pass successfully released 1.64 mg/mL tNiVG per 5.0×10^6 insect cells with a purity of 45% at high sample volume.

Comparative evaluations of three immobilised affinity chromatography methods HisTrap™ FF 1 mL prepacked column, Ni SepFast™ MAG 1 mL adsorbent and conventional method for the recovery of tNiVG from *Sf*9 cells homogenate were investigated. The adsorption efficiency of applied clarified and unclarified feedstock onto HisTrap™ FF 1 mL prepacked column and Ni SepFast™ 1 mL MAG adsorbent was performed at 20 sodium phosphate mM, 500 mM sodium chloride, 20 mM imidazole and 5% glycerol containing pH 8 buffer. A single–step elution of bound tNiVG was performed with 20 mM sodium phosphate containing 250 mM sodium chloride, pH 7 buffer in the presence of 200 and 300 mM imidazole from HisTrap™ prepacked column and SepFast™ MAG adsorbent, respectively. Both IMAC operations achieved 94% purity and 92% recovery yield of tNiVG. Additionally, the unclarified feedstock application onto IMAC shortened the total processing time by about 13–fold as compared to the conventional method.

Followed by a single–step purification strategy, SepFast^{TM} Supor Q column of strong anion–exchange chromatography (AEC) was used to purify tNiVG produced in *Sf*9 insect cells. The preferred conditions of buffer to bind and to elute tNiVG were 50 mM sodium carbonate, pH 9 and 50 mM sodium citrate, pH 5. The use of elution buffer without sodium chloride separated the loosely bound tNiVG from the tightly bound major host proteins and subsequently avoided the desalting step as one of the further downstream processes. The developed method has recovered 89% tNiVG from the original supernatant with a protein purity of 90%. SDS–PAGE, Western blot and ELISA conformed purity and immunogenicity of single–step salt–free tNiVG (57 kDa). Further, the results of mass spectrometry confirmed the identity of tNiVG.

Information obtained from these studies was useful for development of efficient production and single–step purification of recombinant tNiVG from *Sf*9 cells. The overall recovery yield and purity throughout the studies proposed unit operations as simple, economic and fast method for the development of tNiVG. Along with these, the single–step purified tNiVG could be used as a potential agent for the development of an immunoassay for NiV antibodies.

Abstrak tesis yang dikemukan kepada senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGHASILAN DAN PENULENAN GLIKOPROTEIN VIRUS NIPAH YANG TERDAPAT DALAM SEL SERANGGA *Spodoptera frugiperda* 9 (J.E.SMITH)

Oleh

RAKSHA SUNHARE

October 2013

Pengerusi: Profesor Tey Beng Ti, PhD

Institut: Institut Biosains

Glikoprotein virus Nipah (NiVG) ungkapan dalam *Spodoptera frugiperda* 9 [*Sf*9 (J.E. Smith)] mengaruhkan antibodi peneutralan dan boleh digunakan sebagai pengesanan awal bagi melawan jangkitan NiV. Beberapa masalah industry semasa membangunkan glikoprotein rekombinan terpangkas NiV (tNiVG) daripada baculovirus ungkapan sistem vektor (BEVs) daripada sel–sel serangga *Sf*9 telah disiasat dalam kajian ini yang dimasukkan penghasilan, teknik–teknik gangguan sel dan penulenan.

Penghasilan protein rekombinan tNiVG telah dipertingkatkan dengan mengoptimumkan medium pilihan dan keadaan–keadaan penapaian sel–sel *Sf*9. Penghasilan maksimum protein tNiVG telah dicapai dengan menjangkitkan medium bebas serum (MBS) *Sf*900 II dibangunkan eksponen pertengahan 5.0×10^6 sel/mL dengan bakulovirus diperkuatkan kuarterner pada kegandaan jangkitan (MOI) 5. Pada keadaan pilihan, kira–kira 1.43 mg protein tNiVG per 5.0×106 sel telah diperolehi setiap 3 hari selepas pasca–jangkitan. Jumlah protein tNiVG yang telah dihasilkan adalah bersamaan 40% daripada jumlah protein selular dan dengan itu

penghasilan maksimum telah dicapai daripada ungkapan sistem vektor sel serangga–bakulovirus. Penggantian *Sf*900 III SFM segar berikutnya (setiap 3 hari) telah mengurangkan waktu penggandaan (22 j) dan telah mencapai ketumpatan maksimum (15.7×10^6 sel/mL) bagi sel–sel *Sf*9 selepas 4 hari. Oleh yang demikian, *Sf*900 III SFM segar telah digunakan untuk mengkaji kesan amplifikasi bakulovirus rekombinan, MOI dan waktu jangkitan terhadap penghasilan protein tNiVG. Lisis kimia, pencairan pembekuan, penghomogenan tekanan tinggi dan kaedah ultrasonikasi gangguan sel telah disiasat untuk membebaskan protein rekombinan tNiVG daripada sel–sel *Sf*9. Antara kaedah–kaedah yang telah dikaji adalah penghomogenan tekanan tinggi dengan dengan laluan tunggal telah berjaya membebaskan 1.64 mg/mL protein tNiVG per 5.0×10^6 sel–sel serangga dengan ketulenan 45% pada jumlah sampel yang tinggi.

Penilaian perbandingan terhadap tiga kaedah kromatografi keafinan pegun turus siap dibungkus HisTrap™ FF 1 mL, penjerap Ni SepFast™ MAG dan kaedah konvensional bagi pemulihan protein tNiVG dari homogenate sel–sel telah diselidik. Kecekapan pengikatan bagi stok suapan dijernihkan dan tidak dijernihkan yang digunapakai ke atas turus siap dibungkus HisTrap™ FF 1 mL dan penjerap Ni SepFast™ MAG telah dijalankan pada 20 natrium foSfat mM, 500 mM natrium klorida, 20 mM imidazole dan 5 % gliserol yang mengandungi penimbal pH 8. Elusi langkah tunggal telah dijalankan ke atas protein tNiVG yang terikat dengan 20 mM natrium foSfat yang mengandungi 250 mM natrium klorida, penimbal pH 7 dengan kehadiran 200 dan 300 mM imidazola daripada turus siapdibungkus HisTrap™ dan penjerap SepFast™ MAG masing–masingnya. Kedua–dua operasi IMAC telah mencapai 94% ketulenan dan 92% hasil pemulihan protein tNiVG. Selain itu,

penggunaan stok suapan tidak dijernihkan ke atas IMAC telah memendekkan jumlah masa pemprosesan sehingga 13–kali ganda jika dibandigkan dengan kaedah konvensional.

Diikuti dengan satu langkah strategi penulenan, turus kromatografi pertukaran anion (AEC) SepFast^{TM} Supor Q yang kuat telah digunakan untuk menulenkan tNiVG yang telah dihasilkan oleh sel–sel serangga *Sf*9. Keadaan penimbal yang dipilih untuk mengikat dan menanggalkan protein tNiVG adalah 50 mM natrium karbonat, pH 9 dan 50 mM natrium sitrat, pH 5. Penggunaan penimbal elusi tanpa natrium klorida mengasingkan protein tNiVG yang terikat secara longgar daripada protein hos utama yang terikat dengan kuat dan seterusnya mengelakkan langkah penyahgaraman sebagai salah satu proses hiliran berikutnya. Kaedah yang telah dibangunkan telah mendapatkan kembali 89% protein tNiVG daripada supernatant dengan ketulenan protein 90%. SDS–PAGE, ujian pemendapan Western dan ELISA mengesahkan ketulenan dan and keimunogenan bagi langkah–tunggal protein tNiVG bebas–garam (57 kDa). Tambahan lagi, keputusan spektrometri jisim mengesahkan identiti protein tNiVG.

Maklumat yang diperoleh daripada kajian ini berguna untuk pembangunan pengeluaran yang cekap dan penulenan langkah tunggal bagi rekombinan protein tNiVG dari sel–sel *Sf*9. Hasil pemulihan dan ketulenan keseluruhan di sepanjang kajian mencadangkan bahawa operasi unit adalah mudah, ekonomi dan merupakan kaedah pantas bagi pembangunan protein tNiVG. Pada masa yang sama, penulenan protein tNiVG satu–langkah boleh digunakan sebagai agen yang berpotensi untuk pembangunan satu imuocerakinan bagi antibodi–antibodi NiV.

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"Failures are part of life. If don't fail, don't learn. If don't learn, we'll never change"
(Source: Unknown Quotes)

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Thanks to Everyone!

Raksha Sunhare
(http://www.studymalaysia.com/student/views.php?code=UPM)

I certify that an Thesis Examination Committee has met on date 21st October 2013 to conduct the final examination of Raksha Sunhare on her thesis entitled "Production and purification of Nipah virus glycoprotein in *Spodoptera frugiperda* 9 (J.E. Smith) insect cell" 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 Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

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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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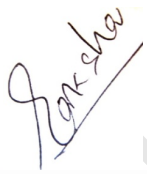
DECLARATION

I declare that the thesis is my original work except for quotation 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.



RAKSHA SUNHARE

Date: 21 October 2013

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