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PURIFICATION OF LONG HELICAL CAPSID OF NEWCASTLE DISEASE VIRUS FROM *ESCHERICHIA COLI*

By

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PURIFICATION OF LONG HELICAL CAPSID OF NEWCASTLE DISEASE VIRUS FROM *ESCHERICHIA COLI*

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Institute: Bioscience

The truncated version of nucleocapsid (N) protein of Newcastle disease virus (NDV) expressed in *Escherichia coli*, $NP_{\Delta c375}$ self-assembles into relatively long herringbone-like particles. This truncated protein is vulnerable to endogenous host cell proteases. In order to limit the degradation and improve the production yield of recombinant $NP_{\Delta c375}$, a bioinformatics programme, PeptideCutter was used to predict the possible endogenous proteases that led to the $NP_{\Delta c375}$ degradation. The size of the degraded bands of the $NP_{\Delta c375}$ of NDV on Western blots was analyzed by using the Quantity One software to predict the potential proteases cleavage sites on $NP_{\Delta c375}$ amino acid sequence. The possible proteases that degrade $NP_{\Delta c375}$ are serine and metalloproteases. Serine and metalloprotease inhibitors, namely phenylmethylsulfonyl fluoride (PMSF) and ethylenediaminetetraacetic acid (EDTA) were therefore employed to inhibit the endogenous proteolytic activities. Combination of the mentioned protease inhibitors at their optimal concentration was found to have a synergistic effect and resulted in about 2.1-fold increase in the $NP_{\Delta c375}$ yield. When tested with enzyme-linked immunosorbent assay (ELISA), the

purified $NP_{\Delta c375}$ treated with protease inhibitors was found to retain its reactivity against chicken anti-NDV antisera. In addition, they assembled into ring-like, short and long herringbone-like structures when examined under a transmission electron microscope (TEM). These imply that both the antigenicity and the particle forming capacity of the $NP_{\Delta c375}$ were preserved after being treated with protease inhibitors.

The conventional sucrose density gradient ultracentrifugation has been stated to be tedious and time consuming in the purification of NDV N protein. With the intention of improving the purification efficiency, the recombinant $NP_{\Delta c375}$ of NDV was purified with a packed bed anion exchange chromatography (AEC) from clarified *E. coli* homogenate. Packed bed AEC permits the purification processes to be completed in a shorter duration with lesser number of unit operations than that of sucrose density gradient ultracentrifugation. There was 76.3% of $NP_{\Delta c375}$ from the clarified *E. coli* homogenate adsorbed to the anion exchanger at the optimal binding pH (pH 6). An optimal desorption of the bound $NP_{\Delta c375}$ was accomplished by applying 50 mM Tris-HCl buffer at pH 7 in the presence of 0.5 M NaCl. The purification of $NP_{\Delta c375}$ of NDV by employing a pre-packed AEC has resulted in a 51.4% recovery of $NP_{\Delta c375}$ from the clarified feedstock with a purity and purification factor of 76.7% and 6.5, respectively. The antigenicity and the self-assembly property of the purified $NP_{\Delta c375}$ were preserved throughout the whole purification process as proven by ELISA and TEM analysis, respectively. The helical structure formed was as long as 490 nm and 22-24 nm in diameter.

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

PENULENAN KAPSID HELIKS PANJANG VIRUS PENYAKIT SAMPAR AYAM DARI *ESCHERICHIA COLI*

Oleh

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Versi terpotong protein nukleokapsid (N) virus penyakit Sampar Ayam (NDV) diekspres dalam *Escherichia coli*, $NP_{\Delta c375}$ gabung sendiri membentuk partikel menyerupai "herringbone" yang agak panjang. Protein terpotong ini adalah terdedah kepada protease endogenus *E. coli*. Dalam usaha untuk menghadkan degradasi dan meningkatkan penghasilan rekombinan $NP_{\Delta c375}$, satu program bioinformatik, PeptideCutter telah digunakan untuk meramal protease endogenus potensi yang meyebabkan degradasi $NP_{\Delta c375}$. Saiz pita $NP_{\Delta c375}$ NDV yang mengalami degradasi pada pemblotan Western telah dianalisis dengan menggunakan Quantity One software untuk meramal tapak pemotongan protease potensi pada jujukan asid amino $NP_{\Delta c375}$. Protease potensi yang bertindak ke atas $NP_{\Delta c375}$ adalah protease serina dan protease metallo. Oleh itu, perencat protease serina dan metallo, iaitu fenilmetilsulfonil fluorida (PMSF) dan asid etilenadiaminatetra asetat (EDTA) telah digunakan untuk merencat aktiviti proteolitik endogenus. Gabungan perencat protease tersebut pada kepekatan optimum telah didapati mempunyai kesan sinergistik dan telah meningkatkan kira-kira 2.1 kali ganda hasil $NP_{\Delta c375}$. Apabila

diuji dengan asai imunosorben bergabung enzim (ELISA), hasil penulenan $NP_{\Delta c375}$ yang telah dirawat dengan perencat protease didapati mengekalkan kereaktifannya terhadap antisera anti-NDV ayam. $NP_{\Delta c375}$ yang ditulenkan juga membentuk struktur cincin dan struktur "herringbone" pendek dan panjang semasa diperiksa di bawah mikroskop elektron (TEM). Ini menunjukkan bahawa kedua-dua keantigenan dan keupayaan pembentukan partikel $NP_{\Delta c375}$ dipelihara selepas dirawat dengan perencat protease.

Pengemparan-ultra ketumpatan sukrosa konvensional adalah menyusahkan dan memakan masa dalam penulenan N protein NDV. Dengan tujuan untuk meningkatkan kecekapan penulenan, rekombinan $NP_{\Delta c375}$ NDV telah ditulenkan daripada homogenat *E. coli* yang telah dijernihkan dengan mengaplikasikan sistem kromatografi pertukaran anion (AEC) pada lapisan terpadat. AEC lapisan terpadat membolehkan proses penulenan disempurnakan dalam jangka masa yang lebih singkat dengan bilangan unit operasi yang kurang berbanding dengan pengemparan-ultra ketumpatan sukrosa. Terdapat 76.3% $NP_{\Delta c375}$ daripada homogenat *E. coli* yang telah dijernihkan terjerap pada penukar anion pada pH penjerapan optimum (pH 6). Kadar elusi optimum $NP_{\Delta c375}$ terjerap tercapai dengan menggunakan larutan penimbal yang mengandungi 50 mM Tris-HCl dan 0.5 M sodium klorida (NaCl) pada pH 7. Penulenan $NP_{\Delta c375}$ NDV dengan menggunakan AEC lapisan terpadat telah membawa 51.4% pemulihan $NP_{\Delta c375}$ daripada suapan yang telah dijernihkan dengan ketulenan dan faktor penulenan 76.7% dan 6.5 masing-masing. Keantigenan dan ciri-ciri gabung sendiri $NP_{\Delta c375}$ yang ditulenkan dipelihara selepas proses

penulenan seperti yang dibuktikan melalui analisis ELISA dan TEM masing-masing. Struktur heliks terbentuk adalah sepanjang 490 nm dan berdiameter 22-24 nm.

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DECLARATION

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

YAP CHEE FAI

Date: 8 January 2013

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