



UNIVERSITI PUTRA MALAYSIA

**STRUCTURE-TO-FUNCTION PREDICTION AND CLONING OF
KPN_02816 (yfgC) HYPOTHETICAL PROTEIN OF
Klebsiella pneumoniae MGH78578 PATHOGEN**

NUR AMALIA HASNI

FBSB 2015 161

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BACHELOR OF SCIENCE (HONS.)

UNIVERSITI PUTRA MALAYSIA

2015

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KPN_02816 (*yfgC*) HYPOTHETICAL PROTEIN OF *Klebsiella pneumoniae*
MGH78578 PATHOGEN**

By

NUR AMALIA BINTI HASNI

Thesis submitted to the Department of Cell & Molecular Biology, faculty of
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Nur Amalia binti Hasni

June 2015

Chair: Dr. Normi Mohd. Yahaya

Faculty: Faculty of Biotechnology & Biomolecular Sciences

Klebsiella pneumoniae is a dangerous pathogen that causes many healthcare-associated infections. In 2006, the complete genome of *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578 was sequenced and 21% of the total 4894 genes in the genome encodes for hypothetical proteins (HPs). In general HPs are referred as proteins of unknown function which they are predicted to be expressed from an open reading frame, but there is no experimental evidence expressed *in vivo*. Analyzing, annotating and characterizing these hypothetical proteins will bring insight to their functions which will lead to new discoveries of their structures, functions and applications. Previously, an HP termed KPN_02816 was identified to be a 'hypothetical protease' with 99% sequence identity to peptidase M48 family from this pathogen. As proteases have been reported to play a role in conferring pathogenicity, this present study further the investigation of predicting the structure and function of KPN_02816 as well as cloning the gene into *E. coli* DH5 α for its production and *in vitro* biochemical characterization. Bioinformatics analyses using CDD, PFAM, MEROPS, PDB, and BLAST programs on the sequence of KPN_02816 sequence showed that the HP has functionally important domains such as peptidase family M48, TPR (tetratricopeptide repeat) domain, TPR₁₄ (tetratricopeptide repeat) and putative zinc dependent protease. Prediction of its 3D structure by I-TASSER revealed that it resembles the crystal structure of APC/C heterotetramer Cut9-Hcn1 (PDB ID: 2XP1A) using the predicted template of x-ray structure of the putative zinc dependent peptidase Q74D82 at resolution 1.7Å (PDB ID: 3C37A). TM-score predicted was 0.929 and the RMSD value is 1.10 Å when model 1 is aligned with 2XP1A. The open reading frame of KPN_02816 was synthesized with the incorporation of *Eco*RI and *Hind*III at 5' and 3' end, respectively and was cloned into pUCIDT AMP cloning vector and subsequently into pET-28b(+) expression vector for production of the protein in *E. coli* BL21(DE3).

Keywords: Hypothetical proteins, peptidase, KPN_02816, structure, cloning

Abstrak tesis yang dikemukakan kepada Jabatan Biologi Sel & Molekul sebagai memenuhi keperluan untuk ijazah Sarjana Muda (Kepujian) Biologi Sel & Molekul

**JANGKAAN STRUKTUR-KE-FUNGSI DAN PENGKLONAN KPN_02816
(yfgC) HIPOTETIKAL PROTEIN PATOGEN *Klebsiella pneumonia*
MGH78578**

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Klebsiella pneumonia ialah sejenis patogen yang berbahaya dan dipercayai mengakibatkan punca jangkitan penyakit penjagaan kesihatan. Pada tahun 2006, genom lengkap *Klebsiella pneumonia* subsp. *pneumonia* MGH78578 telah diujikan dan 21% dari jumlah keseluruhan 4894 gen di dalam genom mengkod protein hipotetikal. Secara umumnya, protein hipotetikal merujuk kepada protein yang tidak diketahui fungsinya dan dipercayai diekspreskan dari satu rangka bacaan terbuka, tetapi tiada bukti uji kaji diekspreskan *in vivo*. Menganalisis, menganotasi dan mengkarakterkan protein hipotetikal akan memberi fahaman mengenai fungsi protein dan membawa ke arah kemungkinan penemuan sasaran ubat baru. Kajian sebelum ini telah menemukan satu protein hipotetikal iaitu KPN_02816 dan dikategorikan sebagai 'protease hipotetikal' dan mempunyai 99% persamaan jujukan dengan keluarga *peptidase* M48. Oleh kerana *protease* telah dilaporkan memainkan peranan dalam menyebabkan kepatogenan, kajian ini berkisar sekitar penyelidikan meramalkan struktur dan fungsi KPN_02816 dan seterusnya mengklonkan gen KPN_02816 ke dalam *E. coli* DH5 α untuk penghasilan dan pencirian biokimia secara *in vitro*. Jujukan KPN_02816 digunakan untuk menganalisa kehadiran domain, dan juga fungsi struktur menggunakan alatan bioinformatik seperti CDD, PFAM, MEROPS, PDB dan BLAST. Analisa ini menrunkaikan bahawa protein hipotetikal ini mempunyai domain yang penting seperti keluarga *peptidase* M48, TPR (ulangan tetratrikopeptida) domain dan TPR_14 (ulangan tetratrikopeptida) dan *protease* kebergantungan zink. Struktur tiga dimensi protein yang diramalkan oleh I-TASSER mendedahkan bahawa ia menyerupai struktur kristal APC/C heterotetramer Cut9-Hcn1 (PDB ID: 2XPJA) menggunakan templat struktur X-ray *peptidase* kebergantungan zink Q74D82 dengan resolusi 1.7Å (PDB ID: 3C37A). Markah TM diperoleh apabila struktur model 1 dan 2XPJA dijajarkan ialah 0.929 dan nilai RMSD 1.10 Å. Rangka bacaan terbuka (ORF) KPN_02816 dihasilkan dengan pengenalan enzim penyekatan *EcoRI* dan *HindIII* di terminal 5' dan 3' masing-masing dan diklonkan ke dalam vector pengklonan pUCIDT AMP dan seterusnya ke dalam vector pengepresan pET-28b(+) untuk penghasilan protein di dalam *E. coli* BL21(DE3).

Kata kunci: Protein hipotetikal, *peptidase*, KPN_02816, struktur, pengklonan

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Approval

This thesis was submitted to the Department of Cell & Molecular Biology, Faculty of Biotechnology & Biomolecular Sciences and has been accepted as fulfillment of the requirement for the degree of B.Sc.(Hons.) Cell & Molecular Biology. The member of the Supervisory Committee was as follows:

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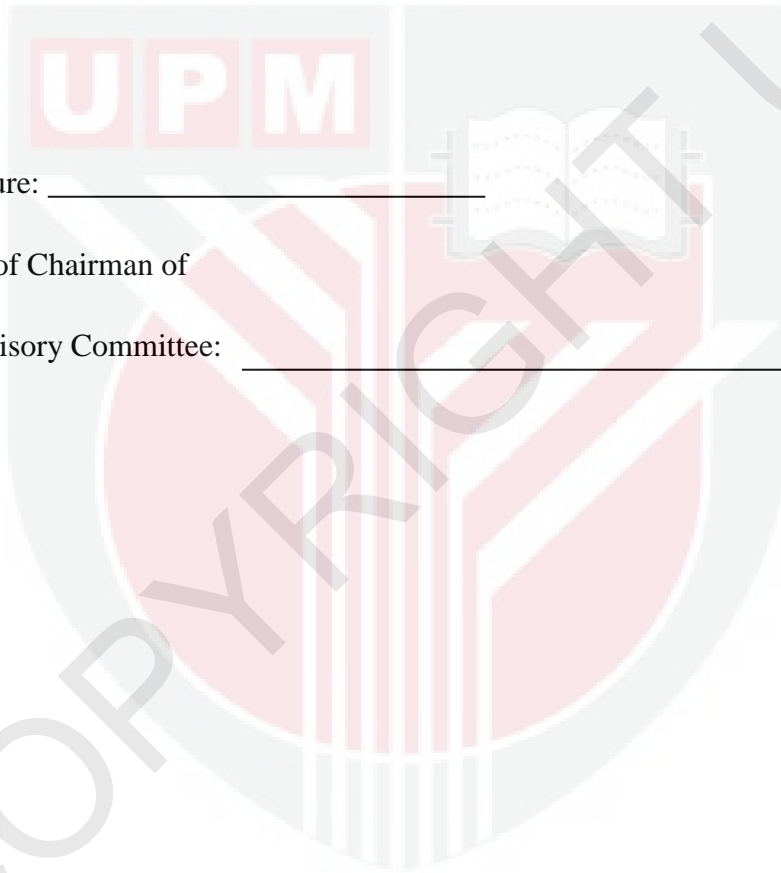


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LIST OF ABBREVIATIONS

bp	base pair
HP	hypothetical protein
nr	non redundant
CDD	Conserved Domain Database
UTI	Urinary Tract Infections
CRKP	carbapenem-resistant <i>K. pneumonia</i>
KPC	<i>K. pneumoniae</i> carbapenemase
ORF	Open Reading Frame
PDZ	PSD-95, Dlg and Zo-1/2
PDB	Protein Data Bank
I-TASSER	Iterative Threading ASSEMBly Refinement
RaCC	Rare Codon Calculator
AMP	Ampicillin
KAN	Kanamycin
LB	Luria Bertani
PCR	Polymerase Chain Reaction
kb	kilobase pair
RE	Restriction enzyme
Zn	Zinc

1.0 Introduction

A member of the Enterobacteriaceae family, together with *E. coli*, *Klebsiella pneumoniae*, accounts for the vast majority of hospital and community-acquired urinary tract infections or UTIs. This type of Gram-negative bacteria is also a frequent cause of nosocomial bloodstream infections and community-acquired pneumonia among alcoholics. The rise of carbapenem-resistant *K. pneumoniae* (CRKP) is disconcerting based on reason that this *K. pneumoniae* carbapenemase (KPC) infection is related with a fatal outcome of about 47-57 % cases. (http://www.cddep.org/projects/resistance_map/klebsiella_pneumoniae_overview).

The genome sequence of *K. pneumoniae* MGH78578 was completely sequenced in 2007 (McClelland *et al.*, 2006). Over the last decade, more than 150 complete genomes of various bacteria, archaea and eukaryotes have been sequenced, and there are many more which are currently in the pipeline. (Koonin & Galparin, 2002). However, despite the availability of complete genome which had been sequenced, it is well known that as many as 30 to 40% of the genes do not have an assigned function (Bork, 2000). These genes are termed as hypothetical genes which encode for hypothetical proteins. These ‘conserved hypothetical’ proteins pose a challenge not just to functional genomics, but also to biology in general (Galperin, 2001). When an open reading frame is marked as ‘conserved hypothetical protein’, this does not mean that the function of the protein is completely unknown. Various bioinformatics tools can be used for the analysis of the hypothetical proteins. The chosen hypothetical protein in this study is KPN_02816 from *K. pneumoniae* subsp. MGH78578 and this chosen hypothetical protein is a hypothetical protease. Protease is chosen as the main subject of interest because of its role in pathogenicity (Dutta & Katarkar, 2013).

1.1 Objectives of this study:

1. To investigate the possible structure and function of KPN_02816 (*yfgC*)
2. To synthesis the hypothetical protein gene into cloning vector with optimized codon sequence
3. To clone the gene encoding the hypothetical protease into an expression vector



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Biodata

Nur Amalia Binti Hasni, the eldest daughter of Hasni Hussain and Rahayu Hanapiah was born on 9th September 1992 at Hospital Besar Alor Star, Kedah. Her early education was at Tadika Kemas Taman Meranti, Changlun Kedah and she was awarded as “Tokoh Pelajar Cemerlang 1998” at that time. She started her primary education at Sek. Keb. Dato’ Wan Kemara, Changlun and she was elected as school prefect for 4 years (2000-2004). In 2003, together with her 5 friends in the school, she was awarded “Anugerah Tunas Mas” which was held at the state level and the award was given by Former Prime Minister of Malaysia, Tun Dr. Mahathir Mohamad. She joined Scout for 3 years and joined the camping and hiking organized by the club. Although her UPSR trial result was not so good, she managed to get 5A’s in the UPSR and further her studies at Mara Junior Science College (MJSC) Pendang for 3 years. She managed to get 8A’s for her PMR. After that, she moved to Mara Junior Science College Beseri for two years. A bit disappointed, she got 6A’s and 3B’s for her SPM. Not too long after that, she got an offer letter from UPM which is the Foundation of Agricultural Sciences but she refused to go there and she chose matriculation program rather than foundation. She passed her matriculation program and fate brought her back to Universiti Putra Malaysia and she studied here in Degree of Bachelor Science (Hons.) Cell & Molecular Biology and despite this course was her fifth choice, she doesn’t regret it after she had go through and learned in depth about this course. Biology was always her passion since she was in primary school. She believed that talking is cheap, exploration and discovery is hard, and once we had discovered something beneficial for others, it will become the best thing ever. So as long as we breathe, take the opportunity to learn, explore and discover new things, as the knowledge is light and a true education is a never-ending process.