CLONING OF INFLUENZA B NS1 GENE IN *Escherichia coli*

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To my beloved parents & my wife:

Thanks for the dim of light when all I see were darkness…

Thanks for giving me the best things in my life …and finally

Thanks for your sacrifices to make me a better person each day….

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ABSTRACT

The non-structural NS1 protein of influenza B virus is a multi-functional virulence protein which is involved in the transport of viral RNA. Inside the host cell, NS1B antagonizes and inhibits the α/β interferon system which is induced as host antiviral response. Moreover, it prevents the activation of double-stranded-RNA activated protein kinase (PKR) by binding to dsRNA and inhibits the maturation of *GAS8* (gene of tumor suppressor). NS1 protein is utilized as a target for diagnostic of influenza viruses in infected animals. pUC57 carrying NS1 synthetic gene of influenza B was attempted to be transformed into *Escherichia coli* strain BL21(DE3). NS1B was extracted and attempted to be cloned into prokaryotic expression vectors pET-32b, pET-32a, pQE-81L and pQE-80L, respectively using restriction digestion enzymes (*Sac*I, *Pst*I and *Hin*dIII). Then, recombinant DNA was attempted to be transformed into *Escherichia coli* strains BL21(DE3) and DH5α.

ABSTRAK

Protein non-struktur NS1 virus influenza B adalah virulensi pelbagai fungsi dan terlibat dalam pengangkutan RNA virus. Di dalam sel perumah, NS1B menghalang sistem interferon α/β yang menyebabkan tindakbalas antivirus oleh perumah. Selain itu, protein NS1 mencegah pengaktifan *double-stranded RNA-aktif protein kinase* (PKR) dengan cara mengikat dsRNA dan menghalang pematangan *GAS8* (gen tumor supresor). NS1 protein digunakan sebagai target untuk diagnostik terhadap virus influenza pada haiwan yang dijangkiti. pUC57 membawa gen NS1 sintetik influenza B diklonkan ke dalam *Escherichia coli* strain BL21(DE3). NS1B diekstraksi dan diklon ke vektor ekspresi prokariotik pET-32b, PET-32a, pQE-81L dan pQE-80L masing-masing dengan menggunakan enzim pembatas (*Sac*I, *Pst*I dan *Hin*dIII). Kemudian DNA rekombinan ditransformasikan ke dalam strain *Escherichia coli* BL21 (DE3) dan DH5α.

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LIST OF SYMBOLS/ ABBREVIATIONS

AMP	-	Adenosine monophosphate
ATP	-	Adenosine triphosphate
bp	-	Base pairs
BSA	-	Bovine Serum Albumin
α/β	-	Alfa / Beta interferon
˚C	-	Degree Celsius
cDNA	-	Clone deoxyribonucleic acid
del NS1	-	Deletion nonstructural 1
dH_2O	-	Deionized water
dNTP	-	Deoxynucleoside triphosphate
DNA	-	Deoxyribonucleic acid
dsRNA	-	double-stranded RNA
E. coli	-	*Escherichia coli*
EDTA	-	Ethylene diamenetetraacetate
ELISA	-	Enzyme linked immunosorbent assay
g	-	Gram
GAS	-	Growth arrest specific gene
GST	-	Glutathione S-transferase
HA	-	Hemagglutinine
His-Tag	-	Histidine tagged
IFN	-	Interferon
IPTG	-	Isopropyl β-D-1-thiogalactopyranoside
IRF	-	Interferon regulator factor

ISG	-	Interferon stimulate gene
kb	-	Kilo base
KDa	-	Kilo dalton
LB	-	Luria Bertani
LPAI	-	Low pathogenicity avian influenza
M	-	Molar
mM	-	Milmolar
MCS	-	Multi cloning site
ml	-	Milliliter
mg	-	Milligram
min.	-	Minutes
µg	-	Microgram
µl	-	Microliter
µm	-	Micromter
$MgCl_2$	-	Magnesium chloride
mRNA	-	Messenger ribonucleic acid
NA	-	Neuraminidase
NEP	-	Nuclear export protein
NES	-	Nuclear export sequence
ng	-	Nanogram
NLS	-	Nuclear localization sequence
NMR	-	Nuclear Magnetic Resonance
NS1	-	Nonstructural 1
OD	-	Optical density
PACT	-	Protein activator of the interferon-induced protein kinase
PCR	-	Polymerase chain reaction
PKR	-	Protein kinase
RNA	-	Ribonucleic acid

RNP	-	Ribonucleoprotein
rpm	-	Rotation per minute
RT-PCR	-	Reverse transcription polymerase chain reaction
SDS-PAGE	-	Sodium Dodecyl Sulphate- Polyacrilamide Gel Electrophoresis
sec.	-	Seconds
SIV	-	Simian immunodeficiency virus
ssRNA	-	Single strand RNA
TAE	-	Tris-acetate-EDTA
Tris	-	2-hydroxymethyl-2-methyl-1,3-propanediol
U6 SnRNA	-	U6 small nuclear ribonucleoprotein
UV	-	Ultraviolet
V	-	Volts

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CHAPTER 1

INTRODUCTION

1.1 Influenza

Influenza is a contagious respiratory viral illness of global importance. The disease was caused by influenza viruses known as flu. The most common symptoms of the flu are chills, fever, sore throat, muscle pains, severe headache, coughing, weakness and general discomfort. Some influenza viruses can cause more severe diseases than the common cold like pneumonia. Influenza viruses spread around the world and can be transmitted through the air by coughs, sneezes, creating aerosols containing the virus. This can also be transmitted by direct contact with infected animals or humans (Metreveli *et al*., 2006; Spickler *et al*., 2009).

1.2 Influenza virus

Influenza viruses have unique features of reverse sense single strand RNA. They have been classified into three distinct types: A, B and C. Influenza B viruses are mainly found in humans. These viruses can cause epidemics in human populations, but have not been responsible for pandemics. Influenza B viruses

comprised of single group of hemagglutinin and neuraminidase antigens since their first isolation in 1940 (Nerome *et al*., 1998). Influenza B viruses are categorized into lineages rather than subtypes and are also classified into strains. Influenza B viruses undergo antigenic drift, though it occurs more slowly than in influenza A viruses (Metreveli *el al*., 2006; Spickler *et al*., 2009). Twelve antigenic variants were distinguished by a panel of monoclonal antibodies appeared to circulate in the 1981–1982 epidemic season in Japan. The evolutionary lineages of influenza B viruses since 1988 have been represented by two epidemic strains B/Victoria/2/87 and B/Yamagata/16/88 (Nerome *et al*., 1998).

1.3 Problem statements of the study

The main problem of this study is to clone NS1B gene in pET-32b, pET-32a, pQE-81L, and pQE-80L vectors for transformation into *Escherichia coli* BL21(DE3) and DH5α.

1.4 Objective of the study

The objective of this research was to clone of NS1B synthetic gene into pET-32b, pET-32a, pQE-81L, and pQE-80L vectors. The recombinant constructs were transformed into *E. coli* hosts.

1.5 Scope of the study

The scope of this study encompassed the cloning of NS1B gene of influenza B into pET-32b, pET-32a, pQE-81L, and pQE-80L vectors, which are subsequently transformed into competent *E. coli* BL21(DE3) and DH5α.

1.6 Significant of the study

Recombinant NS1 fusion protein of high purity is more significant for detection of antigenicity. Successful cloning and overexpression of NS1 gene are useful for specific diagnostic and further applications. This reverse genetic system will allow studies to explore the functions of NS1B domains during the replication cycle and to assess their contributions to the pathogenesis and virulence of influenza B virus.