BIOINFORMATICS ANALYSIS AND MOLECULAR CLONING OF AN EXTRACELLULAR SERINE PROTEASE FROM *ACINETOBACTER BAUMANNII*

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BIOINFORMATICS ANALYSIS AND MOLECULAR CLONING OF AN EXTRACELLULAR SERINE PROTEASE FROM *ACINETOBACTER BAUMANNII*

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I dedicate this work to those interested in molecular science

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ABSTRACT

Drug resistant *Acinetobacter baumannii* topped the list for antibiotic resistant 'critical' pathogens that was released by the World Health Organisation (WHO) in February 2017. The list was intended to guide and promote research and development (R&D) of antibiotics. One of the factors that may contribute to *A. baumannii* virulence are the secretory proteases that this bacteria produces. In order to design effective antibiotocs and treatment targeting secretory proteases from *A. baumannii*, the gene coding for the proteases needed to be cloned to produce recombinant form of the proteins that can be easily expressed and purified in the quantities and purity suitable for functional and structural studies. A secreted serine protease was identified from *A. baumannii*, termed as "SPSFQ". Bioinformatics analysis using BLAST and multiple sequence alignment indicated that the enzyme belonged to serine endopeptidases (E.C. 3.4.21.-) family with a predicted catalytic triad motif of D130/H163/S315. Structure of SPSFQ modelled using the homology modeling software, I-TASSER revealed that the enzyme folding was highly conserved to keratinase 5WSL with seven stranded parallel β sheets flanking by six α helices and four β sheets made of two anti-parallel strands. *SPSFQ* with 1104 bp coding for 368 amino acids was subcloned into pET-22b(+) between *Bam*H1 and *Sal*1 and expressed in periplasmic fraction of *E. coli* BL21 (DE3). Total cell protein with induction condition at 16 ºC and 25 ºC with 1mM IPTG showed two distinct bands around 40 kDa (proenzyme form) and 30 kDa (active form) in western blot. Cell lysate did not show any activity during enzymatic assay probably because of SPSFQ was expressed in inclusion form. As a conclusion, *SPSFQ* was successfully sub-cloned and expressed in *E. coli* BL21 (DE3). Further study will focus on purification and characterization of SPSFQ in order to identify the cellular importance of SPSFQ towards *A. baumannii*.

ABSTRAK

Perintang antibiotik *Acinetobacter baumannii* telah disenaraikan sebagai pathogen yang utama oleh World Health Organization (WHO) pada Februari 2017. Senarai tersebut telah disediakan sebagai panduan untuk penyelidikan dan pembangunan (R&D) antibiotik. Salah satu faktor yang menyumbang kepada kebisaan *A. baumannii* adalah rembesan proteasenya ke luar sel. Rekaan antibiotik dan rawatan yang berkesan berasaskan protease yang dikeluarkan oleh *A. baumannii* memerlukan gen tersebut diklon, diekspres dan ditulenkan untuk pengajian. Satu protease dinamakan "SPSFQ" telah dipencil dari *A. baumannii*. BLAST dan perbandingan urutan menunjukkan *SPSFQ* merupakan "serine endopeptidase" (E.C. 3.4.21.-) dengan asid amino yang aktif pada D130/H163/S315. Struktur protease telah dirangka dengan I-TASSER dan menunjukkan protease mempunyai struktur yang serupa dengan 5WSL (keratinase), iaitu tujuh strand "β sheets" selari diapit dengan enam "α helices" dan empat "β sheets" yang dibentuk dengan dua anti-selari strand. *SPSFQ* yang mengandungi 1104 pasangan bes dan membentuk 368 asid amino telah diklon ke dalam pET-22b(+), antara tapak sekatan *Bam*HI dan *Sal*I dan diekspres dalam ruang periplasmik *E. coli* BL21 (DE3). Jumlah protein sel dengan induksi pada 16 ºC dengan dan 25 ºC dengan 1mM IPTG telah menunjukkan protease ada pada sekitar 40 kDa (enzim yang tidak matang) dan 30 kDa (enzim yang matang) pada SDS-PAGE. Sel yang telah dimusnahkan tidak menunjukkan sebarang aktiviti mungkin disebabkan SPSFQ tidak larut air. Secara kesimpulan, *SPSFQ* telah berjaya diklon dan diekspres oleh *E. coli* BL21 (DE3). Kajian seterusnya akan memberi tumpuan kepada menulenkan dan mencirikan SPSFQ supaya kepentingannya kepada *A. baumannii* dapat dikenalpastikan.

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LIST OF ABBREVIATIONS

AEBSF	-	4-(2 Aminoethyl)-benzenesulfonyl fluoride hydrochloride
APS	-	Ammoniumpersulfate
BLASTp	-	Protein Basic Alignment Search Tool
BSA	-	Bovine serum albumin
Ca^{2+}	-	Calcium ions
$CaCl_2$	-	Calcium chloride
DNA	-	Deoxyribonucleic acid
FV	-	Purified factor V
EC	-	Enzyme Commision
E. coli	-	*Escherichia coli*
et al	-	And others
His	-	Histidine
ICU	-	Intensive care unit
IMAC	-	Immobilised metal-affinity chromatography
IPTG	-	Isopropyl-1-thio-β-D-galactose
LB	-	Luria Bertani
MDR	-	Multi drug resistance
MIC	-	Minimum inhibitory concentration
Mw	-	Molecular weight
NaCl	-	Sodium chloride
NaOH	-	Sodium hydroxide
NCBI	-	National Center for Biotechnology Information
Ni^{2+}	-	Nickel ions
OD	-	Optical density
PCR	-	Polymerase chain reaction

RMSD	-	Root mean square deviation
SDS	-	Sodium Dodecyl Sulfate
SDS-PAGE	-	Sodium dodecyl sulfate polyacrylamide gel
sp.	-	Species
SOC	-	Super optimal broth with catabolite repression
tRNA	-	Transfer ribonucleic acid
TEMED	-	Tetramethylethylenediamine
US	-	United States of America
WHO	-	World Health Organization
XDR	-	Extensively drug resistant
3D	-	Three dimension

LIST OF SYMBOLS

˚C	-	Degree celsius
× *g*	-	Times gravity
µL	-	Microliter
µM	-	Micromolar
µmol	-	Micromole
g	-	Gram
g/L	-	Gram per liter
g/mol	-	Gram per mole
kDa	-	Kilo dalton
L	-	Litre
M	-	Molar
mg	-	Milligram
mL	-	Milliliter
mM	-	Millimolar
rpm	-	Revolutions per minute
U/mg	-	Enzyme unit per milligram
w/v	-	Weight per volume
α	-	Alpha
β	-	Beta
%	-	Percentage

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

On 27th February 2017, World Health Organization had announced "Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics" and carbapenem resistant *Acinetobacter baumanii* was categorized in the critical level together with carbapenem resistant *Pseudomonas aeruginosa* and *Enterobacteriaceae* (World Health Organization, 2017).

Acinetobacter baumannii, a Gram-negative cocci is ubiquitous and can be found across wide geographical areas. In the last decade or so, *A. baumannii* has emerged to be one of the main sources of opportunistic nosocomial infections in healthcare facilities where instrument contaminated with the bacteria that was later used for intubation or other similar application. Infection of such bacteria may cause several types of diseases including pneumonia, sepsis, bacteremia, soft tissue infection and meningitis (Wang *et al.*, 2014). Cases of *A. baumannii* showed a two-fold increase in occurrences in Malaysia from the year 2006 to 2007 (Ministry of Health Malaysia, 2008). Studies had shown that the period of time where patient stays in ICU is proportional to the rate of infection by such multi drug resistant (MDR) bacteria. Where those who underwent surgery are 2.2 times more likely to acquire such infection (Janahiraman *et al.*, 2015).

1.2 Problem Statement

Currently, *A. baumannii* had been identified to be resistant towards disinfectants, able to form robust biofilms, better desiccation tolerance and together with MDR had helped the bacteria to survive in different harsh environment (Kamolvit *et al.,* 2015). The hardier bacteria has rendered common and current treatment options of infections less effective, and will soon be pandrug resistant as available antibiotics are no longer able to control the bacteria. Characterization of the bacteria and its virulence properties are being prioritized by most of the researchers in order to reveal its weakness to speed up new drug designs (Mendez *et al*., 2012).

Extracellular proteome of *A. baumannii* have to be analyzed to provide insight to the infection mechanism. Among the important enzymes that contribute to *A. baumannii* survival, extracellular proteases seems to play a vital role although only two extracellular protease (CpaA and PKF) were characterized (Tilley *et al*., 2014; King *et al*., 2013). The disruption of both CpaA and PKF expression shows significant effect to the *A. baumannii* survival and pathogenicity. Hence, further knowledge on the extracellular proteome of *A. baumannii* is of great importance for the design of next generation antibiotic. Hereby, one of the serine protease gene was isolated from *A. baumannii* and cloned into expression host for characterization, identifying its role for cellular importance and infections.

1.3 Objectives

The objectives of this study include:

i. To analyze *SPSFQ* using bioinformatics tools.
ii. To subclone *SPSFQ* from pGEM into pET-22b(+).
iii. To carry out preliminary expression of recombinant protein in *E. coli* BL21 (DE3) for different IPTG concentration and temperature.

1.4 Scope of Study

The scopes of the study are as below:

i. Bioinformatics analysis of *SPSFQ*.
ii. Subcloning of serine protease gene *SPSFQ* from pGEM into pET-22b(+).
iii. Transformation of *E. coli* BL21 (DE3) with *SPSFQ*/pET-22b(+).
iv. Culture of the recombinant *E. coli* BL21 (DE3) in LB medium with carbenicillin.
v. Sequencing of the recombinant gene cloned into *E. coli* BL21 (DE3).
vi. Preliminary expression of the recombinant *E. coli* BL21 (DE3) with IPTG.
vii. Lysis of the recombinant *E. coli* BL21 (DE3) to extract crude enzyme of SPSFQ.
viii. Screening for enzymatic reaction with plate method.
ix. Using SDS-PAGE to detect the expression of SPSFQ.
x. Using Western blot to verify the expression of SPSFQ.

1.5 Significance of Study

Protease PKF isolated from *A. baumannii* showed inhibition to biofilm formation and enhanced bacterial growth in human serum (King *et al.*, 2013). Extracellular protease CpaA deregulates blood coagulation was also isolated from clinical strain (Tilley *et al.*, 2014). These research had demonstrated the importance of extracellular proteases in growth and propagation of *A. baumannii* in human host. Besides, strains of *A. baumannii* had been reported to gain resistant towards the antibiotic of last resort known as polymyxin (Lean *et al.*, 2014). Hence, understanding of the isolated protease SPSFQ may provide information on whether it can be used as new target for future drug design, providing more treatment options when he bacteria becomes pandrug resistant.

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