UNIVERSITI PUTRA MALAYSIA

EXPRESSION AND CHARACTERIZATION OF A TWO-COMPONENT ALKANESULFONATE MONOOXYGENASE SYSTEM FROM THERMOPHILIC BACTERIUM Anoxybacillus geothermalis STRAIN D9

DURRATUL FATINI BINTI YUSOFF

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By

DURRATUL FATINI BINTI YUSOFF

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

February 2021

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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DURRATUL FATINI BINTI YUSOFF

February 2021

Chair : Raja Noor Zaliha Raja Abd Rahman, D. Engr.
Faculty : Biotechnology and Biomolecular Sciences

The wide distribution of xenobiotic alkanesulfonate compounds in nature without proper handlings has raised health and environmental concerns. Degradation of these compounds happens naturally with the help of microorganisms. Alkanesulfonate monooxygenase system from bacteria carries out biodegradation of alkanesulfonate molecule. It is a two-component enzyme system that involved flavin transfer between flavin mononucleotide (FMN) reductase (SsuE) and alkanesulfonate monooxygenase (SsuD). SsuE supplies reduced FMN ($FMNH_2$) to SsuD as a cofactor. However, most alkanesulfonate monooxygenase systems studied were originated from mesophilic bacteria. Moreover, the stability and characteristic of the thermophile-origin alkanesulfonate monooxygenase system were unclear. Therefore, the expression and characterization of the thermophilic alkanesulfonate monooxygenase system can be applied for the bioremediation of xenobiotic alkanesulfonate pollutants. In this study, the properties of the recombinant alkanesulfonate monooxygenase system and its components from thermophilic *Anoxybacillus geothermalis* D9 were reported for the first time. The study aims to discover the biochemical and biophysical nature of SsuE and SsuD from thermophiles. In this study, *ssuE* and *ssuD* genes from *A. geothermalis* D9 were successfully cloned into an expression vector pET-51b (+) and expressed in *Escherichia coli* strain Rosetta (DE3). The recombinant SsuE and SsuD were purified via affinity chromatography with a molecular mass of 21.7 and 46.3 kDa, respectively. Temperature and thermal denaturation analysis show that SsuD with an optimum temperature of 40 °C is slightly more thermostable than SsuE that is optimal at wide range temperatures (30 – 50 °C). Their stability in a wide temperature ranging from 20 to 50 °C indicates that they are thermostable enzymes. Both proteins also prefer pH 8 but SsuD is more stable in slightly alkaline pH as compared to SsuE. Furthermore, the alkanesulfonate

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monooxygenase system in this study is stable in most organic solvents especially those with high LogP. This system catalyzed redox reactions, thus, their activities were greatly enhanced by the presence of most metal ions. These proteins are moderately stable in non-ionic surfactants and greatly inhibited by anionic surfactants. Physical and structural analysis of this system found that they are mainly made up of α-helices. Besides, *in silico* studies predict SsuE and SsuD exist as dimeric α/β/α flavodoxin and TIM barrel structure, respectively. The computational analysis also predicted the active pockets and interaction of protein residues with the substrate was located in the C-terminal end of β-sheets for both proteins. In general, SsuD characterized in this study exhibits better stability as compared to SsuE which might be due to the differences in their protein structure. In conclusion, the thermostable alkanesulfonate monooxygenase system from *A. geothermalis* D9 with a unique pH stability profile and active in many types of solvents making it an attractive multi-enzyme system to be exploited for bioremediation or industrial purposes.

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

PENGEKSPRESAN DAN KARAKTERISASI SISTEM MONOOKSIGENASE ALKANASULFONAT DUA KOMPONEN DARIPADA BAKTERIA TERMOFILIK *Anoxybacillus geothermalis* STRAIN D9

Oleh

DURRATUL FATINI BINTI YUSOFF

Februari 2021

Pengerusi : Raja Noor Zaliha Raja Abd Rahman, D. Engr.
Fakulti : Bioteknologi dan Sains Biomolekular

Penyebaran sebatian alkanasulfonat xenobiotik secara meluas tanpa pengendalian yang betul telah menimbulkan kebimbangan kesihatan dan persekitaran. Degradasi sebatian ini berlaku secara semula jadi dengan bantuan mikroorganisma. Sistem alkanasulfonat monooksigenase daripada bakteria melakukan biodegradasi molekul alkanasulfonat. Ini adalah sistem dua komponen enzim yang melibatkan pemindahan flavin antara flavin mononukleotida (FMN) reduktase (SsuE) dan alkanasulfonat monooksigenase (SsuD). SsuE membekalkan FMN terturun (FMNH2) kepada SsuD sebagai kofaktor. Walau bagaimanapun, kebanyakan sistem monooksigenase alkanasulfonat yang dikaji berasal dari bakteria mesofilik. Lebih-lebih lagi, kestabilan dan ciri sistem monooksigenase alkanesulfonate asalan termofil adalah tidak jelas. Oleh itu, pengekspresan dan pencirian sistem alkanesulfonat monooksigenase termofilik dapat digunakan untuk bioremediasi bahan pencemar alkanesulfonat xenobiotik. Dalam kajian ini, sifat sistem alkanasulfonat monooksigenase rekombinan dan komponennya daripada termofilik *Anoxybacillus geothermalis* D9 dilaporkan untuk pertama kalinya. Kajian ini bertujuan untuk mengetahui sifat biokimia dan biofizik SsuE dan SsuD daripada termofil. Dalam kajian ini, gen *ssuE* dan *ssuD* daripada *A. geothermalis* D9 berjaya diklon ke dalam vector pengekspresan pET-51b (+) dan diekspres dalam strain *Escherichia coli* Rosetta (DE3). SsuE rekombinan dan SsuD ditulenkan melalui kromatografi afiniti dengan jisim molekul masing-masing 21.7 dan 46.3 kDa. Analisis denaturasi suhu dan terma menunjukkan bahawa SsuD dengan 40 °C suhu optimum sedikit lebih termostabil daripada SsuE yang optimum pada julat suhu yang luas (30 - 50 °C). Selain itu, kestabilan enzim ini dalam julat suhu yang luas antara 20 hingga 50 ° C menunjukkan bahawa SsuE dan SsuD adalah enzim termostabil. Kedua-dua protein juga lebih suka pH 8 tetapi SsuD lebih stabil pada pH sedikit alkali berbanding dengan SsuE.

Selanjutnya, sistem alkanasulfonate monooksigenase dalam kajian ini stabil dalam kebanyakan pelarut organik terutamanya yang mempunyai LogP tinggi. Sistem alkanasulfonat monooksigenase menjadi pemangkin tindak balas redoks, oleh itu, aktiviti mereka ditingkatkan dengan adanya kebanyakan ion logam. Tambahan lagi, protein ini agak stabil dalam surfaktan bukan ionik dan sangat direncat oleh surfaktan anionik. Analisis fizikal dan analisis struktur sistem ini mendapati bahawa kebanyakannya terdiri daripada α-heliks. Selain itu, dalam kajian *in silico* meramalkan SsuE dan SsuD, masing-masing wujud sebagai struktur dimer α/β/α flavodoxin dan TIM tong. Analisis komputasi juga meramalkan poket aktif dan interaksi residu protein dengan substrat terletak di hujung C-terminal helaian-β untuk kedua-dua protein. Secara umum, SsuD yang dicirikan dalam kajian ini menunjukkan kestabilan yang lebih baik berbanding dengan SsuE yang mungkin disebabkan oleh perbezaan struktur protein mereka. Kesimpulannya, pemahaman mengenai sifat sistem alkanasulfonat monooksigenase daripada bakteria termofilik menyediakan beberapa maklumat mengenai enzim unik ini.

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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 Master of Science. The members of the Supervisory Committee were as follows:

Raja Noor Zaliha Raja Abd Rahman, D. Engr.
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Shukuri Mohamad Ali, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Adam Thean Chor Leow, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

____
ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 08 July 2021

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Signature: ______________________________

Name of Chairman of Supervisory Committee: Professor Dr. Raja Noor Zaliha Raja Abd Rahman

Signature: ______________________________

Name of Member of Supervisory Committee: Associate Professor Dr. Mohd Shukuri Mohamad Ali

Signature: ______________________________

Name of Member of Supervisory Committee: Associate professor Adam Thean Chor Leow

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LIST OF ABBREVIATIONS

β	Beta
α	Alpha
x g	Times gravity
w/v	Weight per volume
v/v	Volume per volume
U/mL	Unit per milliliter
U	Enzyme unit
sp.	Species
rpm	Revolutions per minute
RNA	Ribonucleic acid
pM	Picomolar
pI	Isoelectric point
pH	Power of hydrogen
nm	Nanometre
ng	Nanogram
mM	Millimolar
mL	Milliliter
Mg/mL	Milligram per milliliter
M	Molar
LogP	Partition Coefficient
L	Liter
kDa	Kilodalton
kcal/mol	Kilocalorie (energy) per mole
kb	Kilobase

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g	Gram
DNA	Deoxyribonucleic acid
dH2O	Distilled water
bp	Base pair
A600nm	Optical density at wavelength 600 nanometer
Å	Angstrom
µM	Micromolar
µm	Micrometer
µL	Microliter
µg/mL	Microgram per milliliter
µg	Microgram
°C	Degree Celsius
%	Percentage

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CHAPTER 1

INTRODUCTION

1.1 Background study

Detergent pollutions in the marine environment, soil, and water bodies have raised health and ecosystem concerns. One of the main components in detergent is surfactants from linear alkylbenzene sulfonates (LAS) and secondary alkane sulfonates (SAS) molecules. The wide distribution of toxic xenobiotic alkanesulfonates in nature was contributed by various industrial and household activities (Field *et al.*, 1995; Olkowska *et al.*, 2014). Alkanesulfonate monooxygenase has been reported with the ability to degrade alkanesulfonate compounds through the sulfur acquisition process (Thomas *et al.*, 2007; Ellis, 2011). Moreover, this desulfonation process involved two enzymes under *ssu* (sulfonate-sulfur utilization) gene locus which are flavin mononucleotide (FMN) reductase (SsuE) and alkanesulfonate monooxygenase (SsuD) that cooperates for electron and hydride transfer. SsuE is a flavoprotein that carries out the reduction of FMN to $FMNH_2$ with NADPH as its coenzyme while SsuD on the other hand dependent on SsuE for $FMNH_2$ to oxidize the alkanesulfonate molecule (van der Ploeg *et al.*, 2001). In the presence of a dioxygen environment, $FMNH_2$ is having a short half-life thus measuring the monooxygenase activity without flavin reductase is difficult (Zhan *et al.*, 2008). Therefore, the SsuE was expressed, purified, and characterized together with SsuD to investigate the nature of the alkanesulfonate monooxygenase system as well as its helper enzyme (SsuE) from a thermophilic source. In this study, SsuD activity was measure as a two-component system while SsuE activity was measured as an individual enzyme. The most extensive investigations of the two-component alkanesulfonate monooxygenase system were originated from mesophilic Gram-negative bacteria, *Escherichia coli* (Eichhorn *et al.*, 1999).

The advancement of proteins and enzyme studies has shifted the focus on extremozymes with high stability and tolerance in a harsh environment. The properties of extreme enzymes can be beneficial and engineered to meet the high industrial and commercial demands (Pandey *et al.*, 2015). Furthermore, the research focus on the mesophilic alkanesulfonate monooxygenase system, where properties of its individual enzymes from *Anoxybacillus* species remain scarce. So far, the reported structural analysis of SsuE and SsuD are limited to *E. coli* sources (Eichhorn *et al.*, 2002; Thakur *et al.*, 2020). Thermophilic bacterium *A. geothermalis* strain D9 that was previously isolated from seawater with crude oil utilizing ability (Yusoff, 2017) was used as the source for SsuE and SsuD in this study. Apart from that, past research found that *A. geothermalis* D9 produces lipase and alkane hydroxylase during the crude oil degradation

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process. Furthermore, this is the first time desulfonation of alkanesulfonate molecules was reported from this species.

1.2 Problem statement

The two-component alkanesulfonate monooxygenase system involved in the desulfurization of sulfonates is mainly reported to be functional in mesophilic temperature ranges. Moreover, the stability of these thermophiles-origin enzymes under harsh environments such as in the presence of organic solvents and surfactants was unknown. So far, the biochemical and biophysical studies of extreme two-component alkanesulfonate monooxygenase systems are very limited.

1.3 The aim of the study

The main objective of this study is to discover and characterize the properties of recombinant FMN reductase (SsuE) and alkanesulfonate monooxygenase (SsuD) from *A. geothermalis* D9 in biochemical and biophysical aspects. The specific objectives to achieve throughout this study are as follow;

1. To clone and optimize the expression of the two-component alkanesulfonate monooxygenase system from *A. geothermalis* D9 into expression host *E. coli* Rosetta™ (DE3).
2. To purify the SsuE and SsuD via affinity chromatography.
3. To study the properties of purified SsuE and alkanesulfonate monooxygenase system through temperature, pH, co-enzymes, organic solvent, metal ion, and surfactant characterizations as well as their structural insights from *in silico* studies.

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APPENDICES

Appendix A

Gene isolation

Primers design and construction. The *ssuE* and *ssuD* gene isolated from *A. geothermalis* D9 by PCR.

Cloning

SsuE and SsuD were cloned into cloning vector (pMinit 2.0) separately and transformed into cloning host. Cloning of SsuE and SsuD in expression vector pET-51b (+).

Expression

SsuE and SsuD were expressed in *E. coli* Rosetta^{TM} (DE3). Optimization of expression for maximum protein yield.

Purification

- Affinity chromatography using Streptactin sepharose resin.

Characterization of SsuE and SsuD

- Biochemical properties; Temperature, pH, organic solvents, metal ions and surfactants.
- Biophysical properties; T_m and secondary structure determination.

***In silico* studies**

- Homology modeling and docking of SsuE and SsuD with substrate.

Chart 1: Experimental workflow for SsuE and SsuD studies.

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Table I: List of chemicals used in this study.

Chemicals	Manufactures
1-Propanol	Merck, Germany
2-Propanol	Merck, Germany
3-(N-morpholino)propanesulfonic acid (MOPS)	Merck, Germany
40 % Acrylamide Solution, Bis-Acryl/Bis 37.5:1	VWR International, USA
5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB)	Sigma-Aldrich, USA
Acetonitrile	Merck, Germany
Agarose	Genedirex, USA
Ammonium persulfate (APS)	R&M Chemicals, UK
Ampicillin sodium salt	Gold Biotechnology, USA
Benzene	Merck, Germany
Bovine serum albumin (BSA)	Sigma-Aldrich, USA
Bradford reagent	Sigma-Aldrich, USA
Butanol	Merck, Germany
Calcium chloride ($CaCl_2$)	Fisher Scientific, USA
Chloramphenicol powder	Gold Biotechnology, USA
Chloroform	Merck, Germany
Cobalt (II) sulfate ($CoSO_4$)	Fluka, Switzerland
Coomasie brilliant blue G250	Merck, Germany
Copper (II) sulfate ($CuSO_4$)	R&M Chemicals, UK
D-Desthiobiotin	Sigma-Aldrich, USA
Diethyl ether	Merck, Germany
Dimethyl sulfoxide (DMSO)	R&M Chemicals, UK
Dimethylformamide (DMF)	Merck, Germany
Disodium ethylenediaminetetraacetate dihydrate (EDTA)	Sigma-Aldrich, USA
Disodium hydrogen phosphate (Na_2HPO_4)	Friendmann Schmidt, Australia
Ethanol	Merck, Germany
Ethylene glycol	R&M Chemicals, UK
Glacial acetic acid	R&M Chemicals, UK
Glycerol	R&M Chemicals, UK
Glycine	Fisher Scientific, USA
Heptanol	Merck, Germany
Hydrochloric acid (HCl)	QRec, New Zealand
Iron (II) chloride ($FeCl_2$)	Merck, Germany
Isopropyl β- d-1-thiogalactopyranoside (IPTG)	Gold Biotechnology, USA
Lithium chloride (LiCl)	Fisher Scientific, USA
Magnesium chloride ($MgCl_2$)	Merck, Germany
Manganese (II) chloride ($MnCl_2$)	Fluka, Switzerland
Methanol	Merck, Germany
N,N,N',N'-Tetramethylethylenediamine (OmniPur® TEMED)	Merck, Germany
n-Heptane	Sigma-Aldrich, USA
n-Hexadecane	Sigma-Aldrich, USA
n-Hexane	Sigma-Aldrich, USA
Nickel (II) sulfate ($NiSO_4$)	Merck, Germany
Nicotinamide adenine dinucleotide phosphate, reduced tetrasodium salt (NADPH)	Solarbio Life Science, China

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Nicotinamide adenine dinucleotide, reduced disodium salt (NADH)	Sigma-Aldrich, USA
n-Tetradecane	Sigma-Aldrich, USA
Octanol	Merck, Germany
Potassium acetate (KAc)	QRec, New Zealand
Potassium chloride (KCl)	Merck, Germany
p-Xylene	Merck, Germany
Riboflavin 5'-monophosphate sodium salt hydrate (FMN)	Sigma-Aldrich, USA
Rubidium chloride (RbCl)	Merck, Germany
Sodium 1-decanesulfonate ($C_{10}H_{21}NaO_3S$)	Sigma-Aldrich, USA
Sodium acetate	R&M Chemicals, UK
Sodium chloride (NaCl)	Merck, Germany
Sodium dihydrogen phosphate (NaH_2PO_4)	Friendmann Schmidt, Australia
Sodium dodecyl sulfate (SDS)	Merck, Germany
sodium dodecylbenzenesulfonate (SDBS)	Sigma-Aldrich, USA
Sodium hydroxide (NaOH)	QRec, New Zealand
StrepTactin™ Sepharose High-Performance resin	GE Healthcare, Sweden
Toluene	Merck, Germany
Tris	Merck, Germany
Triton™ X-100	Merck, Germany
Tween® 20	Sigma-Aldrich, USA
Tween® 80	Sigma-Aldrich, USA
β-mercaptoethanol	Fisher Scientific, USA

Table II: Strains and plasmids used in this study.

Strains and plasmids	Manufacturers
E. coli 10-Beta	New Englands Biolabs, USA
E. coli BL21 (DE3)	New Englands Biolabs, USA
E. coli Rosetta™ (DE3)	Merck, Germany
E. coli TOP10	Thermo Fisher Scientific, USA
pET-51b (+) DNA	Merck, Germany
pMiniT 2.0	New Englands Biolabs, USA

Table III: Types of media used for culturing bacteria.

Media	Manufacturers
Luria Bertani agar	Merck, Germany
Luria Bertani broth	Merck, Germany
Nutrient agar	Merck, Germany
Nutrient broth	Merck, Germany

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Table IV: The list of consumables and kits.

Consumables and kits	Manufacturers
1 kb DNA Ladder	Genedirex, USA
2 x EasyTaq PCR SuperMix	TransGen Biotech, China
DNA Gel Loading Dye (6 x)	Thermo Fisher Scientific, USA
FavorPrep™ Plasmid Extraction Mini Kit	Favoragen, Taiwan
FlyCut® restriction enzymes	TransgGen Biotech, China
Lambda DNA/HindIII Marker	Thermo Fisher Scientific, USA
NEB PCR Cloning Kit	New Englands Biolabs, USA
PageRuler™ Unstained Protein Ladder	Thermo Fisher Scientific, USA
Pierce™ Unstained Protein MW Marker	Thermo Fisher Scientific, USA
Pink Plus Prestained Protein Ladder	Cleaver Scientific, UK
Qiagen Dneasy Blood and Tissue Kit	Qiagen, USA
QIAquick Gel Extraction Kit	Qiagen, Germany
RedSafe™ Nucleic Acid Staining Solution	iNtRON Biotechnology, Korea
T4 DNA Ligase	Thermo Fisher Scientific, USA

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Appendix B

(a) Buffer recipes for the optimization of expression

Table V: Phosphate and Tris-HCl buffers recipe.

Compositions of phosphate buffer	Amount (50 mM, pH 7.4)
Sodium dihydrogen phosphate (NaH_2PO_4)	1.697 g/L
Disodium hydrogen phosphate (Na_2HPO_4)	10.107 g/L
dH_2O	1 L
Compositions of Tris-HCl	**Amount (50 mM, pH 9.0)**
Tris	6.06 g/L
Hydrochloric acid	Adjust until reach pH 9.0
dH_2O	1 L

(b) Polyacrylamide gel and buffer recipes for SDS-PAGE

Table VI: SDS-PAGE polyacrylamide gel compositions.

Ingredients	12 % of resolving gel	4 % stacking gel
dH_2O	4.40 mL	2.95 mL
Tris-HCl	2.50 mL (pH 8.8)	1.25 mL (pH 6.8)
40 % Acrylamide solution	3.00 mL	0.75 mL
10 % SDS	0.10 mL	0.05 mL
10 % APS	50.0 µL	25.0 µL
TEMED	10.0 µL	7.50 µL

Table VII: 4 X SDS-PAGE sample buffer (Morris formulation).

Ingredients	Amount in 10 mL of the total volume
1 M Tris-HCl pH 6.8	0.25 mL
SDS	1.0 g
0.1% Bromophenol Blue	0.8 mL
Glycerol	4.0 mL
β-mercaptoethanol	2.0 mL
dH_2O	Up to 10 mL

Table VIII: Running buffer for SDS-PAGE analysis.

Compositions	Concentration
Tris (pH 8.8)	3 g/L
Glycine	14.4 g/L
SDS	1 g/L
dH_2O	1 L

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Table IX: Staining buffer for SDS-PAGE analysis.

Compositions	Concentration / Volume
Coomasie blue G-250	2.5 g/L
Methanol	450 mL
Galcial acetic acid	100 mL
dH_2O	450 mL

Table X: Destaining buffer for SDS-PAGE analysis

Compositions	Volume (mL)
Methanol	100
Glacial acetic acid	100
dH_2O	800

(c) Buffer recipes used in affinity chromatography

Table XI: Binding and elution buffers compositions.

Ingredients	Binding buffer (pH 8.0)	Elution buffer (pH 8.0)
Tris-HCl	100 mM	100 mM
NaCl	150 mM	150 mM
EDTA	1mM	1mM
D-Desthiobiotin	-	2.5 mM

(d) Recipes for enzyme assays components

Table XII: Decanesulfonate stock solution (2mL)

Ingredients	Amount in 2 mL
50 mM decanesulfonate ($C_{10}H_{21}NaO_3S$)	0.0244 g/mL
dH_2O	2 mL

Table XIII: DTNB stock solution (5 mL)

Ingredients	Amount in 5 mL
26 mM DTNB	0.0515 g/L
100 mM phosphate buffer pH 7.4	5 mL

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Table XIV: Coenzymes stock solution preparation using extinction coefficient values[a].

Coenzymes	Extinction coefficient ($M^{-1}cm^{-1}$)
FMN	12500
NADH	6220
NADPH	6220

[a]FMN, NADH and NADPH are very sensitive to heat, susceptible to oxidation, and only needed in small amounts. Moreover, the weight needed for each assay is too small and cannot be measured accurately by 4 decimals weighing balance. Therefore, the stock solutions were prepared by adding a small amount of each chemical into an Eppendorf tube separately and resuspended with 50 mM phosphate buffer pH 7.4. The concentration of the stock solution was measured using the extinction coefficient and where the final volume needed in enzyme assay was determined by its final concentration.

Table XV: The final concentration of SsuE and SsuD assays components.

Components	Final concentration	
	SsuE assay	**SsuD assay**
50 mM phosphate buffer pH 7.4	Add accordingly	Add accordingly
FMN	1 μM	1 μM
NADH	0.5 mM	0.5 mM
SsuE	0.6 μM	0.6 μM
SsuD	-	0.2 μM
Decanesulfonic acid	-	2 mM
DTNB	-	1 mM

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Appendix C

Standard curves

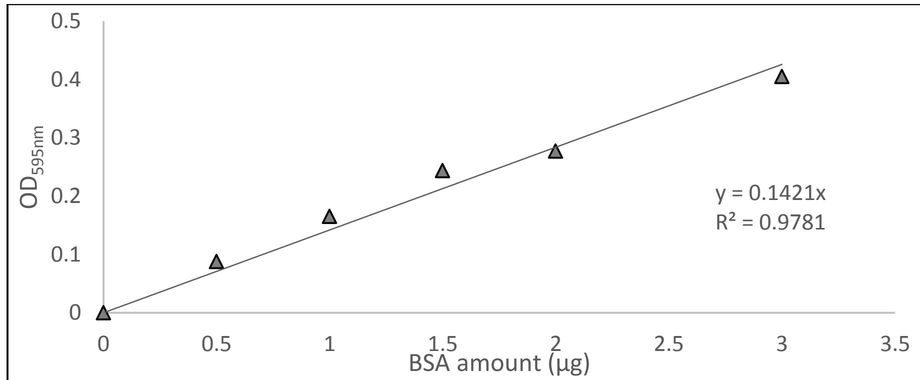

Figure I: BSA standard curve Bradford assay.

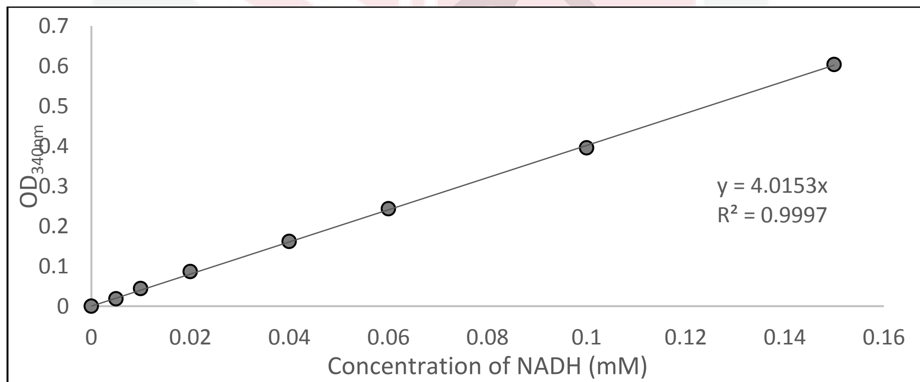

Figure II: NADH standard curve for SsuE assay.

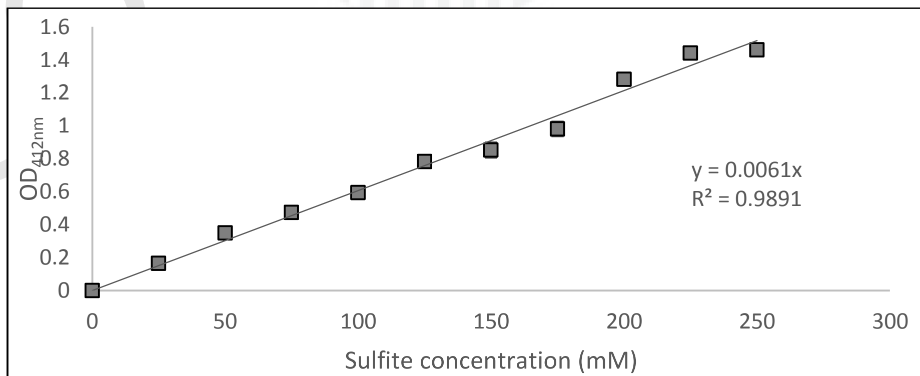

Figure III: Sodium sulfite standard curve for SsuD assay.

Appendix D

Transmembrane and signal peptide predictions for SsuE and SsuD.

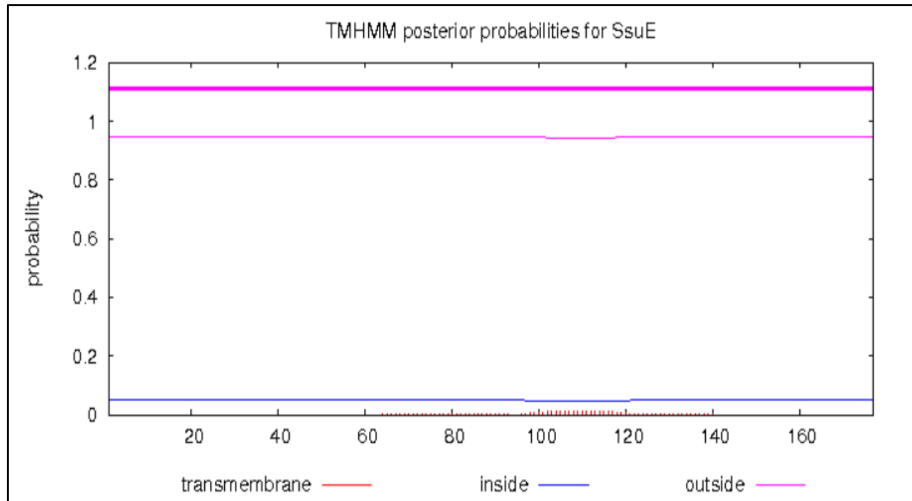

Figure IV: Zero transmembrane helices (TMHs) are predicted for SsuE.

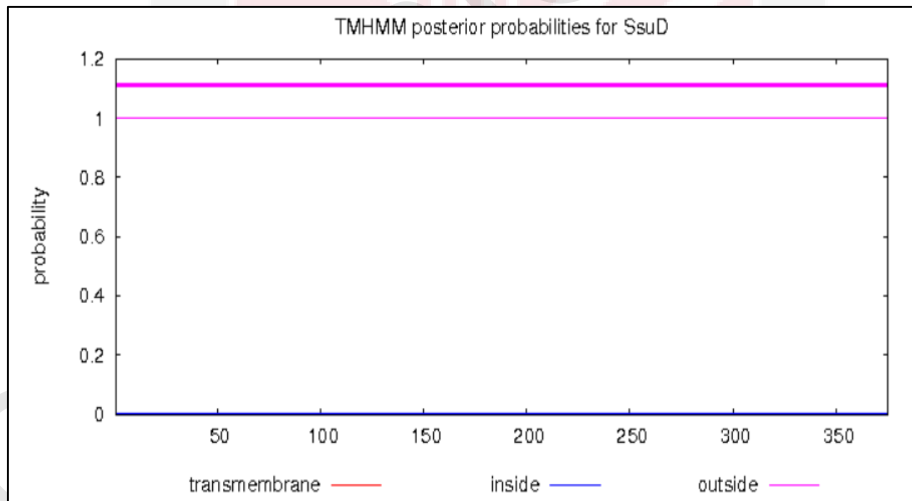

Figure V: Zero transmembrane helices (TMHs) predicted for SsuD.

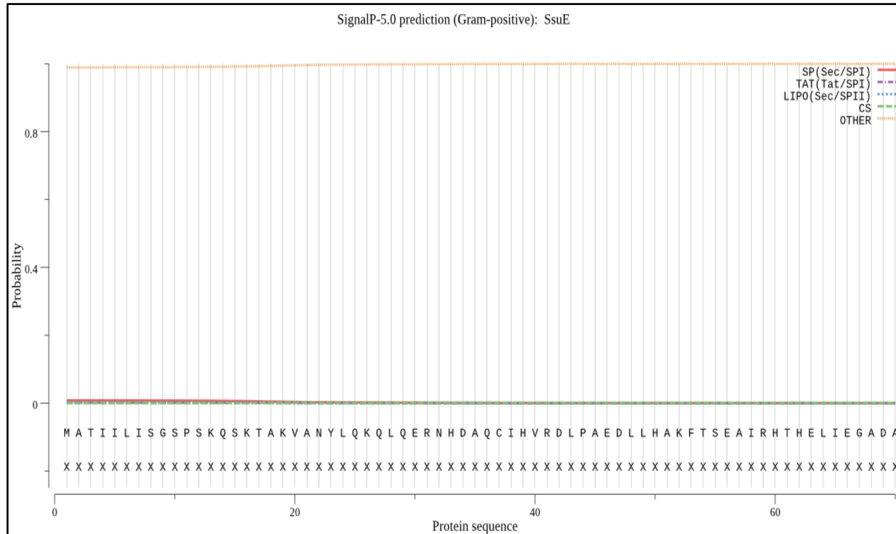

Figure VI: The likelihood of SsuD having Signal peptide (Sec/SPI), TAT signal peptide (Tat/SPI), and Lipoprotein signal peptide (Sec/SPII) are 0.0076, 0.001, and 0.0013, respectively.

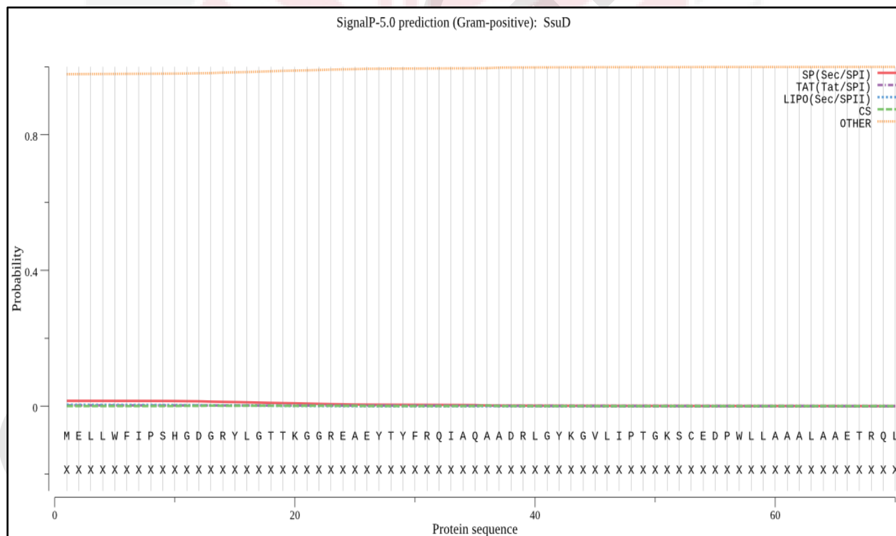

Figure VII: The likelihood of SsuD having Signal peptide (Sec/SPI), TAT signal peptide (Tat/SPI), and Lipoprotein signal peptide (Sec/SPII) are 0.0149, 0.0018, and 0.0033, respectively.

Appendix E

Model verification analysis

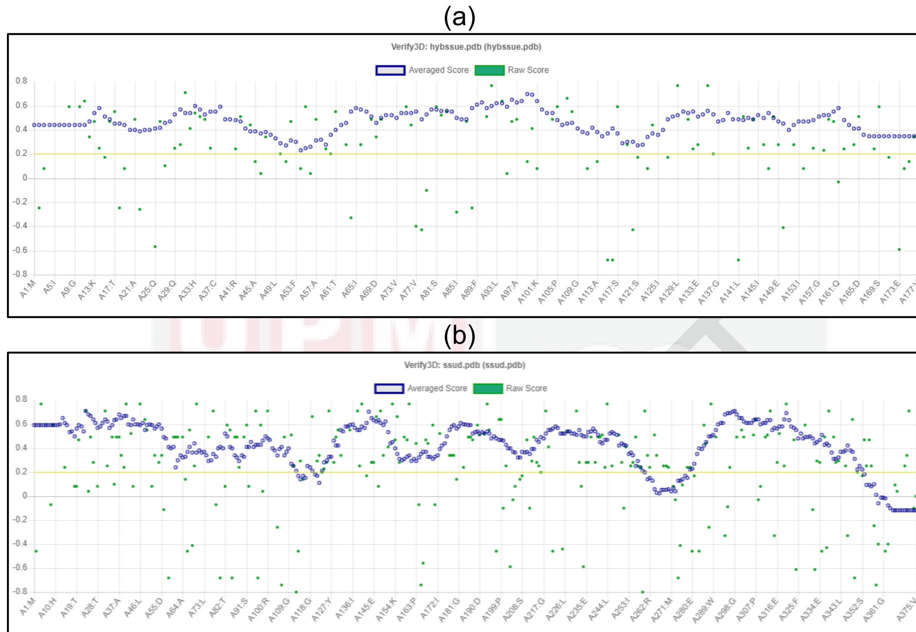

Figure VIII: Verify 3D analysis for SsuE and SsuD predicted models. An acceptable value is at least 80% of residues in a 3D-1D score of ≥ 0.2 **(a)** 100% of the SsuE residues have averaged a 3D-1D score of ≥ 0.2. **(b)** 87.2% of the SsuD residues have averaged 3D-1D scores of ≥ 0.2.

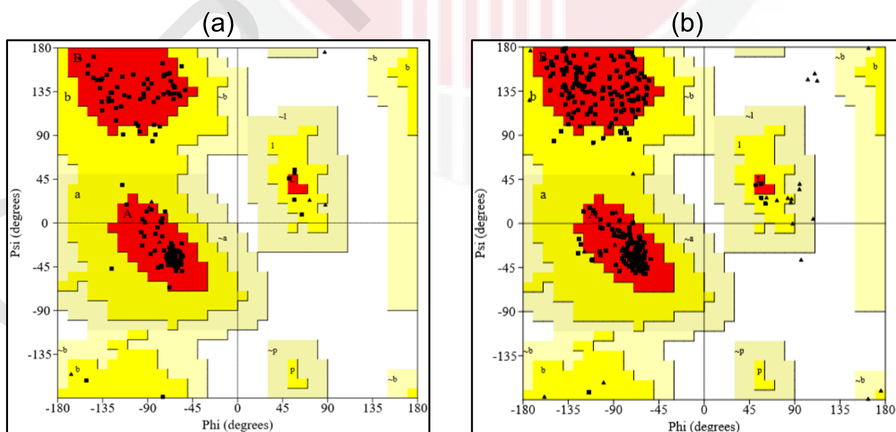

Figure IX: Ramachandran plot for SsuE and SsuD model. (a) About 81.8.0% of SsuE residues in the most favored region [A, B, L]. Another 8.2% residues in additional allowed regions [a, b, l, p]. **(b)** About 95.0% of SsuD residues in the most favored region [A, B, L]. Another 5% residues in additional allowed regions [a, b, l, p].

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(a)

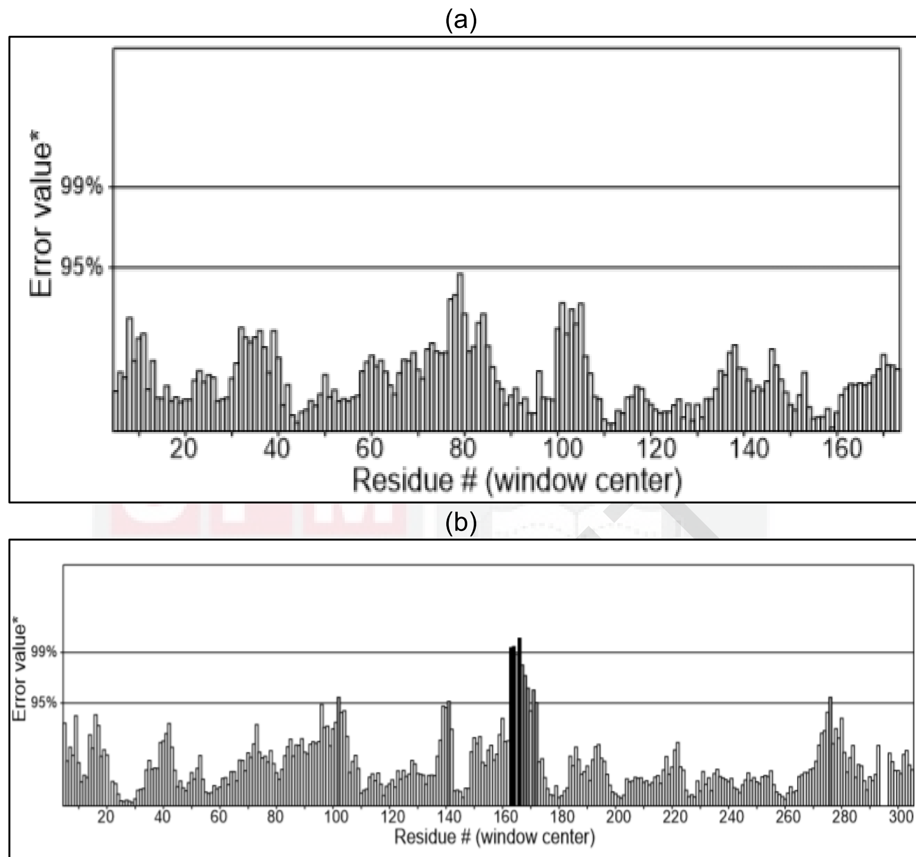

Figure X: ERRAT score for SsuE and SsuD residues. *The lines are drawn to indicate the confidence with which it is possible to reject regions that exceed that error value. Good high-resolution structures have values of 95% and above. **(a)** The overall quality factor for SsuE is 100. **(b)** The overall quality factor of 96.685 for SsuD.

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BIODATA OF STUDENT

Durratul Fatini, daughter of Yusoff Abas and Che Jah Che Latiff, was born on March 28, 1994, in the Federal Territory of Kuala Lumpur, Malaysia. The eighth child of the family obtained her early education in S. K. Selayang Baru I for six years and S. M. Sains Selangor for five years. After sat for Sijil Pelajaran Malaysia in 2011, she was then enrolled in foundation studies under the Asasi Sains Pertanian program at Universiti Putra Malaysia for a year in 2012. After completing a four years course, she was graduated with a Bachelor's degree in Microbiology in 2017 from University Putra Malaysia. In September 2017, she entered the Graduate School at Universiti Putra Malaysia in the Faculty of Biotechnology and Biomolecular Sciences and is currently pursuing a master's degree in enzyme biotechnology.

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