Ain Shams University
Faculty of Pharmacy
Microbiology & Immunology dept.

Identification of Dimerization Interaction of Certain Type VII Secretion System Membrane Proteins in *Staphylococcus aureus*: A new Approach for Virulence Control

A Thesis

Submitted in Partial Fulfillment of the Requirements for the

Master degree

In Pharmaceutical Sciences
(Microbiology and Immunology)

By

Manar Mostafa Ahmed

Bachelor of Pharmaceutical Sciences, 2012
Teaching Assistant, Microbiology and Immunology Department
Faculty of Pharmacy and Pharmaceutical Industries, Sinai University

2018

Ain Shams University
Faculty of Pharmacy
Microbiology & Immunology dept.

Identification of Dimerization Interaction of Certain Type VII Secretion System Membrane Proteins in *Staphylococcus aureus*: A new Approach for Virulence Control

A Thesis

Submitted in Partial Fulfillment of the Requirements for the

Master degree
In Pharmaceutical Sciences
(Microbiology and Immunology)

By

Manar Mostafa Ahmed

Bachelor of Pharmaceutical Sciences, 2012
Faculty of Pharmacy and Pharmaceutical Industries,
Sinai University

Under Supervision of

Prof. Dr. Khaled Mohamed Anwar Aboshanab
Professor of Microbiology and Immunology
Faculty of Pharmacy, Ain Shams University

Prof. Dr. Yasser El-Mohammadi Ragab
Professor of Microbiology and Immunology
Faculty of Pharmacy, Cairo University

Dr. Khaled Ahmed Aly
Assistant Professor of Microbiology and Immunology
Faculty of Pharmacy and Pharmaceutical Industries, Sinai University

2018

Acknowledgments

First, I thank "**Allah**" for granting me the power to accomplish this work.

I would like to express my deepest thanks to **Dr. Khaled Mohammed Aboshanab**, Professor of Microbiology and Immunology, Faculty of Pharmacy, Ain Shams University, for his valuable scientific supervision, constructive advice and continuous guidance throughout the work.

My deepest gratitude and appreciation are expressed to **Dr. Yasser El-Mohammadi Ragab**, Professor of Microbiology and Immunology, Faculty of Pharmacy, Cairo University, for his divine support and for kindly supplying the laboratory facilities whenever needed. His constructive criticism, guided me immensely throughout the work and during the revision of the thesis.

I am also greatly indebted to **Dr. Khaled Ahmed Aly**, Assistant Professor of Microbiology and Immunology, Faculty of Pharmacy and Pharmaceutical Industries, Sinai University, for suggesting the topic of research and for providing continuous scientific supervision and follow up. His valuable time and big effort was greatly appreciated.

I would also like to thank my dear **colleagues** and all the **workers** at the Faculty of Pharmacy, Sinai University for their help and support during this work. Besides, I would like to thank my University Dean Prof. Dr. Soad abo El-Ela, for her help for supplying the equipment for achieving the work.

Finally, my deepest everlasting thanks and appreciation are for my beloved **parents** for their continuous support and encouragement throughout my life.

والحمد لله رب العالمين.....

Manar Mostafa Ahmed

List of Abbreviations

APS	Ammonium persulfate
BCG	Bacille-Calmette-Gue´rin
B. subtilis	*Bacillus subtilis*
BTH	Bacterial two-hybrid
CFP-10	Culture filtrate protein of 10 kDa
COG	Cluster of Orthologous Groups
DMSO	Dimethyl sulfoxide
DSS	Disuccinimidyl suberate
DTT	Dithiothreitol
DUF	Domain of unknown function
Ecc	ESX-conserved component
EDTA	Ethelenediaminetetraacetic acid
ESAT-6	Early secreted antigenic target of 6 kDa
EspG	ESX secretion-associated protein G
ESS	ESAT-6 Secretion System
***essB* C**	C-terminus of *essB* gene
***essB* N**	N-terminus of *essB* gene
***essF* SD**	*essF* soluble domain

ESX	ESAT-6 secretion system
FPLC	Fast protein liquid chromatography
FSD	FtsK/ SpoIIIE domains
FtsK	Filamenting tempreture-sensitive mutant K
IPTG	Isopropyl-β-d-thio-galactopyranoside
L. monocytogenes	*Listeria monocytogenes*
MRSA	Methicillin resistant *Staphylococcus aureus*
MUP	Medical union pharmaceuticals
M. tuberculosis	*Mycobacterium tuberculosis*
NEB	New england biolab
OD_{600}	Optical density at 600nm
PBS	Phosphate buffered saline
PE	Proline-glutamic acid
PMSF	Phenylmethylsulfonyl fluoride
PPE	Proline-proline-glutamic acid
PSI-BLAST	Position Specific Iterated Basic Local Alignment SearchTool
PVDF	Polyvinylidene difluoride
RD1	Region of difference 1
REs	Restriction endonucleases
S. agalactiae	*Streptococcus agalactiae*

S. aureus	*Staphylococcus aureus*
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
Sec	Secretory pathway
T7SS	Type VII Secretion System
Tat	Twine-arginine pathway
TBST	Tris buffered saline with tween 20
TE	Tris- EDTA
TEMED	Tetramethylethylenediamine
TM	Transmembrane
TMHMM	Tied Mixture Hidden Markov Model
Tris-HCl	Trisaminomethane hydrochloride
TSA	Trypticase soy agar
TSB	Trypticase soy broth
WB	Water bath
WT	Wild-type (full-length)
WXG100	Trptophan–Xaa–Glycine motif of 100 amino acids proteins
X-Gal	5-Bromo-4-chloro-3-indolyl- β-D-galactopyranoside

List of Figures

List of Figures