Cairo University
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Molecular Recognition of *Brucella* Based on Matrix-assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry and Molecular Imprinted Polymers

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For the degree of the Master

(Bacteriology, Immunology & Mycology)

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2019

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ABSTRACT

Brucella is an expanding genus of Gram-negative intracellular wide host ranging pathogens. This work aimed at investigating molecular recognition of *Brucella* by MALDI-TOF MS proteomic fingerprinting as well as novel plastic antibodies by developing and characterizing molecularly-imprinted hydrogels to grasp all surface epitopes at one go for whole cell recognition of *Brucella abortus*, *B. melitensis* and *B. suis* known to exist among livestock in Egypt. An MSP library of 11 reference *Brucella* strains was created. A dendrogram for reference strains was plotted to analyze phyloproteomic relations. Based on bacteriologic and proteomic biotyping of 45 field isolates, a map revealed the geographic distribution of *Brucella melitensis* and *B. abortus* from 69 unvaccinated seropositive ruminants in 12 governorates during 2015. The MALDI-TOF MS was re-evaluated as a revolutionary molecular tool for *Brucella* identification reviewing the pros and cons of the technique suggesting recent methods to tackle existing hitches. Effective bacterial recognition using a cell-imprinted polymer (CIP) formed on a 96-well microplate was achieved within 30 minutes. The polymer could discriminate the target strain from other strains with high selectivity reaching approximately 20 folds. It was concluded that bacteriologic and MALDI results fully matched thanks to the limited diversity of *Brucella* isolates and the narrow MSP library. The CIP approach proved valuable for rapid direct *Brucella* recognition and quantification colorimetrically in microtiter plates after full validation.

Key words: *Brucella*, MIPS, hydrogel, electron microscopy MALDI-TOF MS, phyloproteomic dendrogram, MSP.

Dedication

To my precious mother

To my dear brother Dayib

To my dear sister Halima and my brother Hadi

God bless you all and give you good health and happiness.

Acknowledgements

*All thanks to our merciful god **ALLAH**: who gave me patience in all these years of work and gave me the opportunity, ability to finish this work.*

*Foremost, I would like to express my sincere gratitude to my advisor and supervisor **Prof. Dr. Wagih A. Gad El-Said**, Professor Emeritus of Microbiology Faculty of Veterinary Medicine Cairo University, for the continuous support in my study and research, for his patience, motivation, enthusiasm and immense knowledge.*

*My thanks also to **Dr. Mahmoud D. Elhariri**, Assist. Professor of Microbiology, Faculty of Veterinary Medicine, Cairo University, for his support, advice and providing helping facilities for my study.*

*My deepest gratitude and honor to my external supervisor. **Dr. Ashraf E, Sayour,** Senior Microbiologist, Department of Brucellosis Research, Animal Health Research Institute, Dokki, Egypt, for his academic guidance, supervision, encouragement and inspiration. I'm also grateful for his support by providing me the reference strains of Brucella, and helping me in the writing of this thesis. I could not have imagined having a better brother and mentor for my research.*

*I'm thankful as well to **Dr. Hosam E. Sayour**, Senior Bioanalytical Chemist, Department of Chemistry, Animal Health Research Institute, Dokki, for his vast chemical expertise and help in the development and characterization of molecularly imprinted polymers.*

*I'm thankful to my dear **Hadi** for their generous financial support during the years of my study.*

*Finally I'm heartedly thankful to the former Ambassador of the Republic of Djibouti in Egypt, **Mr. Moussa Ahmed**, for enormous helping in my master degree.*

*I'm thankful to **Eng Magdy Ghoneim** for incredible helpful about the weighting of the thesis.*

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LIST OF ABBREVIATIONS

BC	Buffalo cow
BK2	Berkley
BPAT	Buffered plate agglutination test
BSC	Biological safety cabinets
BT	Bitch
BTS	Bacterial Test Standard
C	Cow
CCI	composite correlation index
CIP	Cell-imprinted polymer
CO_2	Carbone dioxide
DNA	Deoxyribonucleic acid
DRS	dielectric relaxation spectroscopy
E	Ewe
ELISA	Enzyme-linked immunosorbent assay
FAC	Fetal abomasal contents
FAO	Food and Agriculture Organization
FB	Febrile blood
FI	Firenze
FPSR	False-positive serological reactions
FTIR	Fourier-transform infrared spectroscopy
FG	Female goat
HCCA	Alpha-cyano-4hydroxy cinnamic acid
HPLC	High-performance liquid chromatography
ID	Identification
IM	Inner membrane
IMAC	Immobilized metal ion affinity chromatography

ISET	Integrated selective enrichment target
IZ1	Izatnagar
k Da	Kilo Daltons
L	Lysis
LG	Lung
LPS	Lipopolysaccharide
LV	Liver
M	Milk
MAbs	Monoclonal antibody
MALDI-TOF MS	Matrix-assisted laser desorption ionization time-of-flight mass spectrometry
MIP	Molecular imprinted polymer
MS	Mass spectrometry
MSN	Mesoporous silica nanoparticles
MSP	Main spectra projection
NH	Native hapten
NIP	Non-imprinted polymer
NL	No lysis
OIE	Office International des Epizooties
OM	Outer membrane s
OMP	Outer membrane protein
OMV	Outer membrane vesicles
P	Placenta
PCR	Polymerase chain reaction
PL	Partial lysis
PPb	Parts per billion
Q	Queen
RBPT	Rose-Bengal plate agglutination test

RC	Rough/Canis
R-LPS	Rough lipopolysaccharide
RPLN	Retropharyngeal lymph node
rRNA	Ribosomal ribonucleic acid
RTD	Routine test dilution
SC	She camel
SDS	Sodium dodecyl sulfate
SIP	surface imprinted polymer
S-LPS	Smooth lipopolysaccharide
SMLN	Supramammary lymph node
SNPs	Single-nucleotide polymorphisms
SP	Spleen
Tb	Tbilisi
UD	Uterine discharge
WB	Weybridge
WHO	World Health Organization

CHAPTER (1)

INTRODUCTION

INTRODUCTION

Brucellosis is an emerging transboundary disease caused by the genus *Brucella* currently encompassing 12 species from terrestrial and marine mammals (**OIE Terrestrial Manual, 2016; Scholz *et al.*, 2016**). The troublesome bio-risk group III brucellae are bacteriologically hard-to-diagnose due to their facultative intracellular nature, slow growth and fastidiousness (**Christopher *et al.* (2010**). The detection of this nasty group of bacteria constitutes one of the exceptions of the postulates established by Robert Koch for the diagnosis of disease etiological agents. The practical diagnosis of brucellosis is mainly indirect by uncovering whatever accessible of the *Brucella* antibodyome rather than the bacteria themselves. Direct detection of *Brucella*, or either its genome, proteome or lipidome is more trustworthy from the diagnostic point of view.

Broadly speaking, molecular recognition is a diagnosis based on the detection of omics, e.g. antibodyomics, genomics, transcriptomics, proteomics, glycomics, lipidomics, metabolomics, regulomics, secretomics, . . . etc. The first reliable microbial classification was achieved by comparative genomic 16S rRNA sequence analysis based on phylogenetic relationship. Compared to the conserved genomics, proteomics reflect more diversity in biomarkers resulting from continuous bacterial microevolution changing the *status quo* of genetic expression to proteins (**Seng *et al.*, 2009**). The bacterial proteome varies in response to disease and the surrounding environmental conditions including exposure to antibiotics allowing for better demarcation (**Shah and Gharbia, 2017**). Phyloproteomic clustering highly resembles taxonomy based on 16S rRNA analysis in bacterial biotyping (**Shah and Gharbia, 2017**) even at the strain level (**Culebras, 2018**).

Mass spectrometry is a group of magical analytical techniques for identifying the molecular mass as well as the chemical structure of compounds. Of the several mass spectrometry formats, the triple quadrupoles, quadrupole-time-of-flight hybrids and matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) are the most common in the clinical sector. MALDI-TOF MS was first introduced by **Karas *et al.* (1987)** for molecular recognition of microorganisms