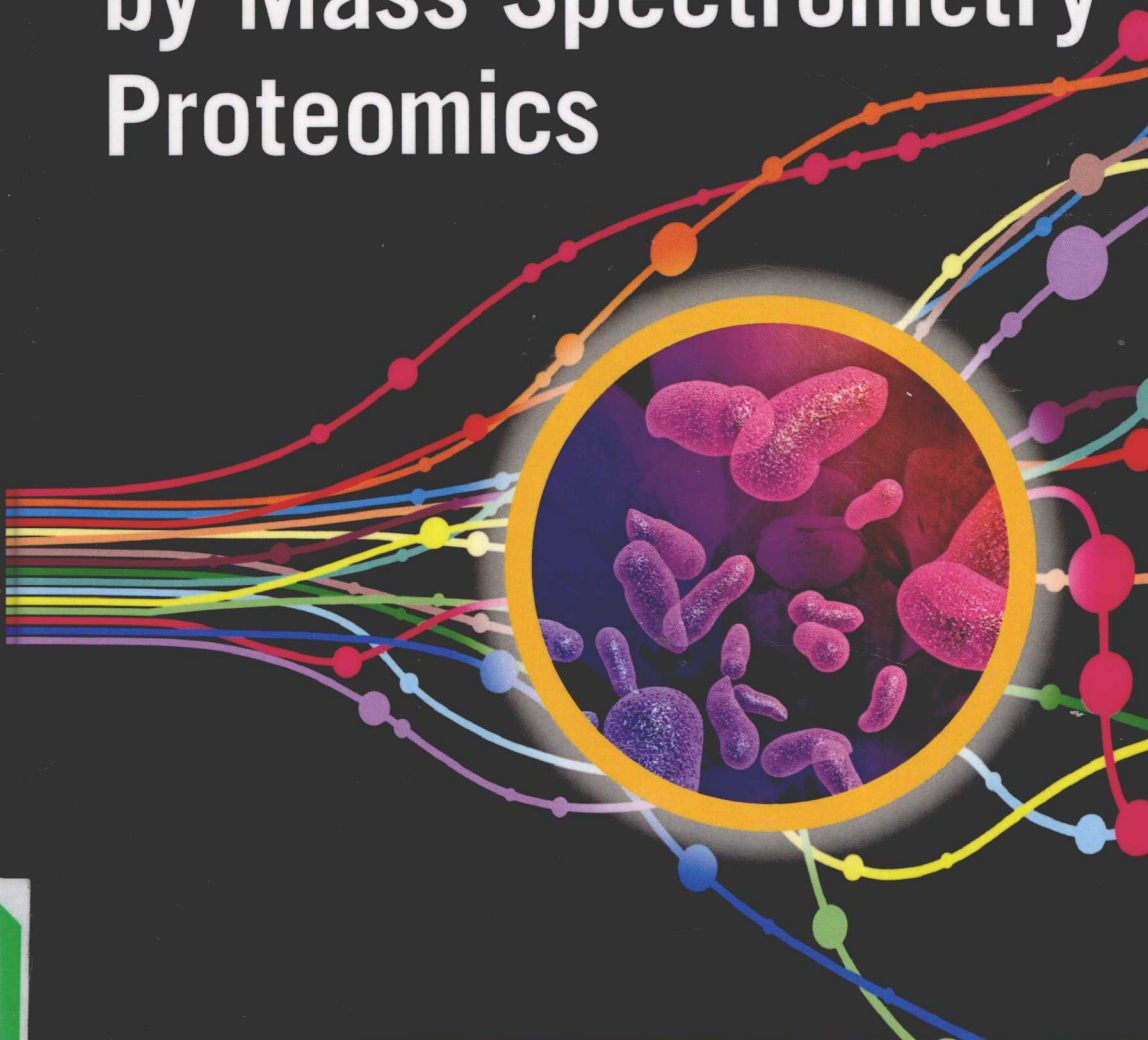
Identifying Microbes by Mass Spectrometry Proteomics



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Identifying Microbes by Mass Spectrometry Proteomics

This work is dedicated to Professor Bjorn F. Hrutfiord, PhD

Preface

Writing a book on mass spectrometry methods for detecting and identifying microorganisms took on the aspect of trying to summarize an entire epoch in a few words. Starting with the first instruments in the 1970s to the present, the discipline of mass spectrometry has evolved and has as many branches as an old oak tree. It became simpler when focused on one simple branch, which shows that microorganisms can be detected, identified, and classified using mass spectrometry, proteomics, appropriate software, and fast computers. The technology works fine. In fact, this process works better than "fine"; it has the ability to detect and classify those microbes that are not sequenced—those not in the genetic databases. Because of the exceptional standardization and organization of the genomic information available, it is a simple process to sort this information by using appropriate software and a computer. We have accomplished tasks in minutes using software which used to take weeks of wet laboratory work. All microbes can be classified according to their degree of match to their taxonomic hierarchy.

Microbe identification is actually very simple when you consider that all that is required is the detection of a few peptides unique to each microbe. Since peptides can be determined by mass spectrometry methods and these can in turn be sorted by software, the unique peptides of all the microbes can be determined. The process includes the following four steps: (1) all the available sequenced microbes are obtained from the national GenBank; (2) software, such as ABOid™, determines all the unique peptides for these microbes; (3) mass spectrometry provides the peptide information for all microbes in their samples; and (4) then back to ABOid™, which sorts out the unique peptides and determines the accurate identification and classification. This is important when considering that the detection of a few unique peptides out of thousands available for a microbe is easier than looking for a specific marker in a complex media. The result is accurate identification and classification over and over again not based on a few markers but based on the detection of a few out of thousands of unique markers (peptides).

This book is a snapshot in a rapidly growing, exciting area of science, and we can expect that updates will be the subject of a new work. It is clear that we are breaking new ground every day, and it will be fun to see where this approach takes us.

Simply detecting and identifying microbes using such an easy system is terrific. The future is exciting. We can expect miniaturized mass spectrometers in the not-so-distant future, and because the software can be ported to multiple platforms, this capability will be handheld and available to everyone. This capability changes

everything. Imagine the fun of being able to go around and identify the microbes in the yard with a youngster. The possibilities are limitless.

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Acknowledgments

I would like to express my gratitude to the many scholars who have shown the motivation and enthusiasm to develop the capabilities of mass spectrometry for more than 40 years. There are numerous scientists and engineers who have made these devices smaller, better, and more useful.

I would especially like to thank those who made this book possible: Patrick McCubbin, who labored to grind and process a wide variety of samples for analysis, for his humor and dedication, which were both welcome and rewarding; Dr. Rabih Jabbour and Dr. Jacek Dworzanski, who operated the mass spectrometers; and Alan Zulich, for his steadfast support. Particular appreciation is given to my son, Harrison Wick, for placing this manuscript into the final format.

Editor

Dr. Charles H. Wick, PhD, is a retired senior scientist from the U.S. Army Edgewood Chemical Biological Center (ECBC), where he has served both as a manager and research physical scientist and has made significant contributions to forensic science. Although his 40-year professional career has spanned both the public sector and the military, his better-known work in the area of forensic science has occurred in concert with the Department of Defense (DOD).

After earning four degrees from the University of Washington, Dr. Wick worked in the private sector (civilian occupations) for several years, leading to a patent, numerous publications, and international recognition among his colleagues.

In 1983, Dr. Wick joined the Vulnerability/Lethality Division of the United States Army Ballistic Research Laboratory, where he quickly achieved recognition as a manager and principal investigator. It was at this point that he made one of his first major contributions to forensic science and to the field of antiterrorism. His team was the first to utilize current technology to model sublethal chemical, biological, and nuclear agents. This achievement was beneficial to all areas of the DOD, as well as to the North Atlantic Treaty Organization (NATO), and gained Wick international acclaim as an authority on individual performance for operations conducted on a nuclear, biological, and chemical (NBC) battlefield.

During his career in the U.S. Army, Wick rose to the rank of lieutenant colonel in the Chemical Corps. He served as a unit commander for several rotations, a staff officer for six years (he was a division chemical staff officer for two rotations), and as a deputy program director of biological defense systems and retired from the position of commander of the 485th Chemical Battalion in April 1999.

Dr. Wick continued to work for the DOD as a civilian at ECBC. Two notable achievements, and one which earned him the Department of the Army Research and Development Award for Technical Excellence and a Federal Laboratory Consortium Technology Transfer Award in 2002, include his involvement in the invention of the integrated virus detection system (IVDS), a fast-acting, highly portable, user-friendly, extremely accurate, and efficient system for detecting the presence of, screening, identifying, and characterizing viruses. The IVDS can detect and identify the full spectrum of known, unknown, and mutated viruses, from AIDS to hoof and mouth disease, to West Nile virus, and beyond. This system is compact, portable, and does not rely upon elaborate chemistry. The second, and equally award winning, was his leadership in the invention of the method for detecting microbes using mass spectrometry proteomics. These projects represent determined ten-year efforts and a novel approach to the detection and classification of microbes from complex matrices.

Throughout his career, Dr. Wick has made lasting and important contributions to forensic science and to the field of antiterrorism. He holds several U.S. patents in the area of microbe detection and classification. He has written more than 45 civilian

and military publications and has received myriad awards and citations, including the Department of the Army Meritorious Civilian Service Medal, the Department of the Army Superior Civilian Service Award, two United States Army Achievement Medals for Civilian Service, the Commander's Award for Civilian Service, the Technical Cooperation Achievement Award, and 25 other decorations and awards for military and community service.

Contributors

Dr. Samir V. Deshpande, DSc, is a senior bioinformatics scientist who, during his 20-year professional career, has designed software solutions to complex problems. His recent and most notable achievement has been the creation of the 100K line code of the successful program called ABOid™, which is credited with the discovery of virus and fungi associated with honeybees.

After earning an MS in electronics from Sardar Patel University, Dr. Deshpande earned distinction as a software engineer for Microsoft. He then joined the U.S. Army Edgewood Chemical Biological Center (ECBC) as a contractor with Science Technology Corporation. Thus began an outstanding career in developing bioinformatic methods for the analysis and classification of microbes. He then earned a DSc from Towson University and continued this career, becoming the leader and bioinformatics expert of the program that invented and discovered the means for detecting and identifying microbes using output from mass spectrometry.

Dr. Deshpande's experience and skill in software analysis along with his experience in design and development with tools like Visual Studio.NET, ASP.NET, Java, MATLAB®, LabView, Perl 5.2, PHP, XML, and C++ and a host of other methods provided the means to determine the complicated interrelationships among microbes. This unique ability resulted in the patented software platform known as ABOid™. This suite of programs allows for the first time a comprehensive means for detecting and classifying microbes to strain level using their unique peptides, a capability that has benefits to all areas of the Department of Defense, the study of infectious disease, and the discovery of new and emerging microbes.

Dr. Deshpande's research interests include the development of proteomic sequences data warehouse, proteomic identification algorithms, and bioinformatics application pipeline development using distributed computing. He has many peer-reviewed publications in reputed international journals. He has given numerous presentations and contributed to the paper that was awarded the Best in Basic Research by DTRA. In the field of data warehousing, data reduction, and archival, his research continues to involve design and creation using databases like Oracle 10g, MYSQL 5.2, and SQL Server 2005. His goal is to further advance this capability so that someday it will function on handheld devices and be useful to the general public.

Dr. Deshpande likes to teach data analysis and statistical software design and development. He enjoys teaching and has helped many students advance in their understanding of the important discipline of bioinformatics.

Dr. Michael F. Stanford, PhD, is a research physicist with more than 25 years of research and technical management experience within the DOD, NASA, and FAA as well as in academia and industry. After receiving his bachelor's degree in physics from Richard Stockton College, Pomona, New Jersey, in 1975, Dr. Stanford began his graduate studies in biophysics at Downstate Medical Center, State University of New York (SUNY), and received his doctorate in biophysics from the

same university in 1982. He received his commission in 1983 as an O-3 (lieutenant) in the navy and served at the Naval Aviation Research Laboratory (NAMRL) in Pensacola, Florida. He served as a radiation specialist officer and concentrated his research on the detection and effects of ionizing and nonionizing radiation on biological systems. During his tenure at NAMRL, he represented the command at national and international conferences, presenting papers on the research in radiation effects ongoing at NAMRL. He retired from the Navy Reserve as an O-5 (commander) in 1997.

Upon completion of naval active duty in 1986, Dr. Stanford accepted a position with the BDM Corporation in Albuquerque, New Mexico, and served as a test and evaluation engineer. There, he participated in the development of the Corps Battle Analyzer (CORBAN) air and ground combat model. He also provided human factors expertise for calculating weapons' effectiveness and probability of kill determination. During this time, his work also focused on researching command, control, communication, countermeasures (C3CM) for the Joint Test Force (JTF) at Kirkland Air Force Base, New Mexico.

From 1988 through 2000, Dr. Stanford supported NASA at the Johnson Space Center in Houston, Texas. He participated in the development of a proton and heavy ion detector for assessing the radiation risk in low earth orbit. Additionally, he participated in the development of the tissue equivalent proportional counter (TEPC), which provided data on the secondary radiation field inside the space shuttle and space station (both these detectors are currently part of the space station and space shuttle suite of detectors). He was also involved in astronaut training on the nature of the space environment and its hazards.

In 2000, Dr. Stanford worked for Northrop Grumman Corporation in support of the Aviation Security Research and Development Laboratory at the FAA (after 9/11 TSA) William J. Hughes Technical Center, Atlantic City, New Jersey. He served as the program manager of the Trace Explosive Detector Program and provided technical expertise during the test and evaluation of technologies procured from multiple vendors.

In 2004, Dr. Stanford joined the Point Detection Team at Aberdeen Proving Ground (APG) and has been involved in the development of new technologies designed to detect and identify a host of biothreats on the battlefield and in the homeland.

Abbreviations

ABC	Ammonium bicarbonate
ABO	Agents of biological origin
ABOid™	Agents of biological origin identification
AHTS	Aerosol-to-hydrosol transfer stages
ANN	Artificial neural network
APC	Agent Containing Particles
APCI	Atmospheric pressure chemical ionization
AP/MALDI	Atmospheric pressure matrix-assisted laser desorption/ionization
ATCC	American Type Culture Collection
BAMS	Bioaerosol mass spectrometry
BEADS	Biodetection enabling analyte delivery system
BWA	Biological warfare agent
CAD	Collisionally activated dissociation
CI	Chemical ionization
CID	Collision induced dissociation
DART	Direct analysis in real time
DESI	Desorption electrospray ionization
DTT	Dithiothreitol
EAM	Energy-absorbing molecule
ECD	Electron capture dissociation
EI	Electron impact (ionization)
EI-MS	Electron impact ionization mass spectrometry
ELDI	Electrospray-assisted laser desorption/ionization
ELISA	Enzyme-linked immunosorbent assay
ESI	Electrospray ionization
ESI-FTICR	Electrospray ionization-Fourier transform ion cyclotron resonance
ETD	Electron transfer dissociation
FAME	Fatty acid methyl ester
FT	Fourier transform
FT-ICR	Fourier Transform-ion cyclotron resonance mass analyzer
GC	Gas chromatography
HCA	Hierarchical cluster analysis
HF-FIFFF	Hollow-fiber flow field-flow fractionation
HPLC	High-performance liquid chromatography
IMAC-Cu	Immobilized copper cations
IRMPD	Infrared multiphoton dissociation
IVDS	Integrated virus detection system
LIMBS	Laser-irradiated magnetic bead system
LIT	Linear ion trap
LIT/FTICR	Linear ion trap/Fourier transform ion cyclotron resonance analyzer

LV	Latent variable
MAB	Metastable atom bombardment
MAB-Py-MS	Metastable atom bombardment ionization pyrolysis mass spectrometry
MALDI	Matrix-assisted desorption/ionization
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MSP	Mass spectrometry and proteomics
NCBI	National Center for Biotechnology Information
PCA	Principal component analysis
PCR	Polymerase chain reaction
Pfa	Probability of false alarms
PLS	Partial least square
PLS-DA	Partial least squares-discriminant analysis
PMF	Peptide mass fingerprinting
PTM	Posttranslational modification
Py	Pyrolysis
Q	Quadrupole
QIT	Quadrupole ion trap
Q/LIT/Q	Quadrupole/linear ion trap/quadrupole
QQ	Two quadrupoles
SASP	Small acid soluble protein
SAX2	Strong anion exchange
SEC	Size exclusion chromatography
SELDI	Surface-enhanced laser desorption ionization
SIM	Selected ion monitoring
SNP	Single nucleotide polymorphism
SRM	Selected reaction monitoring
sub-fmole	10-16-10-17 mole
TFA	Trifluoroacetic acid
TIGER	Triangulation identification for genetic evaluation of risk
TMAH	Tetramethylammonium hydroxide
TOF	Time of flight
TPP	Trans-proteomic pipeline
TQ	Triple quadrupole
VNTR	Variable number tandem repeat
WCX2	Weak cation exchange

Contents

1 Detection and Identification of Microbes Using Mass Spectrometry Proteomics

Charles H. Wick

CONTENTS

1.1 INTRODUCTION

Since they were first discovered scientists have wanted to be able to identify and classify the many different microbes, in particular bacteria, fungi, and viruses. It was an exciting time of discovery during the development of the disciplines of microbiology, mycology, and virology. The results were that in general an academic specialty was offered in the three fields. Sometimes, the bacteriology and virology were offered in the same academic area of microbiology. One reason for this early separation was their size. Fungi were large multi-micron to millimeter-sized organisms, bacteria were of 0.5–2.0 μm, and viruses were nanometer-sized; generally three orders of magnitude