PREFACE

The analysis of microorganisms by mass spectrometry is relatively new by mass spectrometry standards, having begun some 60 years after the first reports of mass spectrometry studies in the last years of the nineteenth century by J. J. Thomson and others. Nevertheless, from a modern perspective, it could be argued that mass spectrometrists have been trying to sell microbiologists (and others) on the idea of classifying bacteria using an instrumental approach since at least the 1960s. Although there were some promising early studies, many in the microbiology world believed that the capabilities of mass spectrometry were overstated then, and perhaps now. The early optimism was not really unwarranted in that mass spectrometry studies came very close to making real headway in the development of rapid and reliable methods for the characterizing of many disease-causing organisms. Two prominent lines of mass spectrometry research illustrate this point. One technique that showed much promise was pyrolysis mass spectrometry (PyMS). This approach was rapid, rugged, and seemed to provide taxonomically specific spectral profiles, especially in well-controlled laboratory settings. On this promise major research efforts were put into the development of PyMS systems, especially for battlefield detection of microbes used in germ warfare. Although these systems showed some promise for the detection of chemical agents, they were less practical for the analysis of microorganisms in real-world settings.

It is easy to look back from today's vantage point and assert that the spectral patterns produced by pyrolysis were not sufficiently dissimilar for different organisms, or that pattern recognition (or computational) approaches were not advanced enough to provide the specificity needed for routine application of PyMS to bacterial characterization. Such criticism misses the important point that these methods did work within certain limitations. Indeed, research

in PyMS and related areas continues today as investigators attempt to increase the diversity of spectral patterns for bacteria using modifications of traditional Currie-point pyrolysis electron impact mass spectrometry, such as metastable atom bombardment and advanced methods for assembling libraries of pyrolysis data. Another approach that very nearly succeeded was the application of laser desorption ionization mass spectrometry to bacteria. Early pioneers in the field were attempting "whole-cell" studies using electron impact and laser desorption ionization, prior to the advent of modern desorption ionization techniques. These studies are the direct linear predecessors of techniques such as "whole-cell MALDI-TOF MS." Had these investigators been working after, rather that before, the introduction of the now-common techniques developed to study proteins and other biopolymers, their work would have been as successful as today's. Unfortunately, their laser desorption studies of bacteria were attempted without the benefit of a matrix to facilitate the interaction between the sample and the laser, or to promote the desorption/ionization process.

Much of the very important early work in this field is documented in a landmark *ACS Symposium Series* book edited by Catherine Fenselau. The editors and authors of the present book hope that they can likewise reflect much of the important work conducted in the last decade. We anticipate that many in the microbiology community will be skeptical about current claims that mass spectrometry can play an important role in the analysis of bacteria, especially rapid characterization. Several chapters in this book were prepared by microbiologists. Even though they have had a hand in the development of mass spectrometry approaches to bacterial analysis, they are nevertheless microbiology-centered investigators, and their contributions should provide both microbiology background to the mass spectrometry specialist and a perspective on mass spectrometry approaches to the microbiologist. This book includes an introduction to basic concepts in mass spectrometry, and a brief history of the development of mass spectrometry methods for analysis of bacteria. The most commonly used mass spectral method for the rapid analysis of bacteria is whole-cell MALDI time-of-flight (TOF) mass spectrometry. In addition to coverage of the widely used whole-cell MALDI-TOF MS approach, a newer approach involving very high resolution Fourier transformation mass spectrometry (FTMS) is also reported. Although many analyses are based on spectral comparisons, this book reflects the parallel development of proteome-based approaches to bacterial identification. By either approach it is now relatively routine to classify target bacteria down to the strain level using mass spectrometry. A number of taxonomically important proteins have now been identified, many of them being ribosomal proteins. These are detected because they are both abundant and easily detected analytes in MALDI mass spectrometry studies.

The most common criticism of all instrumental (chemical) methods for characterizing bacteria is the relatively large number of bacteria required. Typically 100,000 bacteria or more or needed for analysis. Not withstanding that

pre-analysis amplification of bacteria is a common procedure for most methods (including standard methods used by regulatory agencies and hospitals), this remains a valid criticism, especially for those developing rapid analysis methods. Several investigators are working on approaches to concentrate bacteria or bacterial biomarkers prior to MS analysis without resort to lengthy re-growth of cells in culture media. Another criticism of mass spectrometry methods is the cost of instrumentation. For mass spectrometry to receive widespread adoption by microbiologists, a smaller footprint and reduced cost would be very desirable. Considerable progress has been made in both of these areas and is reported some of the following chapters.

Electrospray (ESI) ionization mass spectrometry also plays in important role in bacterial characterization. Because it typically includes a chromatographic separation step, the approach is not considered as rapid as MALDI approaches, which do not incorporate a separation. However, compared to the times needed to grow bacteria in culture prior to analysis, the time frame is not lengthy, and the addition of chromatographic separation provides many opportunities to increase specificity. ESI/MS has been used to characterize cellular biomarkers for metabolic, genomic, and proteomics fingerprinting of bacteria, and these approaches are reported in two chapters.

Finally, methods developed for rapid detection of microorganisms by mass spectrometry may also produce biomarker data that can be used in the design of better molecular probes. As noted above, many of the strain-differentiating proteins have been identified, and this information can be used to infer information about differences in the corresponding genes at the strain level. One chapter reports the use of strain-specific protein biomarkers, detected in mass spectrometry studies of *Vibrio* strains from a human outbreak in the Pacific Northwest that eventually led to an understanding of the genomic changes and have rendered existing gene-based probes less effective over time. This is but one example of the many mechanistic and biochemical advances that will likely come from the development of methods for bacterial speciation. It simply reflects a link between the recognition of the molecular species that differentate cells, and the elucidation of biochemical pathways that follows the determination of molecular structures.

JACKSON O. LAY, JR.
CHARLES L. WILKINS

Fayetteville, Arkansas