Characterization of highly pathogenic microorganisms by MALDI-ToF mass spectrometry and chemometrics

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Identification of microorganisms specifically of vegetative cells and spores by matrix-assisted laser desorption/ionization time-of-flight (MALDI-ToF) mass spectrometry is an emerging new technology. The technique provides specific biomarker profiles which can be employed for bacterial identification at the genus, species, or even at the subspecies level holding the potential to serve as a rapid and sensitive identification technique in clinical or food microbiology and also for sensitive detection of biosafety level (BSL)-3 microorganisms. However, the development of a mass spectrometry based identification technique for BSL-3 level microorganisms is hampered by the fact, that no MALDI-ToF compatible inactivation procedure for microorganisms, and particularly for bacterial endospores, has been evaluated so far.

In the presentation we describe a new methodology for effective inactivation of microorganisms which is compatible with the analysis of microbial protein patterns by MALDI-ToF mass spectrometry. The main challenge of this work was to define the conditions that ensure microbial inactivation and permit at the same time comprehensive analysis of microbial protein patterns. Among several physical, chemical and mechanical inactivation procedures, inactivation by trifluoroacetic acid (TFA) proved to be the best method in terms of bactericidal capacity and information content of the mass spectra. Treatment of vegetative cells by 80% TFA alone for 30 min assured complete inactivation of microbial cells under all conditions tested. For spore inactivation, the "TFA inactivation protocol" was developed which is a combination of TFA treatment with basic laboratory routines such as centrifugation and filtering. This MALDI-ToF compatible sample preparation protocol is simple and rapid and assures reliable inactivation of vegetative cells and spores of highly pathogenic BSL-3 microorganisms.

The TFA inactivation protocol can be applied to reproducibly produce mass spectra from microorganisms. The high level of spectral reproducibility and the applicability of mass spectrometry for microbial typing will be demonstrated by means of examples from the genera *Yersinia* and *Bacillus*.