Improved proteomic biotyping of clinically significant Mycobacteria using a novel MALDI-TOF mass spectrometry sample preparation strategy

Suresh Babu Naidu Krishna

Durban University of Technology, Durban, South Africa

Introduction*:* Identification of mycobacteria to the genus and species level using phenotypic and biochemical methods is time- consuming and unreliable. MALDI-TOF MS has been used to distinguish various microorganisms, such as bacteria and yeast, in a timely and efficient manner. To date, a standard ethanol/formic acid sample preparation protocol is the recommended sample preparation protocol. It has been observed that the employment of the standard ethanol/formic acid sample preparation protocol yielded less than desirable proteomic profiles that were incapable of accurate discrimination of mycobacteria to a species level. The high lipid content of the lipid-rich complex of the cell envelope is thought to make cellular protein extraction difficult, preventing organism identification by MALDI-TOF MS proteomic profiling.

Aims/Objectives: To evaluate the newly developed chloroform-methanol-ethanol-formic acid (CMEFA) sample preparation protocol for the extraction of proteins from well characterized clinical mycobacterial isolates for MALDI-TOF MS analysis.

Methods: CMEFA protocol was used to extract proteins from ATCC derived mycobacterial strains (n=26) and from well characterized clinical mycobacterial isolates (n=110) for MALDI-TOF MS analysis. The spotted MTP 384 ground steel target plate was processed using a Autoflex III smart beam MALDI-TOF MS instrument (Bruker Daltonics, Germany) and analysed using the Flex control software. Additional analysis was performed using theBiotyper 3.0and the ClinProTools 2.2 software (Bruker Daltonics, Germany).

Results: A new and independent main spectral profile reference library (CMEFA-MSP) representing clinically relevant American Type Culture Collection (ATCC) mycobacterial strains and clinical isolates was established and subsequently used to unequivocally identify 110 blind-coded clinical mycobacterial isolates to the species level that displayed log score values of ≥2.3. MALDI-TOF MS identified all 110 mycobacterial isolates to the genus and species level with 100% accuracy. The CMEFA protocol generated unique and highly reproducible mass spectra profiles for all the 110 mycobacterial strains.

Discussion and Conclusions: The optimized CMEFA sample preparation protocol with the delipidation step was simple and provided mass spectra that were unique and highly reproducible for individual ATCC mycobacterial strains and was deemed suitable for the purpose of biotyping of clinical mycobacterial isolates. MALDI-TOF MS when used in conjunction with the CMEFA sample preparation protocol has potential as a simple and cost-effective alternative for the unambiguous identification of clinically important mycobacteria.

Keywords: Biotyping, Chloroform-methanol ethanol-formic acid (CMEFA); MALDI TOF mass spectrometry, Mycobacteria, Tuberculosis.