Differentiation of the Species in the *Mycobacterium tuberculosis* complex using Matrix Assisted Laser Desorption Ionization Time of Flight

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**ABSTRACT**

Background: Members of the *Mycobacterium tuberculosis* complex (MTC) are closely related genetically, making differentiation between them difficult. Speciation of the MTC is important for patient management, disease containment and epidemiological response. Our algorithm for the speciation of MTC is the comparison of genomic deletion patterns (RD1, RD9, RD4, RD13, and RDmic) generated by Real Time-PCR followed by analysis of melting temperatures. We explored the use of Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) as an alternative method to differentiate MTC.

**RESULTS**

Methods: A distinct MALDI-TOF spectral database was created for *M. tuberculosis*, *M. africanum*, *M. bovis* and *M. bovis* BCG using ATCC reference strains. Clinical isolates (10 *M. tuberculosis*, 5 *M. africanum*, 10 *M. bovis*, 10 *M. bovis* BCG) previously characterized by multi-loci sequencing were used to challenge the database. Total bacterial extracts were harvested and analyzed on a Bruker Daltonics microflex mass spectrometer. The highest identification (ID) cutoff score with a least a 5% difference from the next highest ID was used to differentiate strains to the species level.

Results: In a blinded study using the in-house database, 100% (35/35) of Mycobacterial isolates were identified to the MTC (ID > 1.75). 86% (30/35) were identified to the correct species based solely on highest ID score. However, only 15% (5/35) could be differentiated to the species level when the percent difference was examined.

Conclusions: We have demonstrated that it is not possible to differentiate the closely related MTC species using MALDI-TOF MS with the current software. This does not rule out the possibility that the species in the MTC could be differentiated using an alternate algorithm focusing on the few peaks that are different amongst the species.

**INTRODUCTION**

Differentiating between the members of the *Mycobacterium tuberculosis* complex (MTC) is a lengthy process that involves specific DNA probes (GeneProbe®), followed by a multi-step PCR. Any technique that would speed up the process and maintain the same specificity would be beneficial for clinical microbiology laboratory. MALDI-TOF MS is a proteomic method for rapid microorganism identification (ID). We tested the hypothesis that MALDI-TOF MS could replace our current algorithm for MTC ID in a shorter amount of time and for a fraction of the cost while maintaining high specificity.

**MATERIALS AND METHODS**

Database: A custom mycobacterial MALDI-TOF MS database was created using American Type Culture Collection (ATCC) strains and archived clinical isolates previously characterized by multi loci sequencing. This in-house library was then challenged in a blinded fashion using new patient isolates. Identification of patient isolates was first performed using our current practice (i.e., DNA probe analysis followed by species specific real-time PCR for MTC). Extraction: Strains were plated from 7H9 Middlebrook broth to 7H11 agar plates and colonies were harvested after ~14 days of growth. The MALDI-TOF MS protein extraction was performed as previously described with minor changes. (1) Briefly, isolates were brought to a 0.5 McFarland standard in 500 µl of sterile water and incubated at 97°C for 15 minutes. Suspensions were then centrifuged at 13,000 rpm for 2 minutes and supernatant completely removed. Cell pellets were washed twice using 500 µl of sterile water and re-suspended in 300 µl of sterile water. 500 µl of pure ethanol was then added. Samples were again centrifuged at 13,000 rpm for 2 minutes and the supernatant was completely removed. 50 µl of 70% formic acid was added to the pellet and samples were mixed by vortexing for 1 minute. 50 µl of pure acetonitrile was added, with the suspension mixed by vortexing. Samples were centrifuged at 13,000 rpm for 2 minutes, the supernatant was removed and transferred to new tubes for MALDI-TOF MS. MALDI-TOF MS: 1.75 µl of bacterial extract was spotted and allowed to air dry on an MSP 96 polished steel target (Bruker Daltonics). Samples were overlaid with 1.75 µl of matrix (saturated a-cyano-4-hydroxy-cinnamic acid in 50% acetone/2.5% trifluoroacetic acid), dried at room temperature, and analyzed immediately. Mass spectra were acquired on a microflex LT MALDI-TOF spectrometer (Bruker Daltonics) in linear positive ion mode using FlexControl 3.0 software (Bruker Daltonics). Sample data were collected over an m/z range of 2,000-20,000 Da, without gating, using a detector gain of 2600V. Each spot was measured by collecting approximately 800 laser shots at 60Hz in groups of 100 shots per locus, and spectra were combined into a sum spectrum. The Bruker Bacterial Test Standard was used as a calibrator for each sample run. Organisms were identified by comparing their characteristic mass spectra with reference spectra from the integrated database provided by the manufacturer as well the ARUP Rapidly Growing Mycobacterial database. Based on previous studies in our laboratory a cutoff score of >2.1 was used for accurate identification.

**REFERENCES**

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