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ORIGINAL ARTICLE

Rapid identification of *Mycobacterium avium* clinical isolates by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

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KEYWORDS
Matrix-assisted laser desorption ionization time-of-flight mass spectrometry;

Background: Rapid and accurate discrimination of *Mycobacterium avium* from other mycobacteria is essential for appropriate therapeutic management and timely intervention for infection control. However, routine clinical identification methods for *M. avium* are both time consuming and labor intensive. In the present study, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) was used to identify specific cellular protein pattern for rapid identification of *M. avium* isolates.
Methods: A total of 40 clinically relevant *Mycobacterium* strains comprising 13 distinct species were enrolled for the MALDI-TOF MS identification. A 10-minute extraction-free examination

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Mycobacterium avium;
Rapid identification

procedure was set up to obtain mass spectral fingerprints from whole bacterial cells.
Results: The characteristic mass spectral peak patterns in the *m*/*z* (mass/charge ratio) range of 5–20 kDa can be obtained within 10 minutes. The species-specific mass spectra for *M. avium* is identified and can be differentiated from as *Mycobacterium* strains. This technique shortens and simplifies the identification procedure of MALDI-TOF MS and may further extend the mycobacterial MALDI-TOF MS database.
Conclusion: Simplicity and rapidity of identification procedures make MALDI-TOF MS an attractive platform in routine identification of mycobacteria. MALDI-TOF MS is applicable for rapid discrimination of *M. avium* from other *Mycobacterium* species, and shows its potential for clinical application.
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Introduction

Mycobacterium avium represents one of the main agents causing mycobacterial diseases other than tuberculosis and leprosy.[1] Besides pulmonary, soft tissue, and lymph node infections,[2] recent findings indicate that it also causes hypersensitive pneumonitis-like disease.[3] Although the risk of healthy individuals infected by *M. avium* is regarded as low, the prevalence is increasing in immunodeficient patients such as those with HIV infection.[2]

Many different laboratory methods have been developed for rapid identification of *M. avium* from other NTMs, including serological test detecting the sugar residue compositions of surface glycopeptidolipids,[4,5] high-performance liquid chromatography (HPLC) analysis of mycolic acid,[6] multilocus enzyme electrophoresis,[7,8] Gen-Probe assay,[9] and 16S rRNA-based analysis.[10] More recently, polymerase chain reaction-based rapid identification methods were also described.[10–12] We have previously used the fluorescein-labeled antibody combined with fluorescence-activated cell sorting for rapid identification of clinically significant *Mycobacterium* strains to the genus level, including *M. tuberculosis* and some NTM strains.[13] However, some potential drawbacks still exist, especially in being unable to differentiate mycobacterial species due to the lack of a species-specific antibodies.

Bacterial identification based on peptide spectra obtained by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) shows advantages.[14] It allows rapid identification of the protein profile directly from intact and lysed bacteria,[14–16] and is widely used in bacterial identification, including staphylococci,[15] *Haemophilus*,[17] *Helicobacter*, and *Campylobacter* spp.[18] MALDI-TOF MS also examines bacteria from blood culture positive broth samples almost in real-time.[19,20]

MALDI-TOF MS was previously tested to identify *Mycobacterium* species, including multiple strains of individual species.[20–22] However, so far there was no extensive comparison study of detecting *M. avium* from clinical isolates. The present work aimed to evaluate the potentiality of using MALDI-TOF MS as a tool for rapid identification of clinical *M. avium* from other commonly isolated *Mycobacterium* species. We have developed a 10-minute sample preparation and identification procedure, without further extraction, from the whole bacterial cells of clinically relevant mycobacteria in a total of 40 *Mycobacterium* strains and 13 *Mycobacterium* species. We have established an *M. avium*-specific spectrum profile that is capable of accurately discriminating other clinical *Mycobacterium* strains. MALDI-TOF MS displays great potential as a powerful tool for rapid identification of clinical isolates of *M. avium*.

Methods

Mycobacterial strains and culture media

The mycobacterial strains used in this study were cultured on Lowenstein–Jensen medium (L-J medium; BD Difco). Strains tested comprised 28 *M. avium* (including one standard strain *M. avium* ATCC 700736 and 27 other clinical isolates), three nonpigmented, slow-growers (*M. tuberculosis* H37Rv ATCC 27294, *Mycobacterium nonchromogenium* JATA 45-01, and *Mycobacterium intracellulare* JATA 52-01), two slow-growers, pigmented under light (*Mycobacterium simiae* KK 44-02 and *Mycobacterium kansasii* KK 11-05), four slow-growers, pigmented without light (*Mycobacterium scrofulaceum* JATA 31-01, *Mycobacterium gordonae* JATA 33-01, *Mycobacterium flavescens* JATA 67-01, and *Mycobacterium szulgai* JATA 32-01), and three rapid-growers (*Mycobacterium phlei* KK65-01, *Mycobacterium fortuitum* JATA 61-01, and *Mycobacterium chelonae* JATA 62-01).[23] All bacterial strains were obtained from the Department of Laboratory Medicine, National Taiwan University Hospital (Taipei, Taiwan). All *M. avium* isolates were initially identified by conventional physiological and biochemical methods. Isolates were further confirmed as *M. avium* by gas chromatography–mass spectrometry[24] and by polymerase chain reaction and restriction fragment length polymorphism analysis of *hsp65*, a species-specific stress protein gene of *Mycobacterium* species.[25] Final confirmation of *M. avium* was achieved by hybridization with nucleic acid probe complementary to the rRNA of the *M. avium* (AccuProbe Culture Confirmation kit for *M. avium*, Gen-Probe).

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