

Direct analysis and identification of pathogenic *Lichtheimia* species by matrix-assisted laser desorption ionization-time of flight analyzer-mediated mass spectrometry.

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Abstract

Zygomycetes of the order Mucorales can cause life-threatening infections in humans. These mucormycoses are emerging and associated with a rapid tissue destruction and high mortality. The resistance of Mucorales to antimycotic substances varies between and within clinically important genera such as *Mucor*, *Rhizopus*, and *Lichtheimia*. Thus, an accurate diagnosis before onset of antimycotic therapy is recommended. Matrix-assisted laser desorption ionization (MALDI)-time of flight (TOF) mass spectrometry (MS) is a potentially powerful tool to rapidly identify infectious agents on the species level. We investigated the potential of MALDI-TOF MS to differentiate *Lichtheimia* species, one of the most important agents of mucormycoses. Using the Bruker Daltonics FlexAnalysis (version 3.0) software package, a spectral database library with m/z

ratios of 2,000 to 20,000 Da was created for 19 type and reference strains of clinically relevant Zygomycetes of the order Mucorales (12 species in 7 genera). The database was tested for accuracy by use of 34 clinical and environmental isolates of *Lichtheimia* comprising a total of five species. Our data demonstrate that MALDI-TOF MS can be used to clearly discriminate *Lichtheimia* species from other pathogenic species of the Mucorales. Furthermore, the method is suitable to discriminate species within the genus. The reliability and robustness of the MALDI-TOF-based identification are evidenced by high score values (above 2.3) for the designation to a certain species and by moderate score values (below 2.0) for the discrimination between clinically relevant (*Lichtheimia corymbifera*, *L. ramosa*, and *L. ornata*) and irrelevant (*L. hyalospora* and *L. sphaerocystis*) species. In total, all 34 strains were unequivocally identified by MALDI-TOF MS with score values of >1.8 down to the generic level, 32 out of 34 of the *Lichtheimia* isolates (except CNM-CM 5399 and FSU 10566) were identified accurately with score values of >2 (probable species identification), and 25 of 34 isolates were identified to the species level with score values of >2.3 (highly probable species identification). The MALDI-TOF MS-based method reported here was found to be reproducible and accurate, with low consumable costs and minimal preparation time.

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