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Identification of moulds by MALDI-TOFmass-spectrometry Biotyper - comparison and optimization of protocols

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Background: Conventional fungal culture and microscopic techniques commonly used for species identification are laborious and time consuming. Identification by MALDI-TOF mass-spectrometry provides very rapid and cost-effective workflows for bacterial identification. However, fungal identification by MALDI-TOF remains challenging due to complicated extraction protocols and insufficient databases.

Material/methods: A range of clinical relevant molds were included for this study: *Aspergillus* spp., *Rhizopus* spp., *Rhizomucor*, *Lichtheimia* spp., *Microsporum* spp., and *Trichophyton* spp.. Based on the previously published NIH extraction protocol, we used different growth times at 3, 5, 7, and 14 days on solid media and compared the MALDI-TOF identification scores (Biotyper, Bruker Daltonik, Germany). A combination of morphology and sequence based identification served as gold standard. In addition, further extraction procedures were compared: a full-protein extraction including sonication (“Covaris protocol”, USA) or cell disruption by high speed shaking (“TissueLyser protocol”, Qiagen, Switzerland). Further, we compared three different databases: the public available NIH mold database (“NIH-DB”), and the two commercial one from Bruker - the filamentous fungi library 1.0 (“FFL-DB”) and the general Bruker Daltonik database (“BDAL-DB”).

Results: Using the NIH extraction method and NIH-DB, we could reliably identify the species for only a few isolates within 3 days: *Aspergillus fumigatus* showed a median score of 1.98 (IQR 1.96-2.04), whereas he NIH-DB had difficulties to identify *A. niger* isolates at this time-point. *Rhizopus oryzae*,

Rhizomucor, and *Lichtheimia corymbifera* showed optimal detection after 3 days (median 1.91, 2.27, 2.05), whereas at later time-points identification scores were much lower or not even reliable.

In contrast, dermatophytes such as *Trichophyton* and *Microsporum* spp. could not be identified at early time-points. The median scores of *Trichophyton* and *Microsporum* spp. increase from 1.59 and 1.17 at day 3 to 1.83 and 1.40 at day 14, respectively.

Next, we compared different sample preparation protocols to improve identification of all dermatophytes with the NIH-DB. We used the NIH-, Covaris-, and TissueLyser-based protocols and observed median scores of 1.23, 1.17 and 1.33 at day 3. Finally, we combined the NIH-, FFL-, and BDAL-DB and compared the NIH-, Covaris-, and TissueLyser-based protocols. Significant higher median identification scores could be reached: 1.61, 1.54, and 1.80, respectively.

Conclusions: Fast, simple and cost-effective protocols for the MALDI-TOF identification of fungi are required. Each fungi showed optimal time-points for identification over 14 days. Most *Aspergillus*, *Rhizopus*, and *Lichtheimia* spp. could be identified at early time-points. The TissueLyser preparation together with a combined database resulted in the highest median scores for dermatophytes, however due to a lack of proper database entries only 40% could be reliably identified with a correct species name. MALDI-TOF may become a useful tool for the identification of fungal isolates in the future, however currently the most important gap remains insufficient database entries.