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Dermatophytes are keratinolytic fungi responsible for a wide variety of diseases of glabrous skin, nails, and hair. Their identification, currently based on morphological criteria, is hindered by intraspecies morphological variability and the atypical morphology of some clinical isolates. The aim of this study was to evaluate matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) as a routine tool for identifying dermatophyte and Neoscytalidium species, both of which cause dermatomycoses. We first developed a spectral database of 12 different species of common and unusual dermatophytes and two molds responsible for dermatomycoses (Neoscytalidium dimidiatum and N. dimidiatum var. hyalinum). We then prospectively tested the performance of the database on 381 clinical dermatophyte and Neoscytalidium isolates. Correct identification of the species was obtained for 331/360 dermatophytes (91.9%) and 18/21 Neoscytalidium isolates (85.7%). The results of MALDI-TOF MS and standard identification disagreed for only 2 isolates. These results suggest that MALDI-TOF MS could be a useful tool for routine and fast identification of dermatophytes and Neoscytalidium spp. in clinical mycology laboratories.

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Fungal isolates. All isolates were obtained by culture of skin, hair, and nail samples received for routine examination on Sabouraud’s dextrose agar slants containing antibiotics, with and without cycloheximide (Bio-Rad, Marne la Coquette, France), at 26°C for 3 weeks, in order to obtain typical morphological characteristics (13).

The reference database was constructed from a set of 50 reference strains belonging to 12 clinically relevant species of dermatophytes and 2 species of Neoscytalidium, namely, Epidermophyton floccosum, Microsporum langeronii, M. canis, M. persicolor, Trichophyton rubrum, T. mentagrophytes var. interdigitale, T. tonsurans, T. soudanense, T. erinacei, T. equinum, T. simii, T. violaceum, N. dimidiatum, and N. dimidiatum var. hyalinum. All these strains were obtained from clinical samples at Saint Louis Hospital; the species identification was based on usual macroscopic and microscopic criteria (13, 28) and was confirmed by sequencing the 18S ribosomal DNA (21).

To evaluate the database as a tool for routine identification by MALDI-TOF MS, we performed a 6-month prospective evaluation study, during which 360 dermatophyte isolates belonging to 7 different species and 21 isolates of N. dimidiatum or N. dimidiatum var. hyalinum were collected from patients with dermatomycosis (tinea capitis, n = 87; onychomycosis, n = 124; tinea pedis, n = 92; tinea corporis, n = 69; tinea manuum, n = 9). These 381 isolates were identified by means of both conventional culture criteria and MALDI-TOF MS.

MALDI-TOF MS. (i) Sample preparation. After a 3-week incubation period, the colony surface was scraped with a sterile scalpel and harvested in 10 μl of formic acid (70%, vol/vol). Then, 1 μl of this mixture was deposited on the target plate (Andromas, Paris, France) and allowed to dry at room temperature. One microliter of matrix solution (saturated solution of α-cyano-4-hydroxyphenylacetic acid, 50% acetonitrile, 2.5% trifluoroacetic acid) was then added and allowed to cocrystallize with the sample. The strains used for database construction were deposited in 10 replicates, while isolates used to test the database were deposited in 2 replicates.

(ii) Instrument type. All the samples were processed using the Andromas system (Paris, France), including a mass spectrometry apparatus combined with control software and various databases for identification of microorganisms. Before this study, no database was available for identification of dermatophyte and Neoscytalidium species. For each sample, positive ions were extracted with an accelerating voltage of 20 kV in linear mode. The spectra were analyzed and compared over an m/z range of 3,000 to 20,000 with Andromas software (Paris, France).

(iii) Data analysis. The Andromas software identified the number of common peaks between the spectra of the tested isolate and the species-specific spectral fingerprints of the reference strains in the database (i.e., dermatophyte and Neoscytalidium database). For each isolate, all peaks with an intensity greater than 0.1 were retained and were compared with the peaks for the species-specific spectral fingerprints of each reference strain, taking into account possible variations in the m/z value of ±10. Then, the percentage of common peaks was obtained with the following formula: 100 × (number of peaks common between the peaks of the tested isolate and the peaks of the species-specific spectral fingerprint/total number of peaks specific to the species-specific spectral fingerprint). Acceptable identification of a tested strain corresponds to the species having ≥66% of peaks in common with the reference strains in the database. Only the first- and second-best matches were retained. A difference of at least 10% between the first and the second match was required. When the identification was not acceptable (i.e., <66% of peaks are in common with those for the reference strain or a <10% difference exists between the first and second matches), a second run was performed and the results were analyzed in a similar manner.

An external control (Pseudomonas aeruginosa) was used to validate the calibration for each experiment (12).

RESULTS
Reference database. The database for dermatophyte and Neoscytalidium identification was engineered using a previously described strategy (8, 12). A set of reference strains selected as belonging to clinically relevant dermatophyte and Neoscytalidium species and described in Materials and Methods was used to construct the mass spectral database. The MALDI-TOF MS spectra obtained in 10 different runs were analyzed for each reference strain. The majority of ions detected were in the range 4,000 to 14,000 Da. For each reference strain, we retained only the peaks with a relative intensity of greater than 0.1 that were constantly present in all 10 spectra obtained for a given strain. The standard deviation for each conserved peak did not exceed a 6 m/z value. The set of peaks, so-called spectral fingerprints, was specific for each selected reference strain. Figure 1 shows a selection of the specific spectral fingerprints for the 7 most common dermatophyte species, N. dimidiatum, and N. dimidiatum var. hyalinum.

Validation of the database. In order to determine whether this database could be used for the identification of dermatophytes and Neoscytalidium species, we compared the identities of the clinical isolates obtained by the conventional methods with those obtained by MALDI-TOF MS. The database was tested blindly with the 381 clinical isolates described in Materials and Methods.

The two methods agreed for 331 (91.9%) of the 360 dermatophyte isolates (Table 1). Twenty-seven isolates (7.5%) could not be identified, because spectral acquisition failed after 2 runs. The results of MALDI-TOF MS and conventional criteria disagreed for 2 isolates (0.5%): two T. mentagrophytes var. interdigitale isolates were identified by MALDI-TOF MS as T. rubrum and T. tonsurans.

Eighteen (85.7%) of the 21 Neoscytalidium isolates (91.7% of N. dimidiatum var. hyalinum and 77.8% of N. dimidiatum isolates) were correctly identified by MALDI-TOF MS. None of the isolates was incorrectly identified, but spectral acquisition failed after 2 runs for 3 Neoscytalidium isolates.

DISCUSSION
We have developed a MALDI-TOF MS database allowing species identification of commonly encountered dermatophytes. The three dermatophytes usually responsible for tinea pedis and onychomycosis (T. rubrum, T. mentagrophytes var. interdigitale, and E. floccosum) were successfully identified in, respectively, 97.5% (154/158), 97.6% (80/82), and 100% (5/5) of cases. The four dermatophyte species usually responsible for tinea capitis (M. canis, M. langeronii, T. tonsurans, and T. soudanense) were successfully identified in, respectively, 91.7% (11/12), 80.8% (21/26), 88.5% (23/26), and 72% (36/50) of cases, and none was misidentified. Spectral acquisition failed for 19% (5/26) and 28% (14/50) of M. langeronii and T. soudanense isolates, respectively, possibly owing to difficulties in harvesting fungal material from the colony surface, which was very dense or dry. This technical problem was not mentioned in the two previous reports of dermatophyte identification by MALDI-TOF MS, as no M. langeronii or T. soudanense isolates were tested (14, 32).

Concerning Neoscytalidium spp., our database performed better for N. dimidiatum var. hyalinum identification than for N. dimidiatum identification. N. dimidiatum is a pigmented dematiaceous fungus producing a melanin-like pigment in vitro and in vivo, whereas N. dimidiatum var. hyalinum is unable to produce melanin in vitro (27). Recently, Buskirk et al. demonstrated that synthetic melanin and dark fungal pigments can inhibit the de-
FIG 1 MALDI-TOF MS spectra of 7 species of dermatophytes, 1 Neoscytalidium dimidiatum strain, and 1 Neoscytalidium dimidiatum var. hyalinum reference strain.
Currently, only two studies have evaluated performances of MALDI-TOF MS for identification of dermatophytes (13, 32).

<table>
<thead>
<tr>
<th>Group and species</th>
<th>No. (%) of isolates</th>
<th>Accurately identified</th>
<th>No spectral acquisition</th>
<th>Misidentified</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dermatophytes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. floccum</td>
<td>5</td>
<td>5 (100)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T. rubrum</td>
<td>158</td>
<td>154 (97.5)</td>
<td>4 (2.5)</td>
<td>0</td>
</tr>
<tr>
<td>T. mentagrophytes var. interdigitale</td>
<td>82</td>
<td>80 (96.3)</td>
<td>0</td>
<td>2 (2.4)</td>
</tr>
<tr>
<td>T. soudanense</td>
<td>50</td>
<td>36 (72)</td>
<td>14 (28)</td>
<td>0</td>
</tr>
<tr>
<td>T. tonsurans</td>
<td>26</td>
<td>23 (88.5)</td>
<td>3 (11.5)</td>
<td>0</td>
</tr>
<tr>
<td>T. erinacei</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M. langeronii</td>
<td>26</td>
<td>21 (80.8)</td>
<td>5 (19.2)</td>
<td>0</td>
</tr>
<tr>
<td>M. canis</td>
<td>12</td>
<td>11 (91.7)</td>
<td>1 (8.3)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>360</td>
<td>331 (91.9)</td>
<td>27 (7.5)</td>
<td>2 (0.5)</td>
</tr>
<tr>
<td><strong>Neoscytalidium spp.</strong></td>
<td></td>
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<tr>
<td>N. dimidiatum</td>
<td>9</td>
<td>7 (77.8)</td>
<td>2 (22.2)</td>
<td>0</td>
</tr>
<tr>
<td>N. dimidiatum var. hyalinum</td>
<td>12</td>
<td>11 (91.7)</td>
<td>1 (8.3)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>18 (85.7)</td>
<td>3 (14.3)</td>
<td>0</td>
</tr>
</tbody>
</table>

*a Corresponding to the isolates for which spectral acquisition failed after 2 runs.

Using the Voyager DE Pro MS system (Applied Biosystems, Darmstadt, Germany) and the SARAMIS spectral database (AgNoStec, Potsdam, Germany), Erhard and coworkers succeeded in identifying five dermatophyte species (T. rubrum, T. interdigitale, T. tonsurans, M. canis, and Arthroderma benhamiae) (14). They achieved a high confidence level since 19 out 20 isolates were correctly identified. However, this collection was limited. Recently, Theel and coworkers tested a larger collection of isolates to evaluate performances of the Bruker Biotyper MALDI-TOF MS system (32). Using the MicroFlex LT system (Bruker Daltonics, Bremen, Germany) and the MALDI Biotyper library (version 3.0) in combination with a supplemented library containing an additional 20 dermatophyte spectra, the authors succeeded in identifying isolates to the genus level but not the species level. Indeed, from a total of 171 dermatophyte isolates, 159 (93%) were correctly identified to the genus level and only 102 (59.6%) were correctly identified to the species level. In comparison, using the Andromas system, we obtained a correct identification to the species level for 331/360 (91.9%) of dermatophyte isolates. These results showed that the Andromas system represents a powerful tool to discriminate dermatophyte species and is suited for use on the vast majority of routine samples that are commonly processed in mycology laboratories.

Our results show that this technique could replace conventional methods for dermatophyte identification in the near future, not only in specialized research facilities but also in clinical laboratories. This system will become more powerful as the database is expanded by including other dermatophyte species and other fungi with similar morphologies.

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REFERENCES


