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MALDI-TOF MS-Based Identification of Melanized Fungi is Faster and Reliable After the Expansion of In-House Database

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Purpose: Invasive fungal infections caused by melanized fungi are a growing concern. Rapid and reliable identification plays an important role in optimizing therapy. Matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF MS)-based identification has emerged as a faster and more accurate diagnostic technique. However, lack of a protein extraction protocol and limited database restricts the identification of melanized fungi by MALDI-TOF MS. The study is designed to standardize protein extraction protocol, to enrich the existing, and to create an in-house database for the rapid identification of melanized fungi.

Experimental design: In this study, 59 sequence-confirmed, melanized fungi were used to expand and to create an in-house database using a modified protein extraction protocol. A total of 117 clinical isolates are further used to validate the created database.

Result: Using existing Bruker database, only 29(24.8%) out of 117 moulds could be identified. However, all the isolates are identified accurately by supplementing the Bruker database with the created in-house database. MALDI-TOF MS takes significantly lesser time for identification compared to DNA sequencing.

Conclusion and clinical relevance: An expanded database with modified protein extraction protocol can reduce significant time to identify melanized fungi by MALDI-TOF MS.

1. Introduction

Dematiaceous fungi are ubiquitous, melanin-producing, heterogeneous group of eukaryotes.[1–3] They usually grow as saprophytes and are known plant pathogens which rarely affect humans. However, in the past few decades, the melanized fungi (more than 100 species) causing infections are increasingly reported from both immunocompetent and compromised patients.[2–4] The spectrum of clinical manifestations caused by these fungi in a human host ranges from allergic reactions to systemic life-threatening infections with high mortality.[1–3,5] In addition, few of them (*Cladosporium* and *Rhinocladiella*) also exhibit neurotropism and infect the central nervous system even in immunocompetent patients.[6,7] The major problem in the management of phaeohyphomycosis is limited knowledge regarding newer species, along with antifungal susceptibility pattern.[2,4,8,9]

Traditionally, the melanized fungi are identified based on phenotypic features in routine clinical settings.[2,3,8,9] However, in many cases, only a genus level identification is possible with the phenotypic method. The limitations of phenotypic identification are the presence of multiple morphotypes and the lack of sporulation.[2,10,11] In culture, these fungi usually exist as an anamorph, which can be either heterothallic or homothallic, or heterothallic/homothallic.[2,8] In addition, some fungi form anamorphs, teleomorphs, or both. The emergence of molecular techniques (DNA sequencing) has improved the identification of melanized fungi, especially when it is unable to produce spores.[2,4,12] Although the molecular techniques transformed the taxonomy and epidemiology of melanized fungi, the major problem with DNA based identification is the limited availability of database and lack of inter-laboratory concordance.[2,10,13,14] Therefore, molecular characterization can be correlated with phenotypic features for reliable identification and rare fungal species should always be confirmed by DNA sequencing.[2,10,14]

In the past few years, matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF

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MS) emerged as a rapid diagnostic technique in clinical microbiology.[15,16] Multiple studies have reported an MALDI-TOF MS-based identification of bacteria, yeast, and hyaline fungi.[15–18] However, only a few studies have reported a MALDI-based identification of melanized fungi due to the lack of a standardized protein extraction protocol and a limited database availability.[4,19,20] In addition, the present Bruker database contains very few entries of melanized fungi. Therefore, this study was conducted to improve the protein extraction protocol, expansion of the existing database, and creation of an in-house database for the identification of melanized fungi by MALDI-TOF MS in routine clinical settings.

Clinical Relevance

Invasive phaeohyphomycosis (IP) is caused by dematiaceous fungi, which are also known as melanized fungi. It is ubiquitously present as a saprophyte. The exact incidence and prevalence of IP across the world are unknown. The disease affects immunosuppressed and immunocompetent patients almost equally. The most important invasive infection in this group is cerebral phaeohyphomycosis, which is 100% fatal if left untreated. Among the fungi causing phaeohyphomycosis, few fungi are predominantly neurotropic including *Cladophialophora bantiana* (>70% cases), *Exophiala dermatitidis*, and *Rhinocladiella mackenziei*. A review of cases of cerebral phaeohyphomycosis by *C. bantiana* revealed nearly half of the world cases are reported from India. The major challenges in the management of those cases are delay in the diagnosis, lack of knowledge about the disease, and high mortality rate (65.2%), despite antifungal therapy. Moreover, the dematiaceous group of fungi is slow growing and sometimes unable to produce spore for identification. The molecular identification of these fungi is faster, but the presence of pigment in the DNA sometimes inhibits the amplification. MALDI-TOF MS, recently introduced in our laboratories for routine identification of bacteria and yeasts, can also be used for mould identification. In this study, we standardized the protein extraction protocol and created an in-house database for rapid and reliable identification of medically important melanized fungi.

2. Experimental Section

2.1. Fungal Isolates

All the isolates used in this study were obtained from National Culture Collection of Pathogenic Fungi (NCCPF) of PGIMER, Institute of Microbial Technology (IMTECH), Chandigarh and L V Prasad Eye Institute, Hyderabad. A total of 59 melanized fungi were used to enrich the existing database and to create an in-house database. Among these 59 isolates, 55 were sequence confirmed clinical isolates (Table S1, Supporting Information). Another 117 clinical isolates of melanized fungi isolated from different samples were used for the validation of the created database. Identification of those isolates was done by conventional morphological and DNA sequencing of the internal transcribed spacer (ITS) and 28S ribosomal DNA genes.

2.2. PCR Sequencing-Based Identification

The pure mycelial growth from Sabouraud's dextrose agar (SDA) was placed in a mortar and ground properly with liquid nitrogen to make fine powder. 1–2 mL lysis buffer (50 mM EDTA, 100 mM Tris HCl, 3% SDS) was added and mixed in the fine powder. Seven hundred microliters of the suspension was placed in 2 mL sterile microcentrifuge tube (MCT) and an equal volume of phenol, chloroform, and isoamyl alcohol (25:24:1) was added followed by precipitation with ethanol. The purity of extracted DNA was checked by NanoDrop 2000/2000c Spectrophotometers (Thermo Scientific, USA). 100 ng of DNA was amplified by using the primers specific for ITS and 28S region with Eppendorf 5331 MasterCycler Gradient Thermal Cycler (Eppendorf, USA). Sequencing of the amplified product was done with an automatic genetic analyzer (ABI3130, Applied Biosystems, Foster City, CA) by using BigDye Terminator ready reaction kit v3.1. The sequences of ITS and 28S region were The FASTA sequence of ITS and 28S region was blast in NCBI BLAST-n platform (https://www.ncbi.nlm.nih.gov) by using the non-redundant database.[4]

2.3. Protein Extraction Protocols

The protein extraction protocol was followed for melanized fungi was similar with some modifications of our previously standardized protocol.[18] Two growth condition (in solid and liquid medium) were compared and solid media method used for this study. In brief, all the isolates were cultured on SDA and incubated for 3 to 7 days at 28 °C. By using teasing needle, very small pieces of mycelial growth was harvested and placed in 1.5 mL double distilled water (DDW) in a 2 mL microcentrifuge tube and vortexed well to disperse the mycelial clump and produce thin and smaller structure. It was then centrifuged for 10 min at 13 000 rpm. The supernatant was completely removed and replaced with 1 mL of 70% ethanol and was incubated at room temperature for 5 min. The suspension was centrifuged at a high speed and the supernatant was discarded. The pellet was properly dried in a vacuum concentrator (Savant DNA Speed-Vac concentrator, Thermo Fisher Scientific, USA). 50–80 μL of 98% formic acid (FA) was added to resuspend the pellet and incubated for 5 min at room temperature. An equal amount of acetonitrile (ACN) and 0.5–0.75 mm sterile acid-washed glass beads (Sigma–Aldrich, Saint Quentin Fallavier, France) was added and vortexed vigorously for 10–15 min. The suspension was centrifuged at 10 000 rpm for 5 min and 4–6 μL of the supernatant was spotted (1.5–2 μL each time for 3–4 times) on to the surface of a 96-target polished steel plate and allowed to dry. The dry spot was overlaid with 1 μL matrix solution (50 μL ACN, 47.5 μL DDW, 2.5 μL trifluoroacetic acid, and 1 mg α-cyano-4-hydroxycinnamic acid) and subjected to MALDI-TOF MS analysis.[18]

2.4. Mass Spectrum Acquisition and Analysis

A Microflex LT mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) was used for the MALDI-TOF MS based identification of melanized fungi. A Flex control software (Flex control 3.4, Bruker Daltonics) was used for the acquisition of spectra for the creation and validation of in-house database. Spectra of each isolate were acquired within a mass range of 2–20 kDa with a laser frequency of 60 Hz. The spectrum was generated by 40 laser shots steep randomly hitting six different positions of a single spot. A total of 240 laser shots were generated. Bruker Bacterial Test Standard (BTS 8255343) was used for internal calibration of the instrument. Automatic smoothing, normalization, baseline subtraction, and peak selection of acquired spectra was performed by the MALDI BioTyper software (MALDI Biotyper 3.1, Bruker Daltonik GmbH, Bremen, Germany). For each sample, the most significant spectra were selected and compared with the existing Filamentous fungal library (version 1.0) and MALDI BioTyper database (version 4.0). According to Bruker instructions, a score of more than, or equal to 2 means that the identification has been confirmed up to species level. Between 1.7–1.99 means reliable up to genus and below 1.7 indicates no reliable identification.

2.5. Expansion of Bruker MALDI Biotyper Database

For the database creation, samples were prepared according to the previously explained protocol. In brief, 24 spectra of each isolate were acquired by spotting at eight different wells onto the MALDI target plate and each spot was measured three times. The consistency and quality of all 24 spectra were checked by a Flex Analysis software (version 3.4) by analyzing the number of consistent peak and similarity between the peak intensity of all the spectra. After the check, at least 20 best spectra were imported into MALDI Biotyper software and processed (Adjust mass, smooth, subtract baseline, Normalize, pick peaks, and batch processing) prior to include into the database. Then the spectra of each isolate were saved into the Bruker Biotyper database as the main spectra profile (MSP) of newly included isolates.[16–18]

2.6. Creation of Composite Correlation Index Matrix and Main Spectra Profile Dendrogram

The interrelationship between the representative spectra of 26 different species were analyzed by composite correlation index (CCI) matrix. It was constructed by selecting all the representative spectra and by using a tool present in MALDI Biotyper. A CCI value '1' indicates the complete correlation between spectra, whereas '0' means no correlation is present. A CCI matrix was constructed by using the CCI values. In CCI matrix, closely related spectra appeared as a color contrast between yellow and dark red, whereas distantly related spectra produce a contrast between green and dark blue. The color intensity of the CCI matrix determined the relationship between individual melanized fungal isolates.[21]

The relationship between all the isolates used for the in-house database construction was reconfirmed by constructing the MSP dendrogram. The MSP dendrogram was constructed by using a special tool in Bruker MALDI Biotyper software. MSPs of all 59 isolates were used to make the dendrogram. The distance between isolates was represented by different clades in dendrogram and it was normalized up to a value of 1000.[4,21]

2.7. Statistical Analysis

SPSS (IBM SPSS statistics 22) software was used for all the statistical analysis performed in this study. The efficiency of PCR sequencing and MALDI-TOF MS based identification measured was performed by using Cohen's kappa analysis using GraphPad software (GraphPad Prism 6). The statistical analysis was executed by employing two-sided tests with $p \leq 0.05$ indicating significance level.

3. Results

For expansion of existing Bruker database and to create an in-house database of melanized fungi, 59 clinical isolates including 26 species were used (Table S1, Supporting Information). Out of 26 species, five species (*Epicoccum nigrum*, *Lasiodiplodia theobromae*, *Medicopsis romeroi*, *Rhytidhysteron rufulum*, and *Roussoella percutanea*) could not be identified by conventional methods due to lack of sporulation. Using the DNA sequencing of ITS and 28S ribosomal RNA genes, all the isolates were identified up to species level. As there was no significant difference between the spectra obtained from solid and liquid media method and additional subculturing in liquid media added extra time, we used solid media method for protein extraction in this study (**Figure 1**). When compared DNA sequencing versus MALDI-TOF MS-based identification after sufficient amount of growth in solid media, DNA extraction to sequencing required 24 to 48 h, whereas MALDI-TOF MS based identification completed within 3–4 h. The total identification time of melanized fungi required 5–9 days for DNA sequencing and 3–7 days for MALDI-TOF MS from patient sample (**Figure 2**; Table S2, Supporting Information).

Among 26 species, eight species including *Cladosporium spp.*, *Curvularia lunata*, *Curvularia pallescens*, *Alternaria alternata*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Epicoccum nigrum*, and *Scytalidium lignicola* were present in the existing Bruker database. We expanded this existing database with five species including *Alternaria alternata* ($n = 2$), *Chaetomium globosum* ($n = 3$), *Cladosporium cladosporioides* ($n = 2$), *Epicoccum nigrum* ($n = 2$), and *Scytalidium lignicola* ($n = 2$). An in-house database was created with 21 species including *Acrophialophora fusispora* ($n = 3$), *Alternaria tenuissima* ($n = 1$), *Bipolaris australiensis* ($n = 1$), *Bipolaris hawaiiensis* ($n = 1$), *Bipolaris spicifera* ($n = 2$), *Cladophialophora bantiana* ($n = 4$), *Cladophialophora carrionii* ($n = 3$), *Cladosporium sphaerospermum* ($n = 2$), *Colletotrichum truncatum* ($n = 1$), *Exophiala jeanselmei* ($n = 1$), *Exserohilum rostratum* ($n = 4$), *Fonsecaea pedrosoi* ($n = 7$), *Humicola fuscoatra* ($n = 1$), *Lasiodiplodia theobromae* ($n = 3$), *Medicopsis romeroi* ($n = 4$), *Nigrospora sphaerica* ($n = 4$), *Papulaspora equi*

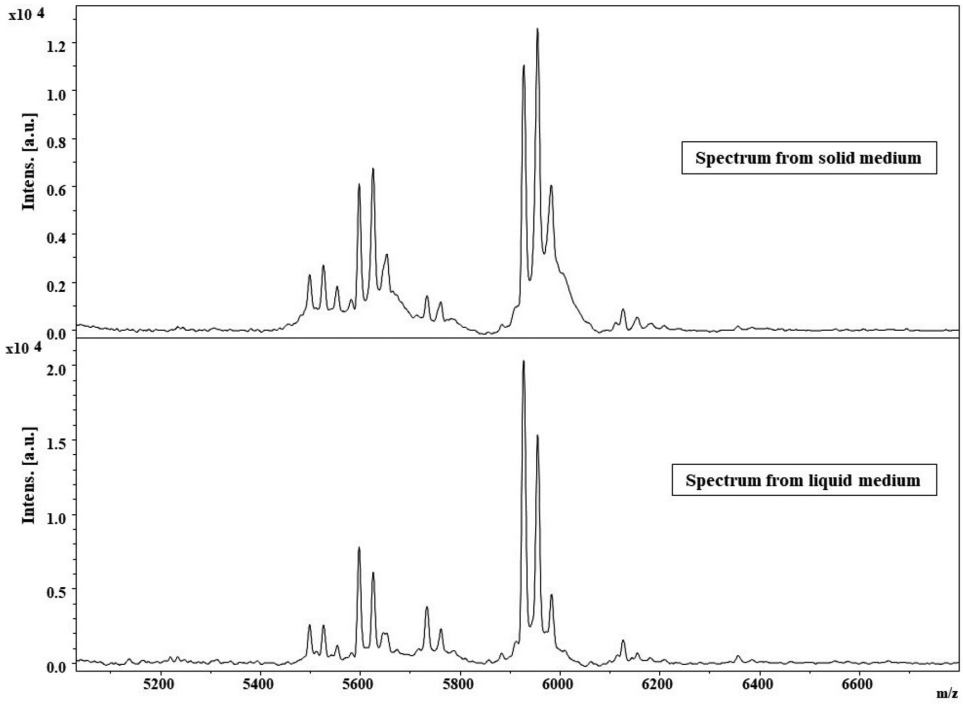

Figure 1. Comparison between the sprctra obtained from solid and liquid media method. There is no significant difference between the spectra obtained by these two methods.

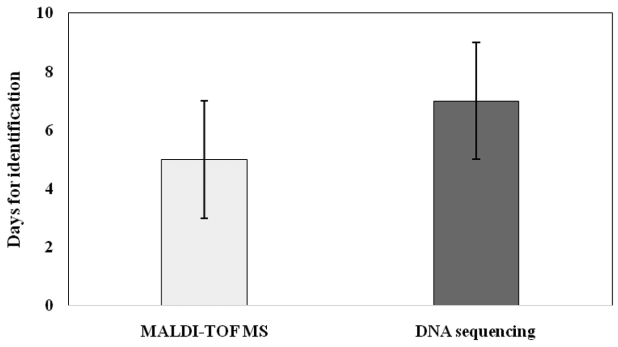

Figure 2. A bar diagram depicting the average time required for identification of melanized fungi by MALDI-TOF MS and DNA-sequencing-based method.

($n = 1$), *Phialophora verrucosa* ($n = 2$), *Rhytidhysteron rufulum* ($n = 1$), *Roussoella percutanea* ($n = 1$), and *Ulocladium spp.* ($n = 1$), which were absent in the existing database. The specificity of MALDI-based identification was checked using the direct visual observation of MALDI-TOF MS spectra, CCI matrix analysis and constructing MSP dendrogram. The comparison of the MALDI spectra of different species showed differences in m/z ratio and intensity of spectra (Figure S1, Supporting Information). Similarly, the CCI matrix analysis of the spectra also showed a low similarity between the MSPs of different species (Figure S2 and Table S3, Supporting Information).

In addition, the clustering of isolates confirmed by DNA sequencing in MSP dendrogram with respective reference strains of different fungal species also indicates the sufficient discrimination between all the species by MALDI-TOF MS (**Figure 3**).

A total of 117 clinical isolates from different patient samples (corneal scraping, corneal button, skin tissue, nasal tissue, sputum, brain abscess, nail clipping, pus, etc.) were used for validation of created database. Twenty-nine (24.8%) isolates belonging to eight species including *Cladosporium spp.*, *Curvularia lunata*, *Curvularia pallescens*, *Alternaria alternata*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Epicoccum nigrum*, and *Scytalidium lignicola* were identified by existing Bruker database. Using both in-house database and existing database, 75 (64.1%) isolates were identified up to species level with log scores ≥2.0 and 42 (35.9%) isolates were identified with log scores ranged from 1.75–1.99, respectively (**Table 1**).

On comparing MALDI-TOF MS with DNA sequencing, *Acrophialophora fusispora* ($n = 6$), *Alternaria alternata* ($n = 5$), *Cladophialophora bantiana* ($n = 6$), *Epicoccum nigrum* ($n = 4$), *Fonsecaea pedrosoi* ($n = 10$), *Lasiodiplodia theobromae* ($n = 4$), *Nigrospora sphaerica* ($n = 7$), *Papulaspora equi* ($n = 5$), and *Phialophora verrucosa* ($n = 4$) were identified up to species level accurately by MALDI-TOF MS with receiver operating characteristic (ROC) cut off log score of more than or equal to 1.74. Whereas, *Alternaria tenuissima* ($n = 4$), *Bipolaris spicifera* ($n = 5$), *Chaetomium globosum* ($n = 6$), *Cladophialophora carrionii* ($n = 4$), *Cladosporium cladosporioides* ($n = 4$), *Exophiala jeanselmei* ($n = 4$), *Exserohilum rostratum* ($n = 6$), and *Scytalidium lignicola* ($n = 5$) were identified reliably with cut off value of more than or equal to 1.81. A very significant correlation was observed between MALDI-TOF MS and DNA sequencing with kappa value 1.0 (value '0' = no agreement and value '1' = high agreement) (**Table 2**).

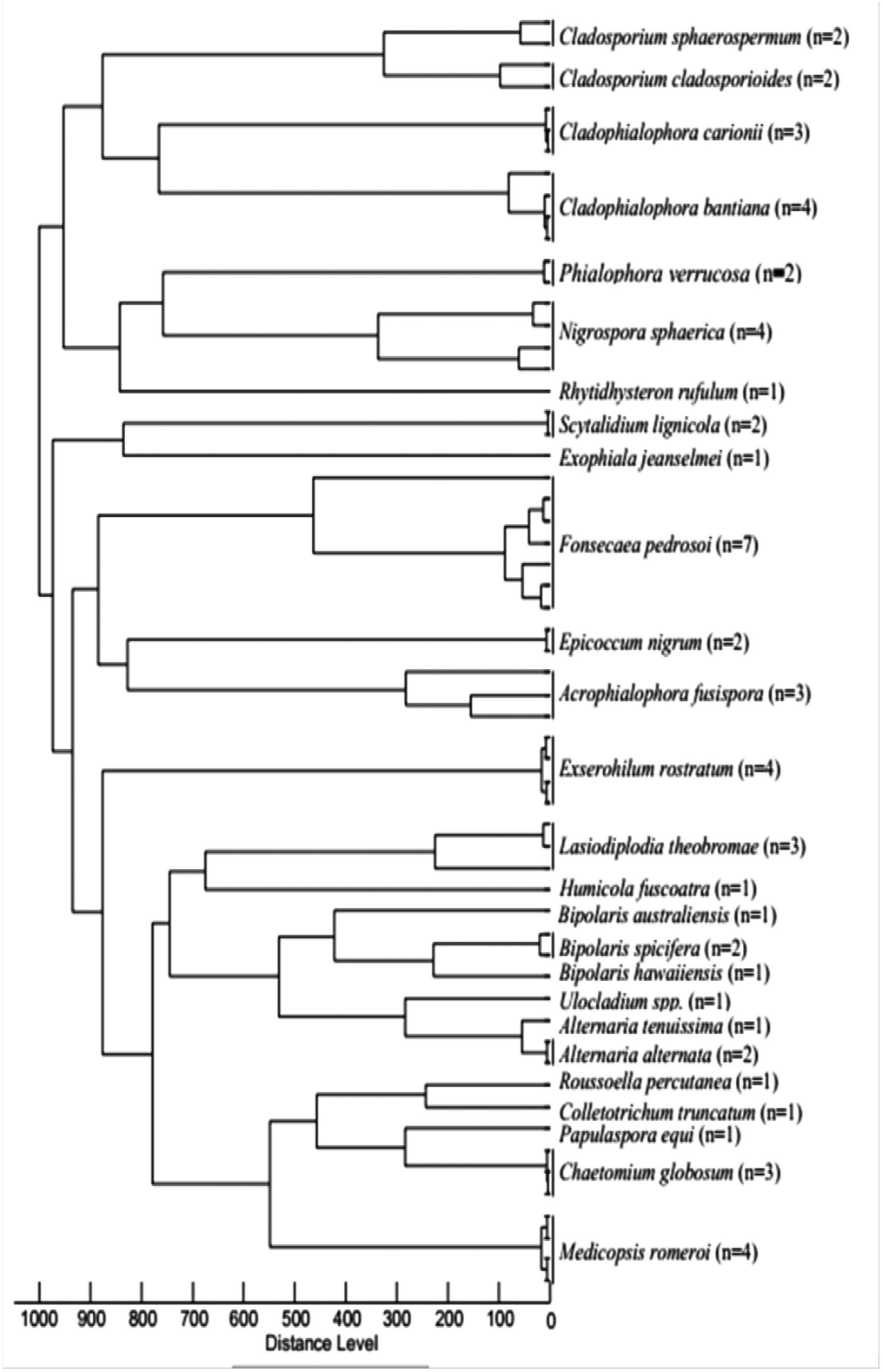

Figure 3. MSP dendrogram of 59 isolates used for database creation. Cluster analysis showed main spectrum profile of all the 59 isolates belongs to 26 species of melanized fungi.

Table 1. Species-wise distribution of isolates across various MALDI log scores for validation set by existing Bruker database plus in-house database.

S. No.	Identification	No. of Isolates	1.7–1.99	2.0–3.0
1	*Acrophialophora fusispora*	6	2	4
2	*Alternaria alternata*	5	2	3
3	*Alternaria tenuissima*	4	1	3
4	*Bipolaris australiensis*	3	3	0
5	*Bipolaris hawaiiensis*	2	1	1
6	*Bipolaris spicifera*	5	2	3
7	*Chaetomium globosum*	6	0	6
8	*Cladophialophora bantiana*	6	3	3
9	*Cladophialophora carrionii*	4	1	3
10	*Cladosporium cladosporioides*	4	1	3
11	*Cladosporium sphaerospermum*	3	0	3
12	*Cladosporium spp.*	1	0	1
13	*Colletotrichum truncatum*	3	1	2
14	*Curvularia pallescens*	2	1	1
15	*Curvularia lunata*	2	2	0
16	*Epicoccum nigrum*	4	1	3
17	*Exophiala jeanselmei*	4	2	2
18	*Exserohilum rostratum*	6	1	5
19	*Fonsecaea pedrosoi*	10	4	6
20	*Humicola fuscoatra*	2	1	1
21	*Lasiodiplodia theobromae*	4	1	3
22	*Medicopsis romeroi*	3	2	1
23	*Nigrospora sphaerica*	7	2	5
24	*Papulaspora equi*	5	4	1
25	*Phialophora verrucosa*	4	2	2
26	*Rhytidhysteron rufulum*	2	0	2
27	*Roussoella percutanea*	2	0	2
28	*Scytalidium lignicola*	5	1	4
29	*Ulocladium spp.*	3	1	2
	Total	117	42	75

The accuracy of MALDI-TOF MS and DNA sequencing-based identification was checked before and after in-house database creation. The overall accuracy of the MALDI-TOF MS was comparable to DNA sequencing. The accuracy of the MALDI-TOF MS was 100% after expansion of the database (**Figure 4**).

4. Discussion

The advent of advanced diagnostic techniques and altered fungal epidemiology, the number of pathogenic fungal species are rapidly increasing with time.[1–6] Melanized fungi, once considered as a plant pathogen, are now known to infect human beings. These infections can occur in both immunosuppressed and competent individuals with high morbidity and mortality.[1–3,6,7] More than 100 species of melanized fungi are known to cause infection in a human host.[1,3] The reliable and rapid identification is very crucial for proper management of the patients infected with melanized fungi. The European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and European Confederation of Medical Mycology (ECMM) recommended histopathology, culture and sequencing for accurate species identification, especially for unusual or newly described pathogens.[9] However, the conventional and molecular (DNA based) methods of diagnosis have several limitations.[2,10,11,13,14] The conventional methods of fungal identification depend on sporulation, whereas many melanized fungi grow very slowly and never sporulate in artificial media.[2,4,12] In this study, *Epicoccum nigrum*, *Lasiodiplodia theobromae*, *Medicopsis romeroi*, *Rhytidhysteron rufulum*, *Roussoella percutanea* failed to sporulates. Although PCR based sequencing is the gold standard for the identification, sometimes pigmented DNA (melanin) inhibits the amplification and limited sequence database may reduce the efficiency of identification of melanized fungi.[4,18] The PCR inhibition by pigmented DNA was also observed in this study. The turnaround time (TAT) of DNA sequencing-based identification required 5–9 days depending upon the growth. In the recent past, MALDI-TOF MS emerged as a reliable and rapid diagnostic technique in microbiology. We identified all the clinical isolates of melanized fungi by MALDI-TOF MS within 3–7 days.[16,17]

In the past few decades, several case reports have been published on invasive fungal infections caused by melanized fungi.[5,19,22–25] However, extensive multicentric studies containing a large number of clinical isolates identified by MALDI was only published by Singh et al.[4] Compared to Singh et al. included 14 genus and 20 species, we used 20 genus and 26 species of melanized fungi to create the in-house database. In addition, while Singh et al. included 72 isolates, 117 isolates were used in this study for the validation of the in-house database.[4] They were isolated from a wide range of infections such as fungal corneal ulcer, fungal sinusitis, chronic obstructive pulmonary disease, chromoblastomycosis, onychomycosis, brain abscess, transplant infections, and skin infections. Moreover, some rare human pathogen including *Colletotrichum truncatum*, *Epicoccum nigrum*, *Fonsecaea pedrosoi*, *Humicola fuscoatra*, *Nigrospora sphaerica*, *Papulaspora equi*, *Phialophora verrucosa*, *Roussoella percutanea*, and *Ulocladium spp*. were included for the first time in the MALDI database. The addition of a large number of rare melanized fungi in the MALDI database will further contribute for the reliable identification.

The major problems with the MALDI-TOF MS based identification of filamentous fungi are the lack of uniform standardized protein extraction protocol and limited database availability.[4,18] Multiple MALDI protocol exists regarding protein extraction of moulds including the fungal growth spotted directly on the plate, growth from solid and liquid media with off plate extraction.[16–18] Among these, the solid and liquid media method had maximum precision and accuracy.[18] However, production of pigment in filamentous fungi is a concern with solid media method, and the liquid media method is time consuming.[18,26] The previous study and Bruker recommended the liquid media method for filamentous fungi, but the limitation of this method is a longer turnaround time.[18,26] Melanized fungi are "slow grower" and the liquid method need subculturing from solid agar, which further delays identification time.[1–3,26] Therefore, to reduce the turnaround time, solid media method was used in this study.

Table 2. Performance of MALDI-TOF MS in comparison to DNA Sequencing.

S. No.	Species	No. of isolates	Correctly identified	AUC (95% CI)	ROC	Kappa (95% CI)
1	*Acrophialophora fusispora*	6	6	1.00 (0.99–1.00)	≥1.74	1.00(1.00–1.00)
2	*Alternaria alternata*	5	5	1.00 (0.99–1.00)	≥1.79	1.00(1.00–1.00)
3	*Alternaria tenuissima*	4	4	1.00 (0.99–1.00)	≥1.81	1.00(1.00–1.00)
4	*Bipolaris spicifera*	5	5	1.00 (0.99–1.00)	≥1.83	1.00(1.00–1.00)
5	*Chaetomium globosum*	6	6	1.00 (0.99–1.00)	≥1.86	1.00(1.00–1.00)
6	*Cladophialophora bantiana*	6	6	1.00 (0.99–1.00)	≥1.76	1.00(1.00–1.00)
7	*Cladophialophora carrionii*	4	4	1.00 (0.99–1.00)	≥1.83	1.00(1.00–1.00)
8	*Cladosporium cladosporioides*	4	4	1.00 (0.99–1.00)	≥1.81	1.00(1.00–1.00)
9	*Epicoccum nigrum*	4	4	1.00 (0.99–1.00)	≥1.78	1.00(1.00–1.00)
10	*Exophiala jeanselmei*	4	4	1.00 (0.99–1.00)	≥1.82	1.00(1.00–1.00)
11	*Exserohilum rostratum*	6	6	1.00 (0.99–1.00)	≥1.84	1.00(1.00–1.00)
12	*Fonsecaea pedrosoi*	10	10	1.00 (0.99–1.00)	≥1.78	1.00(1.00–1.00)
13	*Lasiodiplodia theobromae*	4	4	1.00 (0.99–1.00)	≥1.75	1.00(1.00–1.00)
14	*Nigrospora sphaerica*	7	7	1.00 (0.99–1.00)	≥1.79	1.00(1.00–1.00)
15	*Papulaspora equi*	5	5	1.00 (0.99–1.00)	≥1.77	1.00(1.00–1.00)
16	*Phialophora verrucosa*	4	4	1.00 (0.99–1.00)	≥1.77	1.00(1.00-1.00)
17	*Scytalidium lignicola*	5	5	1.00 (0.99–1.00)	≥1.83	1.00(1.00–1.00)

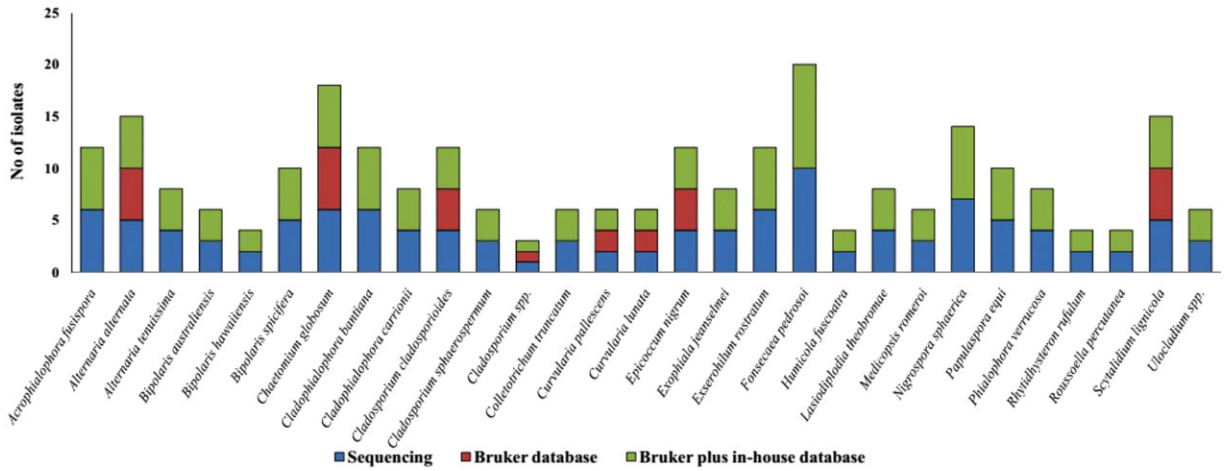

Figure 4. Comparison of MALDI-TOF MS with DNA sequencing before and after database expansion. A stacked column chart depicting the species-specific distribution and performance of MALDI-TOF MS using existing Bruker database (middle column), DNA sequencing (lower column), and MALDI-TOF MS using Bruker plus in-house database (upper column).

The efficiency of MALDI-TOF MS based identification also depends on the availability of an extensive database.[16–18] The under-representation of melanized fungi in MALDI-TOF MS database is one of the limitations. Singh et al. could identify only six species by the existing Bruker database.[4] Similarly, only eight species were identified by existing Bruker database in this study but we enriched the database for melanized fungi with 21 new species.

Supplementation of Bruker database with our in-house database resulted in the identification of all melanized fungi. Identification log score was greater than 1.7 for all isolates. Although Bruker recommended log score ≥ 2.0 for species-level identification, few isolates in this study had log score < 2, though their identifications were confirmed up to species level. This indicates a log score <2 may not be excluded up to species-level identification. A similar finding was also reported in previous studies.[16–18]

With changing fungal epidemiology, many melanized fungi, which have never been reported before, are emerging as human pathogens. The taxonomy is also changing with the advancement of molecular diagnostic techniques. This study showed that MALDI-TOF MS is an accurate, reliable, and rapid diagnostic technique for the identification of emerging clinically important melanized fungi.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

main spectra profile, MALDI-TOF MS, melanized fungi

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