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Review article

Fungal identification based on the polyphasic approach: a clinical practice guideline

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Abstract

Taxonomy includes classification, nomenclature, and identification. Identification assigns unknown fungi to species based on their strain characteristics. Traditionally, fungal taxonomy relied on morphological, physiological, and biochemical traits. However, advancements in molecular phylogeny, especially multilocus sequence analysis (MLSA), have revolutionized fungal taxonomy. MLSA combines phylogenetic and genetic approaches. Although effective, MLSA may not fully reflect biodiversity or distinguish between closely related species. Polyphasic taxonomy integrates genotypic, phylogenetic, chemotaxonomic, and phenotypic data into a consensus classification system. Polyphasic taxonomy was first applied to *Rhodotorula glutinis* in 2001 and is now widely accepted. Phenotypic traits, such as protein profiles and chemotaxonomic markers, analyzed using techniques such as matrix-assisted laser desorption ionization-time of flight mass spectrometry, are effective for yeast and filamentous fungi. Genotypic data from DNA/RNA sequencing, compared with data from databases such as Index Fungorum and MycoBank, aids species identification and synonym verification. Despite its practicality, the polyphasic approach lacks strict guidelines, resulting in varied interpretations.

Key words: Fungi, Matrix-assisted laser desorption-ionization mass spectrometry, Multilocus sequence analysis, Phenotype, Phylogeny, Taxonomy

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Introduction

Traditionally, taxonomy comprises classification, nomenclature, and identification. Classification is defined as the systematic arrangement of organisms into taxonomic groups based on similarity, whereas nomenclature is the process of assigning names to organisms. Identification is defined as the process of accurately determining the species of an unknown fungus based on strain characterization [1]. Historically, fungal identification relied primarily on morphological, physiological, and biochemical traits. However, over the past two decades, advances in molecular phylogeny have profoundly influenced fungal taxonomy. Multilocus sequence analysis (MLSA) combined with phylogenetic analysis is one of the most practical, rational, and widely adopted methods for fungal identification in current taxonomic practice [2].

However, the complexities of fungal classification are addressed using a polyphasic taxonomic approach, which integrates multiple data types and methodologies. While molecular data play a crucial role, they are not always sufficient to provide clear biodiversity patterns or ensure the accurate identification of

taxa. Thus, a more holistic approach to studying biodiversity is warranted. Polyphasic taxonomy, which emerged in bacteriology in the 1970s [3], was first applied to fungal taxonomy in 2001, specifically to the basidiomycetous yeast *Rhodotorula glutinis* [4]. Over the past 15 years, polyphasic taxonomy has been widely used in numerous studies, with molecular phylogenetic analysis often serving as the foundation [5].

Polyphasic taxonomy has been integrated into a consensus classification system using all available genotypic, phylogenetic, chemotaxonomic, and phenotypic information. This approach plays a vital role in characterizing microorganisms with unique properties, such as low morphological complexity and issues with sexual reproduction or self-fertilization [5].

This review aims to describe the development of the polyphasic approach and its modern application in clinical mycology (Table 1).

Table 1. Key approaches in the polyphasic fungal identification

Categories	Contents
Phenotypic-based approach	
Morphological characteristics	- Sporulation organ structures (e.g., conidia, macroconidia). - Colony traits: growth rate, aerial mycelium, odor. - Cultivation conditions (e.g., medium composition, temperature) - Microscopy: with/without staining. DIC microscopy
Biochemical and physiological characteristics	- Chemotaxonomic markers (e.g., polysaccharides, fatty acids). - Metabolite profiling (chromatography). - Protein fingerprints - Multilocus enzyme electrophoresis - Isoenzyme analysis. - Metabolite profiling - Complementary markers (e.g., carbohydrate assimilation, colony color, growth rate)
Parasitism and mating ability	- Pathogenicity, aggressiveness, specialization (geography, ecology) - Mating via sexual offspring.
MALDI-TOF MS	- Reliable for identifying *Candida*, and *Cryptococcus* spp . - Accuracy varies for molds. - Limited database sizes, cross-identification issues, varying cultivation conditions, and inadequate QC strategies.
Molecular-based approach	
Genetic markers and database	- ITS regions, rDNA regions. - Multilocus sequence analysis - GenBank, CBS-KNAW, ISHAM ITS, MycoBank
Analytical tools	- RNA-Seq-based next-generation sequencing - PCR-based methods (RT-PCR, nested PCR). - DNA/RNA probes (e.g., FISH, Northern blot). - Post-amplification methods (Microarray) - Isothermal amplification methods (e.g., LAMP, NASMA) - Multigenic phylogeny
Resource for nomenclature	- MycoBank (http://www.mycobank.org) - Index Fungorum (http://www.indexfungorum.org/names /Names.asp) - the Mycology Online database (https://www.Mycology. adelaide. edu. au/) - Fungal Taxonomy (https://www.fungaltaxonomy.org/) - Clinical Fungi websites (https://www.clinicalfungi.org)

Abbreviations: DIC, differential interference contrast; MALDI-TOF-MS, matrix-assisted laser desorption/ionization-mass spectrometry; QC, quality control; ITS, internal transcribed spacer; PCR, polymerase chain reaction; RT-PCR, reverse transcription PCR; FISH, fluorescence in situ hybridization; LAMP, loop-mediated isothermal amplification;NASBA, nucleic acid sequence-based amplification

Phenotype-based approach

The phenotypic identification of fungi involves examining observable characteristics to determine species or genus. Phenotypic markers include morphological, biochemical, physiological, and biological characteristics such as life-cycle and ecological traits. These markers are the most widely used; however, their assumed relationship to fitness under natural selection remains unverified for many traits (e.g., microscopic fungi) [6].

Morphological characteristics

Morphological characteristics, including spore-forming structures and colony appearance, are widely used in fungal taxonomy. However, these traits often lack consistency without additional biochemical features or phylogenetic analyses. As culture conditions significantly influence fungal characteristics, pure cultures grown under specific media and conditions are more reliable than freeze-dried cultures. Standardized observation protocols for genera such as *Penicillium*, *Alternaria*, and *Colletotrichum* are critical for ensuring consistent and reliable analysis [5,7,8]. Traditional light microscopy often involves the use of dyes to stain cells and increase contrast under a bright-field microscope; however, staining can kill cells and distort structural features. In contrast, differential interference contrast microscopy enhances the contrast of unstained, transparent cells, thereby providing three-dimensional images while preserving natural morphology for detailed visualization [9].

Biochemical and physiological characteristics

Biochemical and physiological characteristics, such as cellular components (e.g., polysaccharides, fatty acids, and mycotoxins), secondary metabolites, protein fingerprints, multilocus enzyme electrophoresis, and isoenzyme analysis, have been prominent methods in fungal taxonomy. These traits are often affected by variability owing to culture conditions [5]. However, their relevance has diminished with the rise of molecular methods owing to limited informativeness. Metabolite profiling using chromatographic assays has been applied to classify genera such as *Fusarium*, *Alternaria*, *Aspergillus*, *Penicillium*, and *Talaromyces*. However, the unstable production of secondary metabolites reduces their reliability [10,11]. Complementary markers, such as carbohydrate assimilation, colony odor, and growth rate, have demonstrated practical value in differentiating fungal species [5].

Parasitism and mating ability

Certain fungal species, especially biotrophic fungi, exhibit a high degree of host specialization, and traits such as pathogenicity are valuable for distinguishing closely related species. Substrate-specific (phylogenetic) and organotropic adaptations may reflect the initial phases of species divergence. Experiments involving investigating pathogenic characteristics of necrotrophic and hemibiotrophic fungi require careful planning to ensure reliable results. This highlights the importance of adopting an approach to studying fungal parasitism that facilitates accurate species identification and ecological evaluation [5].

The mating ability of fungi to produce sexual offspring could serve as an ideal method for species differentiation under the biological species concept. However, the practical application of this approach in mycology is limited because of technical challenges and the inherent absence of sexual reproduction in many fungal species [5,12].

Matrix-assisted laser desorption/ionization (MALDI-TOF) mass spectrometry (MS)

MALDI-TOF MS is a reliable alternative to conventional DNA-dependent methods for identifying fungi and bacteria. Proteins within the molecular weight range of 2–20 kDa serve as biotaxonomic markers. Ribosomal proteins dominate this range, providing high signal-to-noise ratios, effective ionization, reliable spectra, and minimal interference from microbial growth conditions [13-15]. MALDI-TOF MS has been successfully applied to identify various fungi, including major yeasts such as *Candida* and *Cryptococcus* [16-18]. However, mold identification remains challenging owing to various factors, including the presence of conidia, varying stages of colony growth, melanin content, and the protocols used for sample preparation. Globally, an increasing number of companies produce commercial mass spectrometry systems, such as the MALDI Biotyper (Bruker Daltonics), VITEK MS (bioMérieux), Axima (Shimadzu), and MicroIDSys (ASTA), which are widely used for clinical fungal identification [5,13,15,19]. To support the adoption and validation of commercial MALDI-TOF MS systems, the Clinical and Laboratory Standards Institute has issued the MM18-A2 guideline document [20].

MALDI-TOF MS has demonstrated considerable accuracy in identifying yeast pathogens, including common species such as *Candida* spp., *Trichosporon spp*., *Geotrichum spp*., *Cryptococcus spp*., and *Malassezia spp*. This technique has consistently achieved approximately 100% identification accuracy for *Candida* spp., including for rare species such as *C. auris* [15, 21]. *Trichosporon* spp. were identified at the species level with 98.6% accuracy compared with that using molecular methods [22]. The identification accuracy of *Cryptococcus* spp. was 96.55%, with further improvements observed with the addition of in-house libraries [23]. Furthermore, subspecies such as *C. neoformans* var. *grubii* and *C. neoformans* var. *neoformans,* as well as molecular types within *C. gattii,* could be distinguished [24, 25]. *Malassezia* spp. have been successfully identified using direct formic acid extraction methods, enabling the identification of 14 species, including *M. furfur*, *M. globosa*, and *M. pachydermatis* [16,26,27].

For filamentous fungi, the accuracy of species-level identification varies widely from 30.2%–100% depending on the database and methodology. Culture media and incubation conditions have minimal impact on the accuracy of *Aspergillus* identification. Recent studies have reported high accuracy for *Aspergillus* spp., often between 80%–98.1% [28-31]. Enhanced identification performance has been achieved through the integration of in-house databases with commercial systems or by upgrading mold databases, thereby addressing challenges such as distinguishing closely related species, such as *A. flavus* and *A. oryzae* [30,32]. The identification rates of other molds, such as *Fusarium*, *Scedosporium*, and *Mucorales*, range from 70%–98% [30,31,33]. For *Fusarium* spp., optimized scoring thresholds and comprehensive reference spectra have yielded correct identification rates of up to 91% [33]. *Scedosporium* spp., including

S. apiospermum and *S. prolificans*, are crucial pathogens in individuals with cystic fibrosis and those who are immunocompromised—have been identified with high sensitivity and accuracy, providing results comparable with those of gene sequencing [31]. Within *Mucorales* order, which includes genera such as *Rhizopus*, *Lichtheimia*, and *Mucor*, species-level identification rates have reached 81.1% using enhanced in-house databases [34].

For basidiomycetes, identification rates using the VITEK MS PRIME substantially increased from 35.3% with the *In Vitro* Diagnostic database to 100% with the Research Use Only database [31]. The MicroIDSys Elite (ASTA) also demonstrated noteworthy results and helped identify 75.0% of basidiomycetes, including *Schizophyllum commune* [30]. Dermatophyte identification rates varied significantly (13.5%–100%) depending on the reference database and methodology. In-house databases have considerably improved accuracy, often exceeding 90%, whereas commercial libraries still lack sufficient representation of dermatophyte diversity [15]. Similarly, MALDI-TOF MS has helped effectively identify black or melanized fungi associated with a wide range of infections, particularly when in-house databases are employed. For example, *Exophiala* spp. were correctly identified at the genus and species levels with 96.8% and 90.5% accuracy, respectively [35,36]. MicroIDSys Elite also demonstrated 84.3% accuracy for dermatophytes while successfully distinguishing *Sporothrix globosa* at the species level [30].

Despite these advancements, MALDI-TOF MS has limitations. These include the size of proprietary databases, cross-identification challenges such as *Trichophyton interdigitale* vs. *T. mentagrophytes* due to overlapping peaks in their protein profiles, species variation (e.g., in genetically closely related species such as *Scedosporium apiospermum* vs. *S. aurantiacum*), issues with multiple species or contaminants, variations in solvent and matrix composition, biological variation influenced by culture conditions (e.g., growth rate, adherence to solid media), underdeveloped quality control strategies, and laboratory technique-related issues, such as smearing, uneven organism spotting, and improper plate cleaning. These limitations emphasize the ongoing need for database expansion and methodological refinement to ensure consistent and accurate identification [14,15,36].

Molecular-based approach

Genetic markers and database

The use of sequencing data has substantially advanced phylogenetic, biological, genetic, and evolutionary research, providing valuable insights into the diversity and interrelationships of various fungal groups [19,37]. DNA markers are widely used for biodiversity analysis and eliminate the need for specialized skills required for traditional identification methods. The internal transcribed spacer (ITS) region is the most widely used method for fungal species differentiation and DNA barcoding. The multicopy nature facilitates easy amplification, and the high variability renders this a reliable and convenient source of information [2]. Currently, the GenBank database contains extensive ITS sequence data from fungal genomes, offering rich resources for comparative analyses [2,38,39]. However, using ribosomal DNA (rDNA) as the sole source

for phylogenetic analysis has produced phylogenetic trees with low resolution, poor bootstrap support, or polytomies [40]. Consequently, MLSA has become the standard practice for identifying fungal species and populations [41]. Currently, ITS and other rDNA regions are primarily used for preliminary identification rather than as primary phylogenetic markers [42].

Several databases are available for effective analysis of sequencing results. GenBank remains the most widely used resource for fungal sequences despite a 20% error rate. Specialized databases, including CBS-KNAW, European Molecular Biology Laboratory, DNA Data Bank of Japan, ISHAM ITS, MycoBank, SILVA, USARIOID-ID, and UNITE, focus on fungal taxonomy, ITS sequences, and microbial data. Tools, such as SmartGene and MicroSeq, are also applied in clinical and research settings. For medically significant fungi, the CBS database and the Westmead Millennium Institute provide additional clinical mycology resources [2,5,20].

Analytical tools

DNA-based analyses offer diverse methods for species differentiation. RNA sequencing is extensively used to analyze gene expression and genome structure, providing critical insights into transcriptional activity and genomic organization. Polymerase chain reaction (PCR) based methods, including conventional PCR, nested PCR, reverse transcription PCR, real-time PCR, serial analysis of gene expression, and barcoding, are fundamental for identifying fungal species and analyzing genetic material [2,19]. DNA/RNA probe-based techniques, such as in situ hybridization, northern blotting, and fluorescence in situ hybridization, play pivotal roles in sequence-specific detection and genetic element identification [2]. Post-amplification methods such as microarray analysis enable large-scale studies of gene expression and genetic variation. Isothermal amplification methods, including rolling circle amplification, loop-mediated isothermal amplification, and nucleic acid sequence-based amplification, simplify DNA amplification by operating at constant temperatures [2,5]. While traditional methods such as amplified fragment length polymorphism PCR are effective for closely related species, their broader taxonomic applications are limited. Since 2014, next-generation sequencing (NGS) has helped identify over 300 fungal infections. The most frequently detected fungi include *Pneumocystis jirovecii* (25%), *Aspergillus* spp. (22%), *Candida* spp. (16%), and *Cryptococcus* spp. (7%). However, excluding *P. jirovecii*, traditional fungal culture methods could detect only 38% of infections, thereby highlighting the superiority of NGS over traditional methods [39].

Multigenic phylogeny provides a robust framework for analyzing genetic relationships among species using multiple genes. Methods such as genealogical concordance phylogenetic species recognition help identify incongruences in gene genealogies but have limitations for dermatophytes [2,20,43]. Coalescence-based delimitation, methods, including Poisson Tree Processes, provide probabilistic tools for defining species boundaries. Comparative genomic analysis enables comprehensive examinations of genetic content across species and enhances the understanding of species relationships and evolutionary processes [5]. Resources for nomenclature include platforms such as MycoBank, Index Fungorum, Mycology Online Database, Fungal Taxonomy, and Clinical Fungi websites [20].

The use of molecular tools for species classification remains challenging owing to the rapid discovery of over 1,300 new species annually and the reclassification of known organisms based on phylogenetic analyses. DNA sequence variations often lead to new names for known fungi or to the creation of new species, even without phenotypic differences. Although this approach is evolutionarily valuable, its complexity hampers clinical application. In practice, phenotypically similar species that respond to the same antifungal treatment are often grouped into species complexes [2,5,20].

Conclusion

Recent advancements in fungal identification have helped integrate morphological traits with chemotaxonomy, ecology, genetics, molecular biology, and phylogenetic studies [19]. The polyphasic approach incorporates diverse data types, including genotypic, phenotypic, chemotaxonomic, and phylogenetic information, into a consensus taxonomy. Techniques such as MALDI-TOF MS actively enhance phenotypic identification by aiding in analyzing protein profiles and chemotaxonomic markers and are highly effective in identifying yeast and *Aspergillus* spp. Genotypic approaches help analyze DNA and RNA sequence data, as well as compare them against established databases such as GenBank, Index Fungorum, and MycoBank to identify fungal species or discover novel species.

Despite its practicality, the polyphasic approach has limitations, including a lack of strict guidelines and variability in interpretation. These challenges highlight the need for standardized methods and comprehensive reference databases. Nevertheless, this approach is particularly valuable when phylogenetic methods are inconclusive, such as when dealing with recently diverged species or limited gene data. This approach also facilitates the development of taxonomic hypotheses, which can be validated through further phylogenetic analysis and supports the creation of robust strain collections.

Ethics statement

This was not a human population study, and thus, approval by the institutional review board and informed consent were not required.

Conflicts of interest

Jayoung Kim has been on the *Annals of Clinical Microbiology* editorial board since August 2024 and has been a guest editor for this special topic. However, she was not involved in the review process of this article. No potential conflicts of interest relevant to this article have been reported.

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None.

References

1. Carroll KC, Pfaller MA et al. eds. Manual of Clinical Microbiology. 13th ed. American Society for Microbiology; 2023: 2275-2282.
2. Persing DH, Tenover FC et al. eds. Molecular microbiology. 3rd ed. American Society for Microbiology; 2016: 489-500.
3. Colwell R. Polyphasic taxonomy of the genus Vibrio: numerical taxonomy of Vibrio cholerae, Vibrio parahaemolyticus, and related Vibrio species. J Bacteriol 1970;104:410-33.
4. Gadanho M, Sampaio J, Spencer-Martins I. Polyphasic taxonomy of the basidiomycetous yeast genus Rhodosporidium: R. azoricum sp. nov. Can J Microbiol 2001;47:213-21.
5. Gannibal PB. Polyphasic approach to fungal taxonomy. Biol Bull Rev 2022;12:18-28.
6. Title PM. eds. Bailey & Scott's Diagnostic Microbiology, 15th ed. Elsevier; 2022:791-813.
7. Frisvad JC and Samson RA. Polyphasic taxonomy of Penicillium subgenus Penicillium. A guide to identification of food and air-borne terverticillate Penicillia and their mycotoxins. Stud Mycol 2004;49:1-174.
8. Frisvad JC, Larsen TO, De Vries R, Meijer M, Houbraken J, Cabañes F, et al. Secondary metabolite profiling, growth profiles and other tools for species recognition and important Aspergillus mycotoxins. Stud Mycol 2007;59:31-7.
9. Pluta M. Nomarski's DIC microscopy: a review. Phase contrast and differential interference contrast imaging techniques and applications 1994;1846:10-25.
10. Smedsgaard J and Nielsen J. Metabolite profiling of fungi and yeast: from phenotype to metabolome by MS and informatics. J Exp Bot 2005;56:273-86.
11. Frisvad JC. Taxonomy, chemodiversity, and chemoconsistency of Aspergillus, Penicillium, and Talaromyces species. Front Microbiol 2015;5:773.
12. Saleh D, Milazzo J, Adreit H, Tharreau D, Fournier E. Asexual reproduction induces a rapid and permanent loss of sexual reproduction capacity in the rice fungal pathogen Magnaporthe oryzae: results of in vitro experimental evolution assays. BMC Evol Biol 2012;12:1-16.
13. Calderaro A and Chezzi C. MALDI-TOF MS: A Reliable Tool in the Real Life of the Clinical Microbiology Laboratory. Microorganisms 2024;12:322.
14. Robert M-G, Cornet M, Hennebique A, Rasamoelina T, Caspar Y, Pondérand L, et al. MALDI-TOF MS in a medical mycology laboratory: on stage and backstage. Microorganisms 2021;9:1283.
15. Sarvestani HK, Ramandi A, Getso MI, Razavyoon T, Javidnia J, Golrizi MB, et al. Mass spectrometry in research laboratories and clinical diagnostic: a new era in medical mycology. Braz J Microbiol 2022;53:689-707.
16. Diongue K, Kébé O, Faye M, Samb D, Diallo M, Ndiaye M, et al. MALDI-TOF MS identification of Malassezia species isolated from patients with pityriasis versicolor at the Seafarers' Medical Service in Dakar, Senegal. J Mycol Med 2018;28:590-3.
17. Fries BC, Goldman DL, Cherniak R, Ju R, Casadevall A. Phenotypic switching in Cryptococcus neoformans results in changes in cellular morphology and glucuronoxylomannan structure. Infect Immun 1999;67:6076-83.

18. Fries B, Lee S, Kennan R, Zhao W, Casadevall A, Goldman D. Phenotypic switching of Cryptococcus neoformans can produce variants that elicit increased intracranial pressure in a rat model of cryptococcal meningoencephalitis. Infect Immun 2005;73:1779-87.
19. Gautam AK, Verma RK, Avasthi S, Sushma, Bohra Y, Devadatha B, et al. Current insight into traditional and modern methods in fungal diversity estimates. J Fungi 2022;8:226.
20. Clinical Laboratory Standards Institute (CLSI). 2018. Interpretive criteria for identification of bacteria and fungi by DNA target sequencing; MM18-A2 approved guideline. CLSI, Wayne, PA.
21. Lee H, Park JH, Oh J, Cho S, Koo J, Park IC, et al. Evaluation of a new matrix-assisted laser desorption/ionization time-of-flight mass spectrometry system for the identification of yeast isolation. J Clin Lab Anal 2019;33:e22685.
22. de Almeida Jr JN, Favero Gimenes VM, Francisco EC, Machado Siqueira LP, Gonçalves de Almeida RK, Guitard J, et al. Evaluating and improving Vitek MS for identification of clinically relevant species of Trichosporon and the closely related genera Cutaneotrichosporon and Apiotrichum. J Clin Microbiol 2017;55:2439-44.
23. Zvezdanova ME, Arroyo MJ, Méndez G, Guinea J, Mancera L, Muñoz P, et al. Implementation of MALDI-TOF mass spectrometry and peak analysis: application to the discrimination of Cryptococcus neoformans species complex and their interspecies hybrids. J Fungi 2020;6:330.
24. Siqueira LPM, Gimenes VMF, de Freitas RS, Melhem MdSC, Bonfietti LX, da Silva Jr AR, et al. Evaluation of Vitek MS for Differentiation of Cryptococcus neoformans and Cryptococcus gattii Genotypes. J Clin Microbiol 2019;57:10.1128/jcm. 01282-18.
25. Buchan BW and Ledeboer NA. Advances in identification of clinical yeast isolates by use of matrix-assisted laser desorption ionization–time of flight mass spectrometry. J Clin Microbiol 2013;51:1359-66.
26. Honnavar P, Ghosh A, Paul S, Shankarnarayan S, Singh P, Dogra S, et al. Identification of Malassezia species by MALDI-TOF MS after expansion of database. Diagn Microbiol Infect Dis 2018;92:118-23.
27. Kolecka A, Khayhan K, Arabatzis M, Velegraki A, Kostrzewa M, Andersson A, et al. Efficient identification of Malassezia yeasts by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). Br J Dermatol 2014;170:332-41.
28. Vidal-Acuña MR, Ruiz-Pérez de Pipaón M, Torres-Sánchez MJ, Aznar J. Identification of clinical isolates of Aspergillus, including cryptic species, by matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). Med Mycol 2018;56:838-46.
29. Li Y, Wang H, Zhao Y-P, Xu Y-C, Hsueh P-R. Evaluation of the Bruker Biotyper matrix-assisted laser desorption/ionization time-of-flight mass spectrometry system for identification of Aspergillus species directly from growth on solid agar media. Frontiers in Front Microbiol 2017;8:1209.
30. Lee H, Oh J, Sung G-H, Koo J, Lee M-H, Lee HJ, et al. Multilaboratory evaluation of the MALDI-TOF mass spectrometry system, MicroIDSys elite, for the identification of medically important filamentous fungi. Mycopathologia 2021;186:15-26.
31. Lee H, Koo J, Oh J, Cho S-I, Lee H, Lee HJ, et al. Clinical Evaluation of VITEK MS PRIME with PICKME Pen for Bacteria and Yeasts, and RUO database for Filamentous Fungi. Microorganisms 2024;12:964.

32. Hedayati MT, Taghizadeh-Armaki M, Zarrinfar H, Hoseinnejad A, Ansari S, Abastabar M, et al. Discrimination of aspergillus flavus from Aspergillus oryzae by matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry. Mycoses 2019;62:1182-8.
33. Triest D, Stubbe D, De Cremer K, Piérard D, Normand A-C, Piarroux R, et al. Use of matrix-assisted laser desorption ionization–time of flight mass spectrometry for identification of molds of the Fusarium genus. J Clin Microbiol 2015;53:465-76.
34. Shao J, Wan Z, Li R, Yu J. Species Identification and Delineation of Pathogenic Mucorales by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry. J Clin Microbiol 2018;56.
35. Hung C-Y, Seshan KR, Yu J-J, Schaller R, Xue J, Basrur V, et al. A metalloproteinase of Coccidioides posadasii contributes to evasion of host detection. Infect Immun 2005;73:6689-703.
36. Keely SP, Cushion MT, Stringer JR. Diversity at the locus associated with transcription of a variable surface antigen of Pneumocystis carinii as an index of population structure and dynamics in infected rats. Infect Immun 2003;71:47-60.
37. Shinohara N, Woo C, Yamamoto N, Hashimoto K, Yoshida-Ohuchi H, Kawakami Y. Comparison of DNA sequencing and morphological identification techniques to characterize environmental fungal communities. Sci Rep 2021;11:2633.
38. Schlick-Steiner BC, Steiner FM, Seifert B, Stauffer C, Christian E, Crozier RH. Integrative taxonomy: a multisource approach to exploring biodiversity. Annu Rev Entomol 2010;55:421-38.
39. Tsang C-C, Teng JL, Lau SK, Woo PC. Rapid genomic diagnosis of fungal infections in the age of next-generation sequencing. J Fungi 2021;7:636.
40. Berbee ML. Loculoascomycete origins and evolution of filamentous ascomycete morphology based on 18S rRNA gene sequence data. Mol Biol Evol 1996;13:462-70.
41. Taylor JW and Fisher MC. Fungal multilocus sequence typing—it's not just for bacteria. Mol Biol Evol 2003;6:351-6.
42. Hibbett D, Abarenkov K, Kõljalg U, Öpik M, Chai B, Cole J, et al. Sequence-based classification and identification of Fungi. Mycologia 2016;108:1049-68.
43. Hilário S, Gonçalves MF, Alves A. Using genealogical concordance and coalescent-based species delimitation to assess species boundaries in the Diaporthe eres complex. J Fungi 2021;7:507.