

Misidentification of *Saprochaete clavata* as *Magnusiomyces capitatus* in Clinical Isolates: Utility of Internal Transcribed Spacer Sequencing and Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry and Importance of Reliable Databases

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***Saprochaete clavata* and *Magnusiomyces capitatus* are human pathogens that are frequently mistaken for each other due to their similar phenotypes and erroneous or limited databases. Based on internal transcribed spacer (ITS) sequences, we propose species-specific carbon assimilation patterns and matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) fingerprints that enable the identification of *S. clavata*, *M. capitatus*, and *Galactomyces candidus* to the species level.**

Saprochaete clavata (de Hoog, Smith, and Guého) de Hoog and Smith 2004 (synonym *Geotrichum clavatum* de Hoog, Smith, and Guého 1986) is an ascomycetous fungus (1, 2). Colonies are white farinose and dry and consist of true hyphae that branch at acute angles and disarticulate into arthroconidia. This species has rarely been isolated from human samples. Its ecology, reservoir, and importance in agriculture and food are unknown (3). This species is closely related to the known human pathogen *Magnusiomyces capitatus* (de Hoog, Smith, and Guého) de Hoog and Smith 2004 (previously known as *Geotrichum capitatum*) (4). *Magnusiomyces capitatus* is reported to have caused invasive infections, especially in patients with hematological malignancies (5, 6), and it

has even been involved in several outbreaks, often associated with contaminated dairy products (7–9). Initially, de Hoog et al. described the new species *G. clavatum* to distinguish strains identified as *G. capitatum* that show distinct growth on cellobiose, salicin, and arbutin (2). However, commercially available strips lack salicin and arbutin and are thus useless for obtaining an accurate identification. Furthermore, the databases that come with the automated platforms for microbial identification based on sugar assimilation patterns or mass spectrometry profiles lack this species (10) or show low discrimination for *M. capitatus* (11, 12).

From analysis of D1/D2 sequence divergence, Phaff et al. mentioned the apparent conspecificity of *G. clavatum*, *Dipodascus spicifer*, and *G. capitatum* (13). In fact, the D1/D2 sequences of the three species have 99% similarity. But, Kurtzman and Robnett considered only *G. clavatum* and *D. spicifer* to be synonymous (14). Based on internal transcribed spacer (ITS) sequence analysis, it was demonstrated that *G. clavatum* differed from *G. capitatum* (96% similarity) and that it belongs to the *Saprochaete*/*Magnusiomyces* clade; therefore, *G. clavatum* was transferred to the anamorph genus *Saprochaete* (1). Despite the use of ITS region sequencing, the majority of clinical isolates of *S. clavata* are identified as *M. capitatus* because nucleotide sequences of *S. clavata* available in public databases, such as GenBank, are either misidentified or too short (163 bp). In order to provide clues for species identification without using ITS sequencing, we analyzed phenotypic characteristics of clinical isolates identified as *Geotrichum* spp. based on profiles generated by routinely available techniques (sugar assimilation pattern [ID32C; bioMérieux] or matrix-assisted laser de-

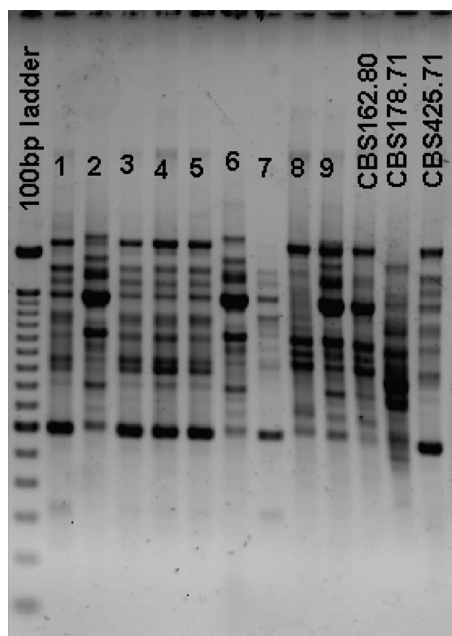


FIG 1 Examples of PCR fingerprints obtained by using M13 primer for clinical isolates of *Saprochaete clavata* (lanes 1, 3, 4, 5, and 7) and *Magnusiomyces capitatus* (lanes 2, 6, 8, and 9) and type strains of *S. clavata* (CBS 425.71), *M. capitatus* (CBS 162.80), and *Galactomyces candidus* (CBS 178.71).

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TABLE 1 ID32C profiles for the 101 *Geotrichum* sp. clinical isolates identified by ITS region sequencing

Species (no. of isolates)	No. (%) of isolates with the indicated profile	ID32C profile
<i>Magnusiomyces capitatus</i> (27)	6 (22.2)	20000100010
	6 (22.2)	32000100030
	3 (11.1)	22000100010
	3 (11.1)	22000100030
	3 (11.1)	32000100010
	2 (7.4)	30000100010
	1 (3.7)	20000100020
	1 (3.7)	22000100011
	1 (3.7)	30400100030
	1 (3.7)	32001100031
<i>Saprochaete clavata</i> (59)	23 (38.9)	30100100031
	16 (27.1)	30100100011
	9 (15.2)	32100100031
	3 (5)	30000100030
	3 (5)	30000100031
	2 (3.4)	30000100011
	2 (3.4)	30100100010
	1 (1.6)	32100100011
	<i>Galactomyces candidus</i> (15)	6 (40)
6 (40)		32003100131
2 (13.3)		32003100031
1 (6.6)		32003100150

sorption ionization–time of flight mass spectrometry [MALDI-TOF MS]).

For the 101 clinical isolates received as *Geotrichum* species since 2003 at the National Reference Center for Mycoses and Antifungals (NRCMA), purity was checked by using a chromogenic medium (BBL CHROMagar; Becton, Dickinson, USA). The urease activity (urea-indole medium; bioMérieux, Marcy l'Etoile, France) and carbon assimilation patterns (ID32C and 50CH; bioMérieux) were determined. The D1/D2 and ITS1–5.8S–ITS2

regions of the ribosomal DNA were sequenced by using universal primers (NL1/NL4 [15] and V9D/LS266 [16, 17], respectively). The sequences of the ITS1–5.8S–ITS2 regions were delimited by the sequences of the primers ITS1 and ITS4 (TCCGTAGGTGAA CCTGCGG and GCATATCAATAAGCGGAGGA, respectively), and the sequences were compared with the nucleotide sequences of the *S. clavata* CBS 425.71 type strain (GenBank accession number KF984489), the *M. capitatus* CBS 162.80 type strain (accession number KF984490), and the *Galactomyces candidus* (teleomorph of *Geotrichum candidum*) CBS 178.71 type strain (accession number KF984491). For each clinical isolate and type strain, a PCR fingerprinting technique was performed with the core sequence of phage M13 (5'-GAGGGTGGCGGTTCT-3') and OPE4 (5'-GTG ACATGCC-3') as a single primer (7, 18). For 19 clinical isolates (15 *S. clavata*, 4 *M. capitatus*) and the 3 type strains, MALDI-TOF fingerprints were obtained using mass spectrometry technology on the Vitek MS automate (bioMérieux) after 24 h and after 48 h of growth on malt extract agar plates with gentamicin and chloramphenicol (Merck) and on Sabouraud agar slants with gentamicin and chloramphenicol (Bio-Rad), and they were analyzed using the Vitek MS version 2.0 reference strain database.

Based on ITS region sequencing, 59/101 isolates were eventually identified as *S. clavata*, 27 as *M. capitatus*, and 15 as *G. candidus*. The delimited sequences of ITS and D1/D2 regions between *S. clavata* (464 bp) and *M. capitatus* (454 bp) have 96% and 99% similarity, respectively. Random amplified polymorphic DNA (RAPD) analysis suggests that species-specific fingerprints may be obtained (Fig. 1). This technique, however, cannot be envisioned as a routine means for species identification. Comparison of ID32C profiles allowed for the differentiation of species-specific carbon assimilation profiles (Table 1), with 10 codes specific for *M. capitatus*, 8 for *S. clavata*, and 4 for *G. candidus*. Using the Vitek MS and its corresponding database, the identifications were confirmed for the 4 clinical isolates and the type strain of *M. capitatus*, but no identifications were obtained for the other 17 isolates in the v2.0 database. However, when analyzing MALDI-TOF MS pro-

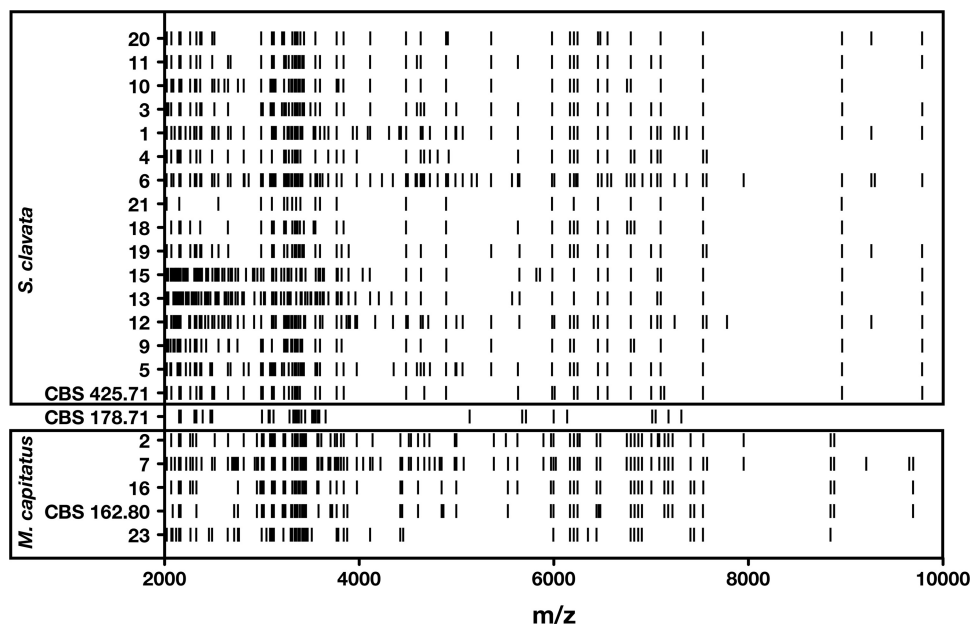


FIG 2 Raw MALDI-TOF MS spectra for 19 clinical isolates and the type strains CBS 425.71 (*Saprochaete clavata*), CBS 178.71 (*Galactomyces candidus*), and CBS 162.80 (*Magnusiomyces capitatus*) after 24-h subculture on 2% malt dextrose agar plates plus gentamicin and chloramphenicol.

files of the 22 isolates, three groups corresponding to the three species can be delineated based on specific peaks (Fig. 2).

These results underline the importance of using specialized databases, such as that of the Centraalbureau voor Schimmelcultures (CBS) (see <http://www.cbs.knaw.nl/collections/BioloMICSSequences.aspx?file=all>), where the taxonomy is more reliable than that in public repositories, as pointed out by Nilsson and colleagues (19). It also shows the importance of incrementing databases according to the latest developments of fungal taxonomy.

Nucleotide sequence accession numbers. Newly determined sequence data from this study have been deposited in GenBank under accession numbers [KF984489](#), [KF984490](#), and [KF984491](#).

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