48 *Chaetomium*

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CONTENTS

48.1 INTRODUCTION

48.1.1 Classification and Morphology

The genus *Chaetomium* consists of dematiaceous (dark-walled) fungi classified in the family Chaetomiaceae, order Sordariales, class *Sordariomycetes*, subphylum Pezizomycotina, phylum Ascomycota, and kingdom Fungi. Of the 35 recognized and 69 unassigned species within the genus, the following are most common: *Chaetomium atrobrunneum* (obsolete synonym: *C. fusisporale* and *C. rectopilium*), *Chaetomium funicola*, *Chaetomium globosum* (obsolete synonyms: *C. caprophilum* and *C. cinnamomeum*), *Chaetomium perlucidum*, *Chaetomium piluliferum* (a teleomorph of *Botryotrichum piluliferum*), and *Chaetomium strumarium* (obsolete synonyms: *Achaetomium cristalliferum*, *Achaetomium strumarium*, *C. spinulosum*, and *C. sulphureum*) [1,2].

Chaetomium species are saprobic ascomycetes with worldwide distribution and are commonly found in dung, straw, paper, bird feathers, seeds, plant debris, soil, and air. Some *Chaetomium* spp. are thermophilic and neurotropic in nature and are among the fungi causing phaeohyphomycosis in humans, with clinical presentations ranging from brain abscess, peritonitis, cutaneous lesions, onychomycosis, to fatal deep mycoses.

Chaetomium species grow rapidly at between 25°C and 35°C, with some species responsible for invasive human disease growing well at 35°C–37°C, and others showing a predilection for the central nervous system growing at up to 42°C–45°C. *Chaetomium* colonies are cottony and white in color initially and become gray to olive with age. The colony reverse is tan to red or brown to black. Hyphae are septate. Perithecia are large, dark brown to black, fragile, and globose to flask shaped and have filamentous, hair-like, brown to black appendages (setae) on their surface. Perithecia have ostioles (small-rounded openings) and contain asci and ascospores inside. Asci are clavate to cylindrical in shape and rapidly dissolve to release their ascospores (4 to 8 in number). Ascospores are one celled, olive brown, and lemon shaped [2].

Features fundamental to the identification of *Chaetomium* species include the presence of hairs or setae covering the ascomata, the size and shape of ascospores, and the presence and position of germ pores. Among the similar *Chaetomium* species (i.e., *C. gangligerum*, *C. raii*, *C. jodhpurense*, and *C. fusisporum*), *C. gangligerum* produces broader ascospores (12–15 µm × 7.5–9.5 µm) and usually forms chlamydospores; *C. raii* has smaller ascospores (10–13 µm × 5.5–7.5 µm), and *C. jodhpurense* and *C. fusisporum* have larger ascospores (14–19 µm × 6–8 µm and 14–17 × 7–8 µm, respectively).

C. strumarium is distinguished by its formation of ascocarps covered with pale, thin-walled, flexuous hairs, a feature contributing to its original placement in the genus *Achaetomium*. Presence of pinkish exudate droplets and/or crystals associated with hyphae or ascocarps, good growth at 42°C, and production of small conidia further separate *C. strumarium* from other species in the *Chaetomium* genus [3].

Furthermore, *C. perlucidum* produces globose (spherical) to subglobose or ovoid ascomata (108–220 µm × 90–200 µm), with undulate hairs and a wide ostiole (30–50 µm in diameter) and eight-spored asci (20–40 µm × 7–18 µm). The ascospores (12.0–15.0 µm × 6–7.5 µm) are elliptical and olive brown and contain a subapical germ pore. These features help differentiate *C. perlucidum* from other invasive species of *Chaetomium* [4].

C. globosum is distinguished by its lack of growth at elevated temperatures (<35°C) and its generation of lemon-shaped ascospores.

48.1.2 Clinical Features

Despite being saprophytic ascomycetes with only occasional involvement in human disease processes, *Chaetomium* species are capable of inducing a broad spectrum of mycoses including onychomycosis, sinusitis, empyema, pneumonia, and fatal disseminated cerebral disease, especially in immunocompromised patients and intravenous drug users [5–9].

Chaetomium atrobrunneum is a notably invasive, neurotropic species, and its ability to grow at elevated temperatures may contribute to its neurotropism [10–13]. Thomas et al. [9] described a case of fatal brain abscess due to *C. atrobrunneum* in a bone marrow transplant patient. The rapid progression of cerebral infection indicates that the brain tissue provides a favorable environment for growth and proliferation of the fungus.

Chaetomium strumarium is another invasive, neurotropic species. Abbott et al. [3] reported three *C. strumarium*-related cases of fatal cerebral mycosis in males with prior histories of intravenous drug use from the United States and Australia. *C. strumarium* was detected by histopathology and isolated from the brain tissue.

Chaetomium perlucidum is recently confirmed as a neurotropic species. Barron et al. [4] documented the first two cases of invasive human mycoses caused by this phaeoid ascomycete. The first case concerned a 45-year-old female patient with acute myelogenous leukemia, who had an unrelated, 4/5 HLA-matched umbilical cord blood transplant. The patient became disoriented and febrile, and computed tomography of the chest revealed a 3 × 2 cm mass in the right lower lobe. After suffering a massive right-sided intraparenchymal hemorrhage, the patient died. Autopsy revealed disseminated invasive fungal infection in the lungs, brain, and myocardium; and cultures from the surgically obtained lung tissue yielded *C. perlucidum*. The second case involved a 78-year-old female with a history of asthma and chronic bronchiectasis. The patient underwent a lobectomy due to worsening symptoms, and cultures from the lung tissue grew *C. perlucidum*. The patient showed no further manifestations of disease after the lobectomy.

Chaetomium globosum is an occasional agent of onychomycosis [14,15]. In addition, Teixeira et al. [16] reported that *C. globosum* was responsible for a systemic infection with enlargement of the axillary and cervical lymph nodes in a chronic myeloid leukemia patient, who underwent an allogeneic sibling-matched bone marrow transplant. Culture of the aspirates from both lymph nodes resulted in the growth of *C. globosum*. The infection was successfully treated with amphotericin B.

48.1.3 Diagnosis

Considerable similarities exist between mycosis caused by *Chaetomium* and *Aspergillus* from radiographical and histopathological standpoints. A melanin-specific stain (i.e., the Masson-Fontana stain) is helpful reputedly for distinguishing the melanin-containing *Chaetomium* from most *Aspergillus* species. However, this is contradicted by reports of melanin from *Aspergillus* (e.g., *Aspergillus fumigatus*) [17], and so the information obtained may have limited value in differentiating the causal fungi.

In vitro culture techniques offer a slow but valuable way to isolate and propagate *Chaetomium* organisms for subsequent macroscopic and microscopic characterization. Inoculation of sterilized plant material with ascospore suspension may enhance induction of mature perithecia, leading to the production of well-developed ascomata on the surface of the substrate.

Molecular methods such as (i) PCR and (ii) sequencing analysis of rRNA and internal transcribed spacer (ITS) regions provide an approach for the rapid and accurate identification of *Chaetomium* species from other fungi.

48.2 METHODS

48.2.1 Sample Preparation

Clinical specimens are examined by microscopy with a melanin-specific stain (i.e., the Masson-Fontana stain). However, as mentioned above, a melanin stain may not be particularly useful. Portions of the samples are inoculated on Sabouraud glucose agar with or without antibiotics. The resulting isolates are identified on the basis of macroscopical and microscopical features.

For DNA extraction, a small amount of fungal pellet is suspended in 600 µL extraction buffer (200 mM Tris–HCl, pH 7.5, 25 mM EDTA, 0.5% w/v sodium dodecyl sulfate [SDS], and 250 mM NaCl) and vortexed for 15 s. The mixture is then incubated at 100°C for 15 min, chilled on ice for 60 min, and centrifuged at 13,000 rpm for 15 min. The supernatant is transferred to a new tube, and the solution is extracted with phenol–chloroform–isoamyl alcohol (25:24:1 v/v). DNA is precipitated with cold isopropanol (–20°C), air dried, and resuspended in 100 µL distilled water.

48.2.2 Detection Procedures

Arzanlou et al. [18] utilized the universal primers ITS1 and ITS4 to amplify the ITS region of the nuclear ribosomal RNA operon, including the 3′ end of the 18S rRNA gene, the first ITS region (ITS1), the 5.8S rRNA gene, the second ITS region (ITS2), and the 5′ end of 28S rRNA gene. Subsequent sequencing analysis allows identification of fungal organisms including *Chaetomium* species.

Procedure

1. PCR mixture (25 µL) is composed of 0.5 U *Taq* polymerase (Bioline), 1× PCR buffer, 0.5 mM $MgCl_2$, 0.2 mM of each dNTP, 5 pmol of each primer, and approximately 10–15 ng of fungal genomic DNA.
2. Amplification is performed on a GeneAmp PCR System 9700 (Applied Biosystems) with primary denaturation at 96°C for 5 min; 36 cycles of 96°C for

30 s, 52°C for 30 s, and 72°C for 60 s; a final extension at 72°C for 7 min.

3. The amplicons are sequenced using BigDye Terminator v. 3.1 (Applied Biosystems,) or DYEnamicET Terminator (Amersham Biosciences) Cycle Sequencing Kits and analyzed on an ABI Prism 3700 (Applied Biosystems).
4. Newly generated sequences are subjected to a Blast search of the NCBI databases, sequences with high similarity are downloaded from GenBank, and comparisons are made on the basis of the alignment of the obtained sequences.
5. Phylogenetic analysis is performed with PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 using the neighbor-joining algorithm with the uncorrected ("p"), the Kimura 2-parameter, and the HKY85 substitution models. Alignment gaps longer than 10 bases are coded as single events for the phylogenetic analyses; the remaining gaps are treated as missing data. Any ties are broken randomly when encountered.
6. Phylogenetic relationships are also inferred with the parsimony algorithm using the heuristic search option with simple taxon additions and tree bisection and reconstruction (TBR) as the branch-swapping algorithm; alignment gaps are treated as a fifth character state and all characters are unordered and of equal weight. Branches of zero length are collapsed and all multiple, equally parsimonious trees are saved.
7. Other measures calculated include tree length, consistency index, retention index, and rescaled consistency index (TL, CI, RI, and RC, respectively). The robustness of the obtained trees is evaluated by 1000 bootstrap replications. Bayesian analysis is performed. The best nucleotide substitution model is determined using MrModeltest v. 2.2. MrBayes v. 3.1.2 is used to perform phylogenetic analyses, using a general time-reversible (GTR) substitution model with inverse gamma rates, dirichlet base frequencies, and the temp value set to 0.5.

Note. Part of the large-subunit 28S rRNA (LSU) gene may be also amplified with primers LR0R and LR5 followed by sequencing analysis.

48.3 CONCLUSION

The genus *Chaetomium* consists of a large group of dematiaceous (dark-walled) fungi that are present in a variety of environments, including dung, straw, paper, bird feathers, seeds, plant debris, soil, and air. Several *Chaetomium* spp. are opportunistic human pathogens, causing phaeohyphomycosis, with clinical manifestations of brain abscess, peritonitis, cutaneous lesions, onychomycosis, and fatal deep mycoses. Since conventional methods for identification of *Chaetomium* spp. require lengthy incubation and demand specialized skill, molecular procedures have been increasingly applied for culture-independent detection and differentiation of *Chaetomium* spp. The use of melanin stains alone may be inconclusive.

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