25 Chrysosporium

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25.1 INTRODUCTION

25.1.1 CLASSIFICATION AND MORPHOLOGY

The genus Chrysosporium (obsolete synonym: Glenospora) belongs to the mitosporic Onygenales group, order Onygenales, class Eurotiomycetes, subphylum Pezizomycotina, phylum Ascomycota, and kingdom Fungi. The mitosporic Onygenales group consists of nine genera: Blastomyces, Chrysosporium, Coccidioides, Emmonsiis, Geomyces, Locaszia, Malbranchea, Myriodontium, and Paracoccidioides. In turn, the genus Chrysosporium is separated into 28 recognized species: Chrysosporium articulatum, Chrysosporium carmichaelii, Chrysosporium chriptopororum, Chrysosporium evolceanui, Chrysosporium filiforme, Chrysosporium fluviale, Chrysosporium flavia, Chrysosporium indicum, Chrysosporium keratinophilum, Chrysosporium lobatum, Chrysosporium lucknowense, Chrysosporium mephiticum, Chrysosporium merdarium, Chrysosporium minutisporum, Chrysosporium ophiodicola, Chrysosporium pannicola, Chrysosporium pilosum, Chrysosporium pseudomerdarium, Chrysosporium queenslandicum, Chrysosporium siglerae, Chrysosporium submersum, Chrysosporium sulphureum, Chrysosporium sychronum, Chrysosporium tropicum, Chrysosporium undulatum, Chrysosporium vallenarensce, Chrysosporium xerophilum, and Chrysosporium zonatum, and 18 unassigned species [1–3]. The telemorphs of Chrysosporium spp. are found in the genera Aphanoascus, Nannizziosis, and Uncinocarpus, family Onygenaceae, and order Onygenales [4,5].

The obsolete species in the genus include Chrysosporium dermatitidis (→ Blastomyces dermatitidis), Chrysosporium pannorum (→ Geomyces pannorum), Chrysosporium pruinosum (→ Sporotrichum pruinum), and Chrysosporium thermophilum (→ Myceliophthora thermophila) [6]. Not discussed here are Chrysosporium parvum (or Chrysosporium parvum var. parvum) and Chrysosporium parvum var. crescents, which are considered as synonymous for Emmonsiis parva and Emmonsiis crescents, respectively. To simplify the presentation and reduce confusion, this chapter focuses on Chrysosporium spp. that are keratinophilic, causing subcutaneous infections, whereas Chapter 29 deals with Emmonsiis spp. that mainly cause pulmonary adiaspiromycosis.

Chrysosporium colonies grow moderately at 25°C, and may appear granular, woody, or cottony, flat, or raised, and folded. Colonies are white cream, yellow, or tan to pale brown on the front, and white to brown on the reverse. Hyphae are septate. Conidia (aleurionidioidea) are hyaline, broad based, one celled, and smooth or rough walled. Conidia are broader than vegetative hyphae and occur terminally on pedicels, along the sides of the hyphae, or in intercalary positions. Conidia usually have an annular frill that is the remnant of the hyphal wall that remains after detachment from the hypha. Arthroconidia are abundant and larger than parent hyphae in diameter.

C. tropicum colonies are moderately fast growing, flat, white to tan to beige, often with a powdery or granular surface texture. Reverse pigment is absent or pale brownish yellow with age. Ameroconidia are hyaline, one celled, and produced directly on vegetative hyphae by nonspecialized conidiogenous cells. Conidia (6–7μm×3.5–4μm) are typically pyriform to clavate with truncate bases and are formed either intercalary (arthroconidia), laterally (often on pedicels), or terminally.

Chrysosporium zonatum (synonym: C. gouri; telemorph: Uncinocarpus orisii) colonies on potato dextrose agar (PDA) at 37°C are flat and coarsely powdery, and appear yellowish white initially but darken by 14–21 days to buff
(grayish or brownish orange) with a light brown reverse. Colony topography and color on PDA are similar at 37°C and 25°C, but darkening of the colony obverse and growth rate are slightly faster at 37°C (73 mm in diameter after 14 days) than at 25°C (66 mm in diameter). Microscopically, the fungus forms solitary aleuroconidia that are borne at the ends of short, typically curved stalks or that are sessile (borne on the sides of the hyphae). Conidia (3.5–13 μm × 2.5–5 μm) are single celled, rarely two celled, smooth to slightly roughened, and clavate (club shaped) to broadly obovoid (egg shaped) and have a rounded tip and a broad, flat basal scar. Intercalary arthroconidia may be formed but are uncommon. Racquet hyphae (hyphae showing swellings near the septa) are common [4]. The teleomorph of *Chrysosporium zonatum* is a heterothallic ascomycete *Uncinocarpus orissi* (synonyms, *Pseudoarachniotus orissi* and *Gymnoascus arxii*) (family Onygenaceae). *U. orissi* ascospores are solitary, globose, and reddish brown and are composed of pale reddish brown ascospores surrounded by thin-walled hyaline racket hyphae and conidia. Pairing of *C. zonatum* with *U. orissi* produces ascocoma-containing ascospores that are oblate (like flattened disks) with truncate ends and appear smooth to slightly pitted (punctate) [4].

*Chrysosporium ophidiicola* colonies attain diameters of 27–29 mm in 14 days at 25°C on potato carrot agar (PCA; 20 g potato, 20 g carrot, 15 g agar, 1 L water). Colonies are white from front and uncolored on reverse, felt, plane, and filbriate, with a poorly defined margin. Sparse tufts of aerial mycelium are present on the submarginal zone. Vegetative hyphae (1.5–2.5 μm wide) are hyaline, branched, septate, smooth, and thin walled. They are often disarticulated at maturity to form cylindrical arthroconidia (7.5–10 μm × 2–3 μm) adjacent to each other. Fertile hyphae arise as lateral branches. Terminal and lateral conidiose are borne on straight or flexuous side branches of variable length (4.5–16 μm) or are sometimes sessile. Conidia (4–9 μm × 2–3 μm) are unicellular, solitary, thin walled, smooth, hyaline to pale yellow, and cylindrical to slightly clavate and are released by rhexolytic dehiscence, with broad and long basal scars. Intercalary, solitary conidia are often present, similar to the terminal and lateral ones. Racquet hyphae are scarce, and chlamydospores are not observed. On potato flake agar at 23°C, colonies are white to pale yellow, with a similarly colored reverse side, are velvety to granular with age, and produce a strong, pungent odor. Conidia are borne on stalks as well as arthroconidia. On PDA (Difco Laboratories), *C. ophidiicola* grows more quickly and produces denser colonies of 31–35 mm in diameter in 14 days at 25°C. Colonies are white to pale yellow, buff after 1 month, and powdery, with droplets of colorless or light yellow exudates at the periphery. On phytone-yeast extract agar (BBL), colonies measure 32–39 mm in diameter in 14 days at 25°C and are white and light yellow at the center, powdery, and dense, with the presence of droplets of colorless exudate at the center and a light brown on the reverse side. On oatmeal agar (30 g oat flakes, 1 g MgSO₄·7H₂O, 1.5 g KH₂PO₄, 15 g agar, 1 L water), colonies are similar to those on PCA, with a very restricted growth at 15°C (5 mm in diameter in 14 days). At 37°C, there is no growth. Colonies produce a strong, pungent (skunk-like) odor after 1 month of incubation in all the media tested. The fungus shows a strong keratinolytic activity. Phenotypically, *C. ophidiicola* is separated from the *Chrysosporium* anamorph of *Nannizziopsis vriesii* by the absence of asperulate fertile hyphae and globose-to-pyriform conidia sometimes grouped in clusters and the presence of an odor in the colonies by *C. ophidiicola* [3].

*Chrysosporium* species are differentiated from each other by the texture of the colony and morphology, location, and size of the conidia. *Chrysosporium zonatum* differs from other members of the genus *Chrysosporium* by its faster growth at 37°C than at 25°C and by forming darker to buff colonies, and clavate, broadly truncate aleuroconidia typically borne on short, curved stalks. *Chrysosporium queenslandicum* (teleomorph: *Uncinocarpus queenslandicus*, or *Apinisia queenslandica* and *Brunneospora reticulata*) is similar, but its colonies do not darken, and intercalary arthroconidia are common [4].

*C. ophidiicola* is separated from *C. mephiticum* by the narrow, cylindrical-to-slightly clavate conidia of *C. ophidiicola*, and the pyriform-to-subglobose conidia of *C. mephiticum*. *C. ophidiicola* is differentiated from *A. mephitalis* by the production of teleomorph during the culture of *A. mephitalis*, and *C. ophidiicola* is distinguished from *Chrysosporium europae* by the characteristic vinaceous, buff-pigmented colonies on phytone-yeast extract agar and the absence of a strong, pungent odor of/from *C. europae*. Some species such as *Chrysosporium pannicola* do not grow at 37°C. *Chrysosporium* differs from *Blastomyces* by being nondormorphic, from *Microsporum* and *Trichophyton* by lacking macroconidia, from *Geomyces* by lacking branched, fertile hyphae on erect conidiophores, and from *Sepedonium* by having hyaline conidia.

### 25.1.2 Clinical Features

*Chrysosporium* spp. are soil saprophyes with broad distribution. They have been isolated in soil, plant material, dung, and birds [6–8]. These organisms may enter hosts through airborne conidia and exposure to soil. Many *Chrysosporium* spp. are keratinophilic filamentous fungi involved in the breakdown of shed keratinous substrates, and may cause skin infections and onychomycosis in humans [9–12]. In addition, *Chrysosporium* species have been occasionally associated with disseminated human mycosis, affecting the brain, lungs, sinuses, liver, and kidneys and leading to sinusitis, pneumonia, pleuritis, pericarditis, and osteomyelitis [13–15].

Roilides et al. [4] reported the first case of *Chrysosporium zonatum* infection in a 15-year-old boy with X-linked chronic granulomatous disease. The patient developed a lobar pneumonia and tibia osteomyelitis after a 2 month prophylactic therapy with γ-interferon. The patient
presented with pain in his right shoulder and distal part of his right tibia, infrequent cough, and fever. A computed tomography (CT) scan of the chest showed a well-demarcated large mass in the right lower lobe, enlarged left hilar lymph nodes, and lingular pneumonia. A tibia x ray was diagnostic for osteomyelitis. A biopsy of the tibia lesion revealed granulomatous tissue and a few short and thick hyphae. *Chrysosporium zonatum* was grown from sputum and biopsy specimens, which was identified by its thermotolerance, darkening colonies (yellowish white to buff) and club-shaped terminal aleurioconidia borne at the ends of short, curved stalks. Therapy with amphotericin B, itraconazole, and then liposomal amphotericin B subdued the infection started in slide culture preparations with pablum cereal agar for 14–21 days. Blocks (1 × 1 cm) of mycelium and agar from cultures of *Chrysosporium* species are excised from the culture plates and transferred to sterile snap-cap polypropylene tubes (12 mm × 75 mm; Fisher Scientific). The mycelial blocks are freeze-dried by using an Edwards Modulyo freeze-dryer. Freeze-dried blocks of agar and *Chrysosporium* mycelium (100 mg) are placed in 1.5 mL tubes and ground to a fine powder by using a 200-μL capacity pipettor tip. The fungal material is rehydrated with 500 μL of DNA extraction buffer (50 mM Tris, 10 mM EDTA, 1% sarcosyl, pH 8.0) with gentle agitation for 10 min. An equal volume of 1:1 chloroform–phenol is added to each tube, and mixed by shaking for 20 min. Then the aqueous and organic phases are separated in a microcentrifuge for 5 min at 14,000 × g. The aqueous phase is pipetted into a clean tube, and 0.1 volume of 3 M sodium acetate (pH 6.0) and 1.3 volumes of ethanol are added. The tube is sealed, and the contents are mixed by inverting the tube several times. Precipitated nucleic acids are pelleted by centrifugation at 14,000 × g for 1 min. Ethanol is decanted, and the pellet is dried by inverting the tube over absorbent paper for 5 min. Nucleic acids are dissolved in 100 μL of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0), and 250 μL of a saturated NaI solution and 10 μL of glass milk (Gene Clean kit, Bio 101) are added to the tube. The tube is inverted periodically, and DNA is adsorbed to the glass milk for 20 min. The glass milk is pelleted and rinsed, and the genomic DNA is eluted into 50 μL of 1/10-strength TE. DNA is stored at −20°C until used [18]. Alternatively, a loopful of *Chrysosporium* mycelium is transferred from SDA to a 1.5 mL tube containing 2.5 mg zymolase (Sigma–Aldrich) in 250 μL zymolase lysis buffer (10 mM Tris/HCl (pH 8.0), 1 mM EDTA) and incubated for 45 min at 37°C. Subsequently, 200 μL is transferred to another tube for DNA extraction using Qiagen kit, resulting in 100 μL of DNA extract [20].

### 25.2 METHODS

#### 25.2.1 SAMPLE PREPARATION

*Chrysosporium* and other fungal isolates are revived from either freeze-dried or frozen (vapor phase of liquid nitrogen) stock and grown at 25°C on petri plates containing pabulum cereal agar for 14–21 days. Blocks (1 × 1 cm) of mycelium and agar from cultures of *Chrysosporium* species are excised from the culture plates and transferred to sterile snap-cap polypropylene tubes (12 mm × 75 mm; Fisher Scientific). The mycelial blocks are freeze-dried by using an Edwards Modulyo freeze-dryer. Freeze-dried blocks of agar and *Chrysosporium* mycelium (100 mg) are placed in 1.5 mL tubes and ground to a fine powder by using a 200-μL capacity pipettor tip. The fungal material is rehydrated with 500 μL of DNA extraction buffer (50 mM Tris, 10 mM EDTA, 1% sarcosyl, pH 8.0) with gentle agitation for 10 min. An equal volume of 1:1 chloroform–phenol is added to each tube, and mixed by shaking for 20 min. Then the aqueous and organic phases are separated in a microcentrifuge for 5 min at 14,000 × g. The aqueous phase is pipetted into a clean tube, and 0.1 volume of 3 M sodium acetate (pH 6.0) and 1.3 volumes of ethanol are added. The tube is sealed, and the contents are mixed by inverting the tube several times. Precipitated nucleic acids are pelleted by centrifugation at 14,000 × g for 1 min. Ethanol is decanted, and the pellet is dried by inverting the tube over absorbent paper for 5 min. Nucleic acids are dissolved in 100 μL of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0), and 250 μL of a saturated NaI solution and 10 μL of glass milk (Gene Clean kit, Bio 101) are added to the tube. The tube is inverted periodically, and DNA is adsorbed to the glass milk for 20 min. The glass milk is pelleted and rinsed, and the genomic DNA is eluted into 50 μL of 1/10-strength TE. DNA is stored at −20°C until used [18]. Alternatively, a loopful of *Chrysosporium* mycelium is transferred from SDA to a 1.5 mL tube containing 2.5 mg zymolase (Sigma–Aldrich) in 250 μL zymolase lysis buffer (10 mM Tris/HCl (pH 8.0), 1 mM EDTA) and incubated for 45 min at 37°C. Subsequently, 200 μL is transferred to another tube for DNA extraction using Qiagen kit, resulting in 100 μL of DNA extract [20].

### 25.2.2 DETECTION PROCEDURES

Pounder et al. [19] described a real-time PCR with SYBR green DNA-binding dye and amplicon melting temperature analysis for fungal detection using pan-fungal primers ITSI forward (5′-TCCGTAGGTTGACCTTCGGG-3′) and ITS4 reverse (5′-TCCGCTTATTGATATGC-3′). The identity of the fungus is verified by subsequent sequencing analysis.

**Procedure**

1. PCR mixture is composed of 1× Lightcycler FastStart DNA Master Hybridization Probes mixture (Roche Applied Science) (containing deoxynucleoside
triphosphates), FastStart Taq DNA polymerase, and 1 mM MgCl₂ (additional MgCl₂ is added to a final concentration of 4.6 mM), 0.4 mM each of ITS1 forward and ITS4 reverse primers, 1x SYBR green (Molecular Probes), and 3μL template DNA.

2. Thermal cycling parameters include 95°C for 10 min; 50 cycles of 95°C for 5 s, 60°C for 20 s, and 76°C for 30 s; and a final extension at 72°C for 2 min.

3. The quality of the amplicon is determined using the derivative of the melt analysis curve (55°C–99°C, 45-s hold at 55°C, 5 s/°C) using the RotorGene 3000 (Corbett Robotics, Inc.).

4. The amplified product is purified for bidirectional sequencing using ExoSAP-IT (USB Corp.). Five microliters of Big Dye Terminator Ready Reaction Mix v. 1.1 (Applied Biosystems) is added to 4 μL of each primer (0.8 pmol/μL) and 3 μL of purified PCR product. Cycle sequencing is performed with a 9700 thermal cycler (ABI), using 25 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. Sequencing reaction products are passed through a Sephadex G-50 fine column to remove unincorporated dye terminators. Purified sequencing reaction products are run on an ABI Prism 3100 Genetic Analyzer with a 50 cm capillary array.

5. Sequences are analyzed with the SmartGene Integrated Database Network software version 3.2.3 vr. SmartGene is a web-based software and database system with reference sequences derived from the National Center for Biological Information (NCBI) GenBank repository.

Note: Sequence-based identifications are defined by percent identity: species, ≥99%; genus, 93%–99%; and inconclusive, ≤93%.

For strains producing discrepant identification between the methods based on phenotypic characteristics and ITS sequence analysis, the D1–D2 region of the large-subunit RNA gene is amplified with primers NL1 (5’-GCATATCAATAAGCGGAGGAAAAG-3’) and NL4 (5’-GGTCCGTGTTTCAAGACGG-3’) and sequenced for species clarification [3].

25.3 CONCLUSION

The genus *Chrysosporium* contains a large number of filamentous saprophytic fungi classified in the mitosporic Onygenales group, order Onygenales. The teleomorphs of the *Chrysosporium* genus are found in the genera *Aphanoascus*, *Nannizziosis*, and *Uncinocarpus*, family Onygenaceae, and order Onygenales. Many members of the genus *Chrysosporium* are thermotolerant and keratinophilic, and occasionally implicated in human diseases, producing clinical symptoms ranging from skin infections, onychomycosis, sinusitis, pneumonia, pleuritis, pericarditis, osteomyelitis, to other disseminate infections.

Microscopic observation of mycotic elements in clinical specimens and in vitro isolation of related fungal strains have been the main approaches for identification and diagnosis of *Chrysosporium* infections. More recently, molecular techniques such as PCR, sequencing, and RFLP analysis have been developed and applied for improved determination of *Chrysosporium* and other fungal organisms. Through examination of nucleotide sequences in the SSU and LSU of ribosomal RNA genes as well as ITS regions, the identity of *Chrysosporium* spp. can be rapidly and unequivocally ascertained.

REFERENCES


AUTHOR QUERIES

[AQ1] Please check if “is separated into” can be changed to “consist of” in the sentence beginning “In turn, the genus…”

[AQ2] Please check the insertion of spelled out form of PDA, that is, potato dextrose agar.

[AQ3] Please check that intergenic spacer has been replaced by internal transcribed spacer.

[AQ4] Please provide accessed date for Ref. [1].