Phaeoacremonium parasiticum Infections Confirmed by β-Tubulin
Sequence Analysis of Case Isolates

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Phaeoacremonium parasiticum is an agent of opportunistic phaeohyphomycosis belonging to a genus encompassing numerous recently described and soon-to-be-described, difficult-to-identify human pathogens. It appears in the literature to be an uncommon etiologic agent, yet we encountered several cases in a single year. Each presented problems in laboratory identification and case management. We present two cases of invasive disease with definite identification and susceptibility results. These cases are analyzed in relation to a brief review of previous cases known to have been caused by this species. Our first case involved a 40-year-old male cardiac transplant recipient with multiple localized skin lesions. The second featured a 31-year-old female with aplastic anemia and prolonged neutropenia who developed disseminated disease, with multiple positive blood cultures and skin lesions. Both patients died despite aggressive surgical and antifungal therapy. Fungal susceptibility testing showed that our isolates appeared to be susceptible to amphotericin B,itraconazole, voriconazole, ravuconazole, and posaconazole. Because phenotypic identification of Phaeoacremonium is not obligatorily problematic, sequence-based confirmation was performed using a recently proposed standard based on use of a segment of the 5′ end of the β-tubulin gene. Sequences from both isolates involved in the cases were over 99% similar to the corresponding sequence of the ex-type isolate of P. parasiticum. The close DNA similarity, corroborated by relevant morphological similarities (e.g., long, thin phialides and tuberculate hyphae bearing warts up to 3 μm high), confirms these two isolates as P. parasiticum.

Phaeoacremonium parasiticum is an unusual cause of human disease. It was first reported in 1974 as Phialoaphora parasitica causing subcutaneous tissue infection in a renal transplant recipient (1). Since the initial description, only a small number of cases have been reported in the world literature, but perhaps underreporting occurs due to incomplete or incorrect identification (20). Also, as the unpublished reference laboratory experience of one of us (R. C. Summerbell) indicates, some subcutaneous cases in otherwise healthy patients are of a nondramatic nature and definitive identification of the etiologic agents is not pursued. Identification of Phaeoacremonium species has traditionally been by morphology. However, a recent study applying modern diagnostic methods (20) to human case isolates in a large culture collection found that misidentification based upon morphology has been common in the past. Evidently, this occurred partly because Phaeoacremonium is very simple in structure with few differential characters distinguishing species and partly because some of the most common Phaeoacremonium species causing human disease were undescribed prior to 2005 and were thus invariably misidentified in case reports. Another factor was that the name Phialoaphora repens had been consistently misapplied in several published reports to refer to undescribed Phaeoacremonium species (20). Though combined molecular and morphological studies revealed new morphological characters that could be used in the reference laboratory to distinguish both previously and newly described species (20), such evaluations tended to involve some difficult judgments as well as special media. It was suggested that the optimal method for identification both at the scientific level and, where convenient, at the diagnostic level was to use sequences of selected regions of the β-tubulin gene, in addition to sufficient phenotypic characterization to corroborate the sequencing result (20). This recommendation for Phaeoacremonium resembles those made (explicitly or by implication) for several other genera of very simply structured phialidic and annellidic opportunistic fungi, such as Exophiala (4), Trichoderma (24), and Fusarium (5, 13). These are all genera where numerous new morphologically poorly distinguishing phylogenetic species causing human infection are being described and where full species identification increasingly relies on sequencing or related molecular studies. Since reliable species identification in this group, though not always immediately therapeutically essential, is vital for any detection of consistent, specific patterns related to disease progression, treatment response, or environmental sources of infectious inoculum, we employed sequence-based identification in the present cases. These are the first reported Phaeoacremonium cases where this was done as part of the clinical study.

The spectrum of disease caused by P. parasiticum is variable and ranges from subcutaneous infections to fungemia and disseminated disease (1, 8, 14). Presumably, the inciting event is traumatic implantation of the fungus, but in many reported cases, no known trauma occurred (9, 26, 27). Of cases reported to date, many involve immunocompromised patients who are organ transplant recipients (1, 6, 9, 12, 15, 27). Although good outcomes have been achieved with surgical debridement and use of the antifungals amphotericin B, azoles, and fluconosine.

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products were analyzed on an ABI Prism 3700 DNA sequencer (Perkin-Elmer, Norwalk, Conn.). A consensus sequence was computed from the forward and reverse sequences with SeqMan from the LaserGene package (DNASTar, Madison, WI). Sequences were deposited with GenBank.

The sequences obtained were compared with the \( \beta \)-tubulin sequences available from GenBank using a BLAST search. The sequences were consequently manually aligned in Sequence Alignment Editor, version 2.0a11 (23), with the ex-type sequence of \( P. \) parasiticum (CBS 860.73, GenBank accession no. AF246803), and the percentage of DNA similarity was calculated. \( P. \) parasiticum (GenBank accession no. AF246803) was also aligned with \( \beta \)-tubulin sequences of the ex-type cultures of \( Phaeoacremonium \) inflatum (GenBank accession no. AF246805) and \( Phaeoacremonium \) rubiginosum (GenBank accession no. AF246802).

**Antifungal susceptibility studies.** One \( P. \) parasiticum isolate from each of the 2 patients was available for susceptibility testing. Sources of isolates included blood and abscess fluid. Isolates were tested utilizing the Clinical Laboratory Standards Institute (previously National Committee for Clinical Laboratory Standards) broth microdilution method M-38A (2). Voriconazole (Pfizer Pharmaceutical Group, New York, NY), itraconazole (Janssen Research Foundation, Beerse, Belgium), posaconazole (Schering Plough, Kenilworth, NJ), ravuconazole (Bristol Myers Squibb), and amphotericin B (Sigma Chemical Co., St. Louis, MO) were obtained as reagent grade powders from their manufacturers, and stock solutions were prepared in dimethyl sulfoxide. All drugs were diluted in RPMI 1640 medium (Sigma Chemical Co., St. Louis, MO) buffered to pH 7.0 with morpholinepropanesulfonic acid buffer and dispensed into 96-well microdilution trays. Trays containing an aliquot of 0.1 ml in each well of appropriate drug solution (2× final concentration) were sealed and stored at \( \sim 70^\circ \)C until use. The final ranges of drug concentrations tested were 0.008 to 8 \( \mu \)g/ml for azoles and 0.016 to 16 \( \mu \)g/ml for amphotericin B. Drug-free controls were included in each tray. In brief, isolates were grown on potato dextrose agar slants at 35°C for 7 days. The slants were covered with 1 ml of sterile 0.85% saline and gently scraped with a sterile pipette. The resulting suspensions were transferred to separate tubes, and heavy particles were allowed to settle. Conidal suspensions were adjusted to optical densities ranging from 0.09 to 0.11 measured at 530 nm. The final inoculum in assay wells was between 0.5 \( \times \) 10\(^4\) and 5 \( \times \) 10\(^4\). The microdilution trays were incubated at 35°C for 72 h. MIC endpoints were determined at 48 and 72 h. The MIC endpoint was defined as the lowest drug concentration that prevented any discernible growth (optically clear) compared to that of the drug-free controls. Quality control was measured by inclusion of the following strains: \( Candida \) parapsilosis ATCC 22019 (American Type Culture Collection, Manassas, VA) and \( Candida \) keusi ATCC 6238. All readings were within the recommended limits based on CLSI methodology. The antifungal activities of amphotericin B, itraconazole, voriconazole, ravuconazole, and posaconazole against patient isolates of \( P. \) parasiticum are shown in Table 1.

**RESULTS**

**Morphology.** Case isolates had moderately rapidly growing (to 10 mm at 25°C at 7 days) unevenly grayish, progressively darkening colonies (Fig. 1E). Typical for \( P. \) parasiticum, micromorphology revealed moderately thin hyphae (3.5-\( \mu \)m in diameter) frequently adored with tuberculate warts up to 3 \( \mu \)m in length. Conidiophores (Fig. 1F) were dark and lightly encrusted, mostly bearing single subcylindrical phialides 12 to 32 \( \mu \)m long with a small, distinct, tubular collarette. Hyaline allantoid, cylindrical or long-ellipsoidal conidia, 3 to 4 \( \mu \)m by 1.5 to 2 \( \mu \)m, were produced in sticky heads. The optimal growth temperature was 25°C, and the maximum was over 37°C. All of these features suggested \( P. \) parasiticum; however, because morphological identification in \( Phaeoacremonium \) is problematic and because undescribed species are still relatively frequently encountered, sequence-based confirmation was also performed.

**DNA isolation and amplification.** In \( \beta \)-tubulin sequencing, CBS 109666 was 99.1% similar (5-base variation) and CBS 109665 was 99.3% similar (4-base variation) to the sequence of
the ex-type isolate of *P. parasiticum* over a total continuous length of 557 bases. Three of the base changes were in an exon, indicating low variation in the functional parts of the sequence. The levels of β-tubulin sequence similarity observed when distinct *Phaeoacremonium* species were compared to one another was much lower than the level observed among these *P. parasiticum* isolates. Groenewald et al. (11) compared β-tubulin sequences of *Phaeoacremonium angustius* and *Phaeoacremonium aleophilum* and found them to be 82.9% similar. Also, the similarity values recorded in comparisons of *P. parasiticum* β-tubulin sequences with those of the phylogenetically closely related *P. inflatipes* and *P. rubrigenum* were 78.4% and 83.2%, respectively. The close DNA similarity of CBS 109666 and CBS 109665 with the ex-type isolate of *P. parasiticum* thus unequivocally confirms these two case isolates as *P. parasiticum*.

FIG. 1. (A and B) Cutaneous lesions of case 1; (C) Gomori methenamine silver stain of case 1 biopsy specimen; (D) periodic acid-Schiff stain of skin biopsy specimen; (E) colony morphology of CBS 109665; (F) typical conidiophores of *Phaeoacremonium parasiticum* CBS 109665. Bar, 10 μm.
Phaeoacremonium species are mainly found in the environment in woody plants as endophytes or as agents of plant disease (20). Three species, including *P. aleophilum*, *P. argentinum*, and *P. parasiticum* have been isolated from soil (21). At least 13 species are currently recognized. As mentioned above, *P. parasiticum* is an unusual cause of human disease, first reported in 1974 as *Phialophora parasitica*, causing subcutaneous tissue infection in a renal transplant recipient (1). Since the initial description, just a small number of cases have been reported in the world literature (Table 2), but identification problems are likely to have hindered reporting (20). Combined morphological/molecular studies have shown that the most reliable phenotypic characteristics for the identification of *P. parasiticum* are its moderately rapidly growing, unevenly grayish, progressively darkening colonies featuring moderately thin hyphae with prominent tuberculate warts up to 3 μm in size; its long, tapering subcylindrical phialides with a small tubular collarette; and its small, mostly allantoid (sausage shaped) conidia. Morphological distinction from a number of other relatively similar *Phaeoacremonium* species has recently been summarized by Mostert et al. (20). The single most distinctive feature of *P. parasiticum* is the large hyphal warts, which are often clearly indicated in past case reports (for examples, see reference 17). Other grayish-colored opportunistic *Phaeoacremonium* species, such as *Phaeoacremonium krajdenii*, lack these prominent warts and often have relatively broad phialides.

The spectrum of disease caused by *P. parasiticum* includes subcutaneous infections (1, 9, 15), eumycetoma (14), arthritis (16, 25), osteomyelitis (26), and disseminated disease including fungemia, endocarditis, and cases with multiorgan involvement (8, 12; this report). Outcomes among reported cases are relatively good, with 7 (63.6%) of 11 alive and 6 (55%) considered cured. No patients with disseminated infection have lived, but all of these patients were severely immunocompromised. Because these outcome data are obtained from case reports, follow-up time of patients is variable. Our own patients, a heart transplant recipient and an individual with aplastic anemia, were both severely immunocompromised, and both died, though one appeared to die of unrelated causes.

It is striking that 36% (4/11) of reports to date involved renal transplant patients with subcutaneous and/or joint infections, mostly involving the leg. In all cases, these patients survived whether or not antifungal therapy was successful. In one case, however, stabilizing the apparently incurable knee infection required curtailing immunosuppressive therapy, with the result that the patient needed to be placed back on dialysis (27; other case details may be found in references 6 and 17).

The ideal treatment for *P. parasiticum* infection is not defined, and the paucity of cases does not allow for meaningful comparisons of antifungal agents. Agents commonly used in reported cases include amphotericin B preparations and azoles, but terbinafine and 5FC have also been administered (14, 16, 27). On the basis of limited susceptibility data, amphotericin B and azoles may be effective (7). Indeed, among our patients’ isolates, low MICs (range, 0.03 to 8 μg/ml) were seen with extended spectrum triazoles and amphotericin B (Table 1). However, susceptibility testing is not standardized with *Phaeoacremonium* isolates and drug MIC breakpoints, and the best method and time of MIC endpoint determination have yet to be established. It is important to interpret MIC data for *P. parasiticum* with caution; clearly, more data on clinical correlation are needed.

Surgical debridement appears to be an important aspect of the treatment of localized *P. parasiticum* infection. Among eight reported cases of localized infection in the world literature (Table 2), seven had surgical intervention (1, 9, 14, 15, 25, 27; our case 2). In two patients, surgical debridement was curative without the addition of antifungal therapy. Appropriate treatment is undefined, but surgical debridement and use of amphotericin B and extended-spectrum triazoles are associated with relatively good outcomes.

### Table 1. In vitro susceptibilities of two *Phaeoacremonium parasiticum* isolates

<table>
<thead>
<tr>
<th>Case Isolate</th>
<th>MIC (μg/ml) of drug at time (h)*</th>
<th>AMB</th>
<th>ITC</th>
<th>VOR</th>
<th>RAV</th>
<th>POS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 CBS 109666</td>
<td>0.5 1 8 0.25 1 0.25</td>
<td>1 1 1 1 1 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 CBS 109665</td>
<td>2 0.25 1 0.25 0.25 0.25</td>
<td>1 0.25 0.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*AMB, amphotericin B; ITC, itraconazole; VOR, voriconazole; RAV, ravuconazole, POS, posaconazole. MIC endpoints for all drugs are defined as the lowest drug concentrations that prevented any discernable growth (optically clear) compared to that of drug-free controls.

### Table 2. Reported human cases of *Phaeoacremonium parasiticum* infection

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Sex</th>
<th>Underlying disease</th>
<th>Infection site(s)</th>
<th>AF treatment</th>
<th>Surgical therapy</th>
<th>Outcome</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
<td>F</td>
<td>Aplastic anemia</td>
<td>Disseminated</td>
<td>Lipid AMB</td>
<td>N</td>
<td>D</td>
<td>This study, case 1</td>
</tr>
<tr>
<td>40</td>
<td>M</td>
<td>Cardiac transplant</td>
<td>Hip, forearm</td>
<td>Lipid AMB, ITC</td>
<td>Y</td>
<td>D</td>
<td>This study, case 2</td>
</tr>
<tr>
<td>45</td>
<td>M</td>
<td>Liver transplant</td>
<td>Disseminated</td>
<td>AMB, ITC</td>
<td>N</td>
<td>D</td>
<td>6</td>
</tr>
<tr>
<td>30</td>
<td>F</td>
<td>None</td>
<td>Foot</td>
<td>ITC, 5FC</td>
<td>Y</td>
<td>A (cure)</td>
<td>14</td>
</tr>
<tr>
<td>30</td>
<td>M</td>
<td>Renal transplant</td>
<td>Leg</td>
<td>None</td>
<td>Y</td>
<td>A (cure)</td>
<td>15</td>
</tr>
<tr>
<td>92</td>
<td>F</td>
<td>Unknown</td>
<td>Disseminated</td>
<td>AMB, TRB</td>
<td>N</td>
<td>D</td>
<td>8, 26</td>
</tr>
<tr>
<td>49</td>
<td>M</td>
<td>None</td>
<td>Knee</td>
<td>AMB, 5FC</td>
<td>N</td>
<td>A (cure)</td>
<td>16</td>
</tr>
<tr>
<td>30</td>
<td>F</td>
<td>None</td>
<td>Joint</td>
<td>KTC</td>
<td>Y</td>
<td>A (cure)</td>
<td>25</td>
</tr>
<tr>
<td>45</td>
<td>M</td>
<td>Renal transplant</td>
<td>Skin/soft tissue</td>
<td>None</td>
<td>Y</td>
<td>A (cure)</td>
<td>1</td>
</tr>
<tr>
<td>41–44</td>
<td>M</td>
<td>Renal transplant</td>
<td>Leg, later also knee</td>
<td>AMB, 5FC, KTC, ITC</td>
<td>Y</td>
<td>A (failed)</td>
<td>6, 17, 27</td>
</tr>
<tr>
<td>61</td>
<td>M</td>
<td>Renal transplant</td>
<td>Leg</td>
<td>FLC</td>
<td>Y</td>
<td>A (cure)</td>
<td>9</td>
</tr>
</tbody>
</table>

* M, male; F, female; AF, antifungal; AMB, amphotericin B; ITC, itraconazole; KTC, ketoconazole; TRB, terbinafine; FLC, fluconazole; 5FC, flucytosine; Y, yes; N, no; A, alive; D, dead.
In summary, P. parasiticum is an uncommon cause of fungal infection, and its appropriate identification may be difficult. Due to the infrequent isolation and difficulty in making a morphological diagnosis even in the hands of an experienced mycologist, these isolates should be sent to a reference laboratory that can provide additional testing such as has been reported in these cases. Although sequencing of β-tubulin is not a method available to most clinical laboratories, our judgment is that it is important for microbiologists to pursue a definitive identification of unusual fungal isolates. Otherwise our knowledge of the pathogenic potential and methods of treatment of rare or uncommon fungi will remain unclear. In any case, the molecular capabilities of many medical facilities are expanding, and the use of such technologies for difficult identifications of fungi and other microorganisms is expected to increase rapidly in the near future.

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