

Subcutaneous Mycetoma of *Pseudallescheria boydii* complexes Revealed by 18S rDNA sequencing

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Abstract

Pseudallescheria ellipsoidea, one of the *P. boydii* complexes, is a ubiquitously isolated saprophytic fungus from soil, poultry, cattle, sewage, and polluted water. It is known to cause various diseases such as subcutaneous mycetoma in immunocompetent individuals and sepsis in immunocompromized patients. We experienced a case of cutaneous *P. ellipsoidea* infection in a 79-year-old man. He was taking medication for diabetes mellitus and heart disease and visited our dermatologic outpatient clinic with violet-colored, nontender, fluctuating, keratotic, conglomerated plaques on the right forearm. Skin biopsy was consistent with foreign body reaction to ruptured epidermal cyst. After skin culture, PCR and sequencing revealed *P. boydii* complexes, with forward sequencing showing 96% homology of *P. boydii*, and reverse sequencing showing homology 100% of *P. ellipsoidea*. After the patient received fluconazole for 5 weeks, discharged with improved conditions. (J Med Life Sci 2013;10(1):50-53)

Key Words : *Pseudallescheria boydii* complexes, Mycetoma, Sequencing

Introduction

Pseudallescheria ellipsoidea is a saprophytic fungus ubiquitously isolated from soil, poultry, cattle, sewage, and polluted water¹. Recently, it is known to cause various diseases such as subcutaneous mycetoma in immunocompetent individuals and disseminate sepsis in immunocompromized patients². Some studies have proposed that *P. ellipsoidea*, *P. angusta*, and *P. fusioidea* are the another name of *P. boydii* with no significant difference in the aspect of clinical manifestation and rDNA sequences³. But, other studies have revealed that it is one of the *P. boydii* complexes which consists of at least six different species (*P. boydii*, *P. ellipsoidea*, *P. angusta*, *P. aurantiaca*, *P. fusioidea*, and *P. minutispora*)⁴⁻⁶. Although the use of voriconazole, these infections may have high mortality and need to further established treatment⁷. Until now, only one case of pulmonary fungal ball of *Pseudallescheria boydii* identified by LSU rDNA D2 region sequencing have been reported in our country⁸. We introduce our experience with a case of cutaneous *P. boydii* complex infection in a immunocompetent patient revealed by 18S rDNA sequencing.

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Case Report

A 79-year-old man visited our dermatologic outpatient clinic, complaining of violet-colored, nontender, fluctuating, keratotic, conglomerated plaques on the right forearm (Fig. 1).

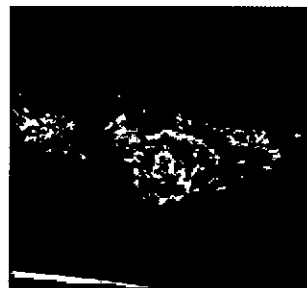


Figure 1. Skin lesion of right forearm.

He had taken the medications for diabetes mellitus and heart disease for several years without any other similar symptoms. Physical examination revealed no specific finding except above right forearm lesion. Skin biopsy done after admission was consistent with foreign body reaction to ruptured epidermal cyst. There was no evidence of tuberculosis infection in acid fast stain and culture of his right forearm lesion. On Gram stain, mold are noted, so, the specimen was cultured in Sabouraud's dextrose agar (SDA, Hanil KOMED, Seoul, Korea) at 30°C. On the 7th culture day, the isolate started to grow. The front surface of the

agar grew cotton like white mycelium, which later turned slightly brown after 14 days incubations (Fig. 2. A, C). The reverse surface were slight yellow at first, then became smoky brown brown after 14 days incubations (Fig. 2. B, D).

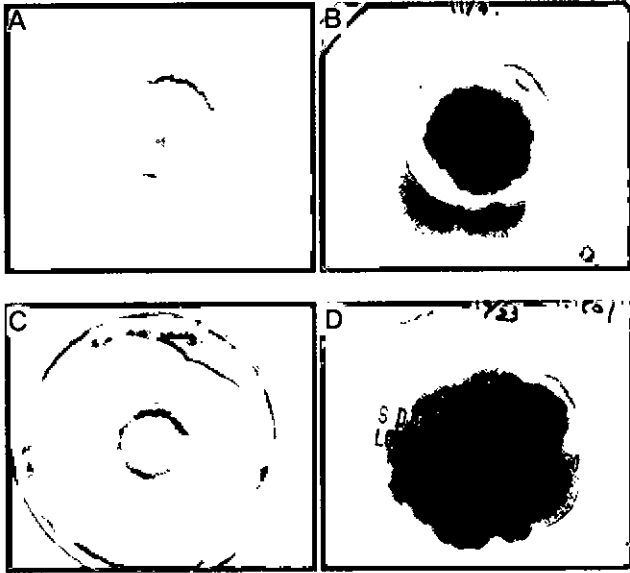


Figure 2. Features on Sabouraud's dextrose agar.
 A. Front image after 7 days incubation
 B. Reverse image 7 days incubation
 C. Front image after 14 days incubation
 D. Reverse image 14 days incubation

Lacto-phenol cotton blue stain for microscopic examination showed septated hyphae with simple long or short conidiophores with conidia having unicellular and oval, and had the larger end toward the apex, and cut off at base (Fig. 3. A, B).

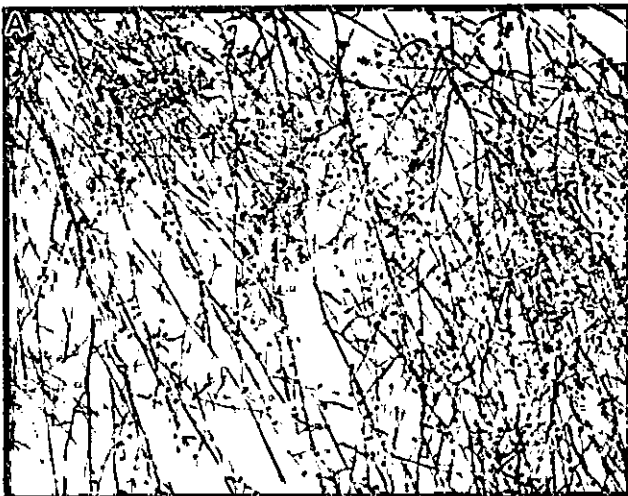


Figure 3. Microscopic findings showing conidiophores with conidia of the fungus on Lacto-phenol cotton blue stain.

A. (LPCB stain, x200)
 B. (LPCB stain, x 400)

Based on the above results, the organism was suspected as *Pseudallescheria* spp. DNAs of fungus were extracted using the bead beater-phenol extraction method. A loopful of culture of each isolate was suspended in 200 μ L of TEN buffer (10m M Tris - HCl, 1 mM EDTA, 100 mM NaCl: pH 8.0), placed in a 2.0 mL screw-cap microcentrifuge tube filled with 200 μ L (packed volume) of glass beads (diameter, 0.1 mm: Biospec Products, Bartlesville, Okla.) and 200 μ L of Phenol:Chloroform: Isoamyl alcohol (25:24:1) (SIGMA chemical co. P-2069). To disrupt the fungus, the tube was oscillated on a Mini-Bead Beater (Biospec Products) for 1 min. To separate the phases the tube was centrifuged (15,000 rpm, 15 min). After the aqueous phase was transferred into another clean tube, 10 μ L of 3 M sodium acetate and 250 μ L of ice-cold ethanol were added to enable the DNA to precipitate, the mixture was kept at - 20°C for 30 min. The DNA pellet was washed with 70% ethanol, dissolved in 60 μ L of TE buffer (10 mM Tris - HCl, 1 mM EDTA, 100 mM NaCl: pH 8.0) and used as a template for PCR. PCR was performed with a set of primers (Forward primer (0817) 5'-TTAGCATGGAATAATRRRAATAGGA-3' and Reverse primer (1536) 5'-ATTGCAATGCYCTATCCCCA-3') for amplification of the partial 18S rDNA sequences. Template DNA (3 μ L) and 20 μ L of each primer were added to a PCR mixture tube (AccuPower PCR PreMix: Bioneer, Daejeon, Korea), which contained 1 U of Taq DNA polymerase, each deoxynucleoside triphosphate at a concentration of 250 μ M, 50 mM Tris-HCl (pH 8.3), 40 mM

KCl, 1.5 mM MgCl₂, and gel loading dye. The reaction mixture was subjected to 35 cycles of amplification (2 min at 94°C, 10 s at 56°C, and 30s at 72°C), followed by a 5-min extension at 72°C (model 9600 thermocycler: Perkin Elmer Cetus). PCR products were electrophoresed on a 1.2% agarose gel and were purified with a QIAEX II gel extraction kit (QIAGEN, Hilden, Germany). For the 18S rDNA analysis, we used Applied Biosystems model 373A automatic sequencer and a BigDye Terminator Cycle Sequencing kit (Perkin-Elmer Applied Biosystems, Warrington, United Kingdom). Sequencing revealed *P. boydii* complexes, with forward sequencing showing 96% homology of *P. boydii*, and reverse homology 100% of *P. ellipsoidea*. After the patient received fluconazole for 5 weeks, discharged with improved conditions.

Discussion

The *Pseudallescheria/Scedosporium* species complex are about to be emerging opportunistic fungal pathogens in some countries. Especially, in Australia, the survey of soil samplings revealed an abundance of the *Pseudallescheria/Scedosporium* in locations associated with high human activity and strains of *S. aurantiacum* were most frequently isolated (54.6%), followed by *S. prolificans* (43%), *P. boydii* (2.1%) and *S. dehoogii* (0.3%)⁹. In some cases, radiographic or histopathologic tests cannot differentiate *Scedosporium* from *Aspergillus*, and the diagnostic confusion lead to complicate the management of *Scedosporium* infection¹⁰⁻¹¹, because the drug of choice for those species is voriconazole, not amphotericin B in case of *Aspergillus* infection. Also, the prevalence of *S. apiospermum* (or *P. boydii*) infection, especially the mycetomatous form could have been underestimated¹¹. So, timely and accurate laboratory diagnosis is essential for proper treatment. Recently, highly species-specific multiplex PCR assay offers a rapid and simple method of detection of the most clinically important *Scedosporium* species in respiratory tract specimens¹². But, even though the sequencing of LSU rDNA D2 region identified the organism as *P. ellipsoidea*, the suggested species in *P. boydii* complex could not be differentiated from each other, so, further studies with β -tubulin genes, calmodulin genes, and the ITS region sequencing might be recommended⁹. We presented a case of cutaneous *P. ellipsoidea* infection in an immunocompetent patient with underlying diabetes mellitus and heart disease, initially suspected by SDA and LPCB stain and revealed by PCR and 18S rDNA sequencing as *P. boydii* complexes. But, Further

studies with sequencing of other regions such as internal transcribed spacer, actin, heat shock protein, and topoisomerase II must be needed to differentiate the species in *P. boydii* complex.

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