Isolation of *Fusarium solani* from a dog: identification by molecular analysis

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A strain of *Fusarium solani* was isolated from a dog showing many cutaneous and submucosal nodules and pyogranulomatous kidney lesions. Clinical isolates from this systemic infection were identified using microscopic examination and confirmed by molecular analysis.

**Keywords**  dog, *Fusarium solani*, isolation, 28S ribosomal DNA

*Fusarium* species are common soil saprobes and plant pathogens which have frequently been reported as etiologic agents of opportunistic infection in humans [1]. In recent years, molecular techniques have greatly improved our ability to identify *F. solani*. The internal transcribed spacer region of the ribosomal DNA has been utilized for the identification of the species [2].

Several species of *Fusarium* have been reported to cause keratomycosis, mycetoma and onychomycosis in humans [1] and have increasingly been reported from disseminated infection in human patients with severe underlying diseases [1,3]. *F. solani* has most frequently been isolated from human infections. Animal cases of *Fusarium* infection have been very few and no cases of systemic infection by *Fusarium* have been reported from dogs. The present study describes the isolation of *F. solani* from a dog.

**Case report**

A 3-year-old male Doberman dog weighing 30 kg was referred to the Veterinary Medical Center, University of Tokyo with a chief complaint of many nodules on the face, tongue and waist. This dog had a history of panniculitis on both sides of the waist, and had been treated with azathioprine, cyclosporine and prednisone for 7 months. Physical examination revealed many 2–3-mm subcutaneous nodules on the face and three 1–2-cm submucosal nodules on the tongue. Microscopic examination of biopsy specimens from the nodules on the face disclosed neutrophils, macrophages and many branching hyaline hyphae (Fig. 1).

The dog was treated with itraconazole (ITZ) at 5 mg kg⁻¹ every day and amphotericin B at 0.15 mg kg⁻¹ IV. However, the nodules did not diminish. After 2 weeks of treatment, the patient was euthanased. Histopathologic examination revealed a granulomatous inflammation with many branching hyphae in skin, tongue and kidneys (Fig. 2).

**Identification of a clinical isolate**

Biopsy specimens from cutaneous nodules on the face were cultured on Sabouraud’s dextrose agar and potato dextrose agar at 24 °C for 2 weeks. Fungal colonies developed within 1 week. The colony of the clinical isolate was flat and white and had a cottony texture after a 2-week incubation on Sabouraud’s dextrose agar at 24 °C (Fig. 3). Microscopic examination of the isolate revealed that microconidia were abundantly produced and disposed in slimy heads on phialides (Fig. 4) These conidia were hyaline, oval and aseptate, measuring 10–15 μm by 2–5 μm. The conidiophores were 50–70 μm in length (Fig. 4). Chlamydospores were also produced. Macroconidia produced on potato dextrose agar were hyaline, moderately curved, mostly three-septate and 40–
50 μm long (Fig. 5). From these findings, the isolates were identified as *F. solani* [3].

Biopsy specimens and, separately, or several hundreds cells of the clinical isolate were lysed with 1 mg of zymolyase-100T (Takara, Kyoto, Japan) and 10 μg of proteinase K per ml in a lysis buffer containing 0.1 mM EDTA, 1% sodium dodecyl sulfate (SDS), 10 mM Tris hydrochloride (pH 8.0) and 0.3% 2-mercaptoethanol at 37 °C for 16 h. High molecular mass DNAs were obtained from these samples by phenol and chloroform extraction. These DNA samples were dissolved in TE buffer (10 mM TrisHCl, pH 8.0 and 1 mM EDTA) for polymerase chain reaction (PCR) amplification.

The genomic DNA (100 ng) samples were amplified in a reaction mixture (30 μl) containing 10 mM TrisHCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.001% gelatin, 200 mM of each of the deoxynucleoside triphosphates, 1.0 Units of *Taq* DNA polymerase and 0.5 μg of a pair of primers. The sequences of the primers for the 28S ribosomal DNA were constructed based on sequences reported [4]: forward primer S-2, 5′-GCATATCAA-TAAGCGGAGGAAAAG-3′; reverse primer S-2, 5′-GGTCCGTGTTTCAAGACG-3′. With these primers, a 600-bp fragment containing the coding sequence of the 28S ribosomal DNA was expected to be amplified. The PCR amplification was carried out for 35 cycles consisting of template denaturation (1 min, at 94 °C), primer annealing (2 min, at 60 °C) and polymerization (3 min at 72 °C). The PCR products were electrophoresed through 2% agarose gel and then stained with ethidium bromide. The PCR products from the samples were sequenced using the dideoxy chain termination method using an ABI PRISM 310 Genetic Analyzer (Perkin-Elmer, CA, USA).
Fig. 5 Macroconidia (arrow) seen as moderately curved structures, mostly three-septate, and 40–50 μm long.

Amplification of the sample DNA with 28S ribosomal DNA primers yielded fragments of 600 bp, consistent with the size of 28S ribosomal DNA from fungal species reported previously [4]. To examine homology relationships between 28S ribosomal DNA of reference strains of *F. solani* complex and that of the samples, we used FASTA database analysis in the DNA databank of Japan (DDBJ). The nucleotide sequences of 28S ribosomal DNA of the biopsy specimen, the clinical isolate, and a reference strain of *F. solani* f. sp. *phaseoli* (DDBJ Accession no. L36629) showed 98% similarity. The nucleotide sequences of 28S ribosomal DNA of the biopsy specimen, the clinical isolate, and the other reference strains of *F. solani* complex showed 96% similarity. Therefore, the isolate was identified as *F. solani* f. sp. *phaseoli* by molecular analysis, suggesting that the molecular analysis presented in this study could be available for the diagnosis of *F. solani* infection by detecting the *F. solani* DNA in biopsy specimens within 2 days. The sequences reported in this paper have been deposited in the GenBank database [Accession no., internal transcribed spacer and 28S ribosomal RNA (28S rRNA) gene of our clinical isolate of *Fusarium solani*, AB075377]. The case isolate was deposited in the Japan Collection of Microorganisms RIKEN, Saitama, Japan as JCM 11488.

Infection of animals by *Fusarium* species have been reported only in horses, cows and cats [5–7]. In humans, steroid treatment, chemotherapy and underlying immunosuppressive diseases, including AIDS, have been reported to facilitate subsequent infections with *Fusarium* [1,3,8]. This dog had also been treated with immunosuppressive drugs, which might have predisposed it to contracting this infection.

References