



CASE REPORT

THE ISOLATION OF FUSARIUM SPOROTRICHIOIDES FROM A DIABETIC FOOT WOUND SAMPLE AND IDENTIFICATION

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ABSTRACT

Fusaria are major opportunist pathogens for immunocompromised patients. In this study, it was concluded as a result of both conventional and molecular identification techniques that the isolate obtained from the patient's diabetic foot was *Fusarium sporotrichioides*. T-2 toxin production of the pathogen was investigated using HPLC and no toxin was determined. For this reason, in laboratory diagnosis, Fusaria should not be considered solely as an environmental contaminant.

Keywords: Fusarium, Diabetic foot, Opportunistic pathogen

DİYABETİK AYAKLI BİR HASTAYA AİT YARA ÖRNEĞİNDEN FUSARIUM SPOROTRICHIOIDES İZOLASYONU VE TANIMLANMASI

ÖZET

Fusarium türleri bağışıklıkları baskılanmış hastalar için önemli fırsatçı patojenlerdir. Bu çalışmada, diyabetik ayaklı bir hastadan izole edilen patojenin, konvansiyonel ve moleküler tekniklerle tanımlanması yapılarak, *Fusarium sporotrichioides* olduğu sonucuna varıldı. HPLC analizleri, patojenin T-2 toksinini üretmediği görüldü. Bu nedenle, laboratuvar tanılarında, *Fusarium*'lar sadece çevresel kontaminantlar olarak değerlendirilmemelidir.

Anahtar Kelimeler: Fusarium, Diyabetik ayak, Fırsatçı patojen

INTRODUCTION

Lower extremity infections are frequent causes of morbidity and mortality in diabetic patients. *Fusarium* species moulds are generally known as plant pathogens, and can lead to opportunistic infections in humans, especially in risk group individuals. In human and animal-based cases of *Fusarium species* capable of producing tricothecene mycotoxins, potential protein synthesis inhibitors are rare. During routine analyses in microbiology laboratories, these pathogens

are generally regarded and interpreted as environmental contaminants¹. In the present report, a fungal pathogen was isolated from the wound of a male patient suffering from type 2 diabetes mellitus and identified as *Fusarium sporotrichioides* by using the conventional and molecular techniques.

CASE REPORT

The case was a 57-year-old male patient with a history of enjoying walking barefoot, frequently on soil or sand, and with type 2

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diabetes mellitus risk factors persisting for 15 years. An ulcerative wound had developed on his right foot (Figure 1). It was learned that sensations of itching on the foot had increased prior to the development of the wound, following which the tissue progressed to a wound and ulcer. At radiological analysis of the patient, admitted to the Marine and Undersea Diseases department of our hospital for hyperbaric oxygen (HBO) therapy due to the development of the diabetic foot, lesions compatible with osteomyelitis in the foot were identified.

Treatment: The patient was treated with fluconazole 100 mg IV (twice a day, for nine days) and after that fluconazole 100 mg p.o (once daily for 26 days) as well as ornidazole 500 mg p.o. (twice daily), cefoperazon-sulbactam 1000 mg IV (once in a day), imipenem-cilastatin 500 mg IV (four times a day) and fusidic acid 500 mg p.o (twice a day).

Mycological study: Smears and tissue samples were taken from different regions of the suppurative foot wound for the purpose of microbiological diagnosis². Following the cultivation in Sabouraud dextrose broth (SDB-Oxoid) for a 48-72 hour period, weak growth with a mould morphology was observed onto Sabouraud dextrose agar (SDA-Oxoid) slants. The mould growth on the culture slants attained specific colony morphology in 4-5 days. Mould growth with the same morphology was determined in fungal cultures belonging to specimens taken from the patient for follow up on days 7 and 30. At direct microscopic examination of the colonies performed with lactophenol cotton blue, conidia with a needle-like appearance and hyphal structures suggested *Fusarium* spp. For identification, subcultures were made onto potato dextrose agar (PDA-Oxoid), incubated at 26 or 37°C and subjected to daily examination. Better growth on potato PDA was observed at 26°C. At differential diagnosis, the base color of the off-white colonies having swollen micelles and a powdery, cotton-like appearance, turned from light yellow to peach in five days. At the end of this period, colony size reached 4-6 cm.

Under microscopic examination of the colonies, differential diagnosis was initiated with the morphological observation of oval microconidia with one or two septa and phialide structures exhibiting polyblastic features, with transparent hyphal structures and macroconidia having a needle-like appearance, slightly bent and lightly curved in places, conidiaferous structures and a large number of transparent chlamydospores of a chain nature (Figure 2). Bearing in mind the morphological features, fungal isolate was defined as *F. sporotrichioides*.

Molecular study: For diagnosis at the species level, PCR was performed by using genomic DNA of fungal isolate cultured in potato dextrose broth (PDB-Oxoid) at 26 °C for five days. The method described by Cenis³ was employed for DNA isolation. The amount of DNA was determined approximately by comparison with a standard DNA specimen in agarose gel. LanspoR1, reverse primer (5-TACAAGAAGACGTGGCGATAT-3) common for *F. sporotrichioides* and *F. langsethiae*, and forward primers, FspoF1 (5-CGCACAACGCAAACCTCATC-3) specific for *F. sporotrichioides*, and FlangF3 (5-CAAAGTTCAGGGCGAAAAC-3) specific for *F. langsethiae*, previously used by Wilson et al.⁴ in the molecular diagnosis of *Fusarium* species, were used for PCR. Some 50-100 ng of genomic DNA was used as a template. The PCR was carried out at 95°C for 3 min, followed by 35 cycles of 30 sec denaturation at 94 °C, 20 sec of annealing at 55°C, and 60 sec of extension at 74 °C. PCR products were loaded on 1% agarose gel and electrophoresed in TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) at 80 V for 60 min. A DNA fragment of 332 bp in size was amplified for *F. sporotrichioides*.

Toxin assay: Corn and rice grains were infected with fungal isolate for isolation of type-A trichothecene T-2 toxin. Infected ground cereal samples were extracted as described by Jimenez et. al.⁵. The extract was analyzed by high-performance liquid chromatography (HPLC) with diode array detection⁶.

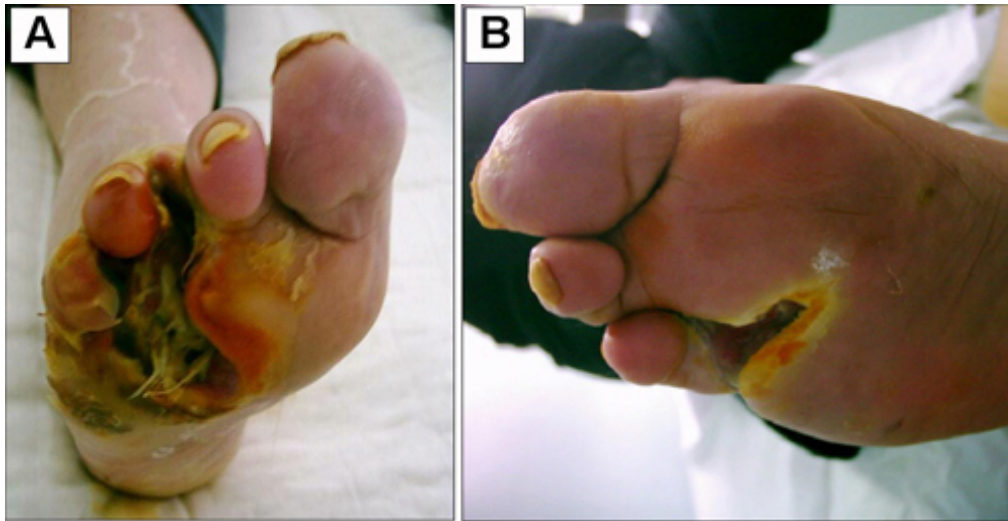


Figure 1: Soft-tissue infection of the foot (A: before treatment, B: after treatment)

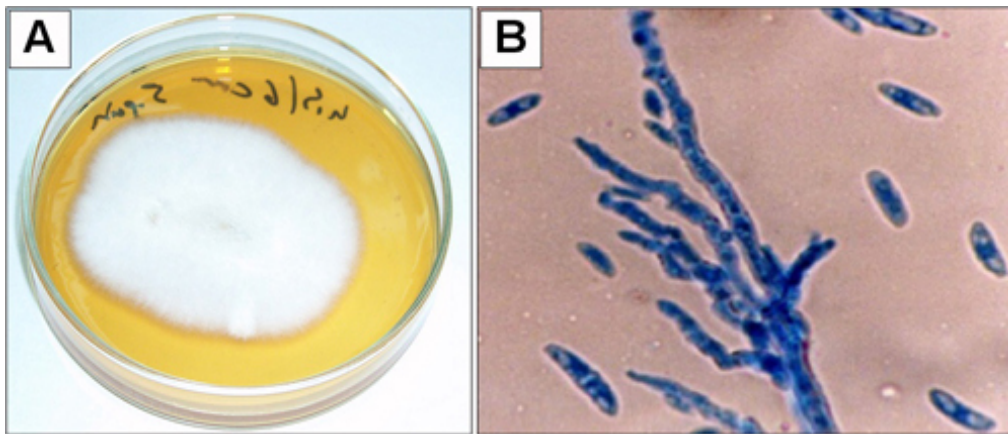


Figure 2: A: macroscopic and B: microscopic morphology of *F. sporotrichioides*.

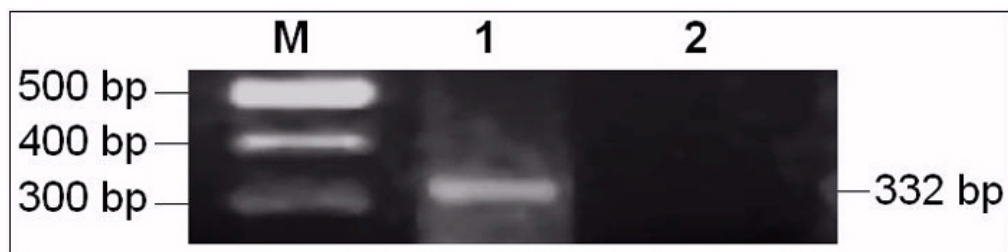


Figure 3: Gel electrophoresis of PCR products amplified by using *FspoF1/LanspoR1* (lane 1; for *F. sporotrichioides*) and *FlangF3/LanspoR1* (lane 2; for *F. langsethiae*) primers. M, 100 bp ladder (Fermentas).



DISCUSSION

In patients with diabetes mellitus, foot infections are common, ranging from chronic bacterial or fungal infections to serious limb-threatening ones. A special consideration should be given to the environmental and opportunistic mycoses. Environmental fungi include *Aspergillus*, *Alternaria*, and *Fusarium* can produce infection and toxin-related diseases. Such patient populations are also at an increased risk for disease caused by other opportunistic fungi such as the yeasts *Candida* and *Cryptococcus* and the dust fungus. *Fusarium* species are very common in the tropical and subtropical areas. *Fusarium* is known to produce infections of the skin, eye and nail. In the present report, a fungal pathogen isolated from the wound of a male patient suffering from diabetes mellitus was identified as *Fusarium sporotrichioides* by using the conventional and molecular techniques.

In typing studies, the determination of specific sequences that can reveal distinctions between species is of great importance. The organization of ribosomal RNA genes is rather well protected in moulds. Three basic RNA genes are separated from one another by the ITS1 and ITS2 sequences. The fact that these regions exhibit variety among different species gave rise to the idea that they could be used as marker sequences for typing studies⁷. Therefore, many researchers have performed *Fusarium* species typing studies together with other moulds using appropriate oligonucleotide primers constituted for these regions⁸. In our study, the method described by Wilson et al.⁴ was applied in the molecular diagnosis of an isolate obtained from the patient's diabetic foot wound and described as *F. sporotrichioides* because of morphological and microscopic analyses. A DNA fragment 332 bp in size definitive of *F. sporotrichioides* was obtained with PCR performed as described above. The DNA fragment expected with the primers used for *F. langsethiae* was not observed, however (Figure 3). Therefore, it was concluded as a result of both microscopic examination and the amplification of a specific region with

PCR that the isolate obtained from the patient's diabetic foot was *F. sporotrichioides*.

F. sporotrichioides species naturally occur in cereals. Moulds of this kind synthesize type-A trichothecenes in particular in various substrates such as barley, corn, wheat or rice⁹. The species *F. sporotrichioides* is capable of producing of T-2 toxin, one of moulds' most toxic mycotoxins. Therefore, in order to determine whether our fungal isolate also produced this toxin, separate inoculations were made on corn and rice from a freshly prepared sample. Analysis of specimens by using HPLC revealed no type-A tricothecene T-2 toxin. In studies regarding the synthesis of type-A trichothecenes in *F. sporotrichioides* isolates culture conditions such as moisture have been reported as major factors affecting the synthesis¹⁰. Therefore, despite the determination of *F. sporotrichioides* as a result of morphological and molecular analyses, T-2 toxin not being determined at HPLC analyses suggests a correlation with the isolate type and culture conditions. Human and animal-based case reports linked to *F. sporotrichioides*, which can be seen all over the world and is present in soil as a saprophyte, are rare. They particularly cause intoxication by invading cereals. In addition, conjunctivitis, keratitis, endophthalmia, maxillary sinus infections, osteomyelitis, septic arthritis, brain abscess, colonization in burn or necrotic injuries, leg region ulcers, deep tissue infections, contact dermatitis and onychomycoses, which can be more frequently observed in patients with suppressed immune systems, are rare *Fusarium*-based opportunist mycoses¹¹⁻¹⁴. Our patient improved and was discharged following antifungal therapy including fluconazole (2x1 for nine days and 1x1 for 26 days) and a total of 52 sessions (130 hours) of HBO therapy in two separate periods over the course of treatment. The fact that *F. sporotrichioides*, described as an opportunistic pathogen as in our patient, can give rise to local infections or colonization, especially in risk group patient wounds, must not be overlooked.



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