Colletotrichum keratoscleritis Diagnosed by 18S rDNA Sequencing

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(Received April 10, 2014; Revised April 17, 2014; Accepted April 24, 2014)

Abstract

The *Colletotrichum*, well-known plant pathogens were increasingly reported to cause human ophthalmic infections in worldwide and until now, only one case of *Colletotrichum* keratitis was reported in our country. Although it is an uncommon cause of keratitis, usually secondary to corneal erosion caused by plant material, but, should be included in the differential diagnosis of fungal keratitis. In our Jeju Province, some peoples may be usually exposed to plants during farming. We introduce here a case of ophthalmic infections, keratoscleritis due to the *Colletotrichum* species in an immunocompetent farmer with underlying diabetes mellitus and hypertension, initially not suspected by SDA and LPCB stain and revealed by PCR and 18S rDNA sequencing as the *Colletotrichum* species. **(J Med Life Sci 2014;11(1):13-17)**

Key Words : *Colletotrichum*, Keratoscleritis, 18S rDNA Sequencing

Introduction

Recently, many cases are requested for microbiologic tests to find the cause of infectious keratitis, other name is corneal infection, because of the importance of the disease. Keratitis could cause to many symptoms such as blurred vision, foreign body sensation, ocular pain, tearing, redness, photophobia, and as spasticity symptoms. If not treated properly, it lead to perforation of the eye and endopthalmitis by the proliferations of cells in the eye, and even to blindness.

Microorganisms that cause corneal infection vary with diverse organisms such as bacteria, fungi, viruses, chlamydia, and amoeba. But, the early detection of casative microorganism and proper treatment can prevent various complications.

According to the national multicenter epidemiological study of the corneal infection, causative bacteria were detected in 63.3% of infectious keratitis, fungus, 11.7%, amoeba, 2.9%[1]. In the epidemiological study of patients with fungal keratitis, *Fusarium*(29.0%), *Aspergillus*(24.6%), Candida(15.9%), *Altenaria*(10.1%), *Acremonium*(5.8%) were isolated[2].

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Another studies showed that, though *Fusarium* and *Aspergillus* are the most common causative agents among the causative fungal keratitis, *Curvularia* or other fungi such as *Acremonium* also are known to cause corneal infection[3,4].

After the first case report of an ocular infection with the fungus *Colletotrichum*[5], the *Colletotrichum*, well-known plant pathogens were increasingly reported to cause human ophthalmic infections in worldwide and until now, only one case of fungal keratitis caused by *Colletotrichum* species revealed by sequencing of the D1-D2 domain of 28S rDNA was reported in our country[6].

We introduce here our experience with a case of ophthalmic infections, keratoscleritis due to *Colletotrichum* species. in a immunocompetent patient revealed by 18S rDNA sequencing.

Case Report

A 68-year-old man who had worked as a farmer for over 20 years visited our ophthalmologic outpatient clinic, complaining of severe pain and blurred vision of left eye. External photography of left eye showed medial thinning and choroid bulging in the left sclera and limbal infiltration in the left cornea and conjunctival chemosis, engorged episcleral and scleral vessels, nummular scleral area of avascularity and necrosis, and small perilimbal corneal infiltration adjacent to scleral lesion (Fig. 1). He had taken the medications for diabetes mellitus and hypertension for

This research was supported by the 2014 scientific promotion program funded by Jeju National University

several years without any other similar symptoms. Physical examination revealed no specific finding except above left eye lesion. His visual acuity decreased and corneal ulcer and inflammation in the left anterior chamber were noted in the slit lamp biomicroscopy examination. On Gram stain of the sample from the left eye, mold are noted, so, the specimen was cultured in Sabouraud's Dextrose Agar (SDA, Hanil KOMED, Seoul, Korea) at 30℃. On the 3th culture day, the isolate started to grow. The front surface of the Sabouraudes Dextrose Agar showed rapidly growing white cottony fungus within 48 hours, which later on turned into mouse gray color colonies after 5 days incubations (Fig. 2. A,C). The reverse surface of the Sabouraudes Dextrose Agar had yellow color pigmentation after 5 days incubations (Fig. 2.B,D). Lacto-Phenol Cotton Blue stain for microscopic examination showed many branching, septate hyaline and phaeoid fungal filaments (Fig. 3.A,B). Based on the above results, definite differentiation of the organism was not possible.

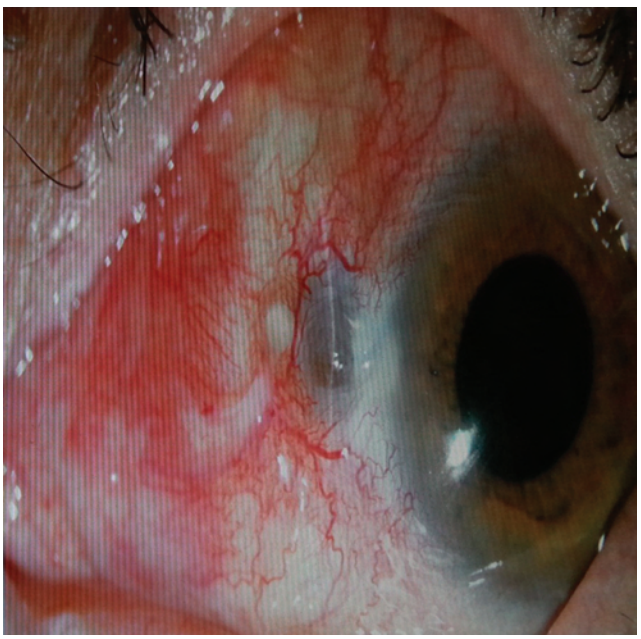
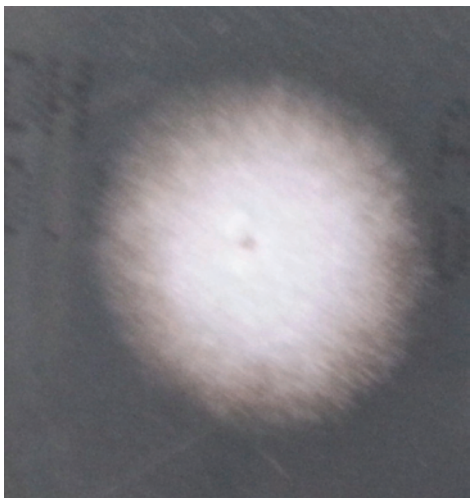
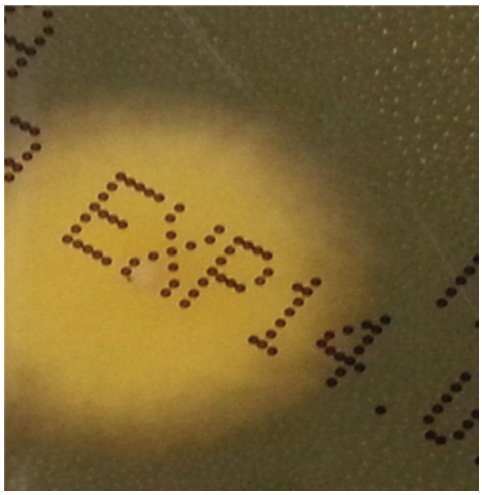


Figure 1. External photography of left eye.
External photography of left eye showed medial thinning and choroid bulging in the left sclera and conjunctival chemosis, engorged episcleral and scleral vessels, nummular scleral area of avascularity and necrosis, and small perilimbal corneal infiltration adjacent to scleral lesion.

DNAs of fungus were extracted using the bead beater-phenol extraction method. A loopful of culture of each isolate was suspended in 200㎕ of TEN buffer (10mM Tris - HCl, 1 mM EDTA, 100 mM NaCl: pH 8.0), placed in a 2.0 mL screw-cap microcentrifuge tube filled with 200㎕ (packed volume) of glass beads (diameter, 0.1 mm: Biospec Products, Bartlesville, Okla.) and 200㎕ 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 ㎕ of 3 M sodium acetate and 250 ㎕ of ice-cold ethanol were added; to enable the DNA to precipitate, the mixture was kept at - 20 ℃ for 30 min. The DNA pellet was washed with 70% ethanol, dissolved in 60 ㎕ 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'-TTAGCATGGAATAATRRAATAGGA-3' and Reverse primer (1536) 5'-ATTGCAATGCYCTATCCCCA-3') for amplification of the partial 18S rDNA sequences. Template DNA (3 ㎕) and 20 ㎕ 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 ㎕, 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℃, 10 s at 56℃, and 30s at 72℃), followed by a 5-min extension at 72℃ (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 Colletotrichum species with forward and reverse sequencing showing 99% homology. After received amnion and conjunctival autograft, the patient took the medications with voriconazole and amphotericin B for 5 weeks, and then improved the conditions.

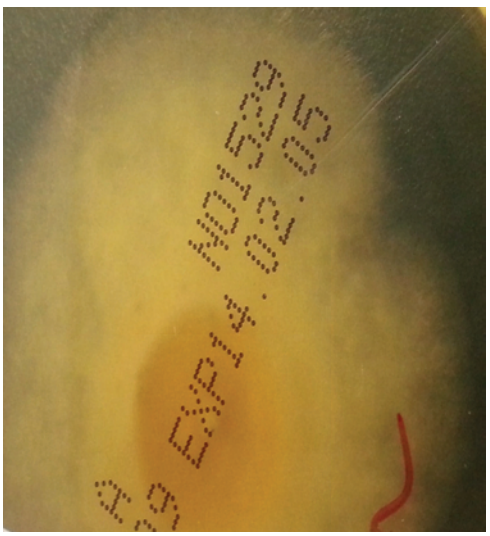


A. Front image after 3 days incubation



B. Reverse image after 3 days incubation

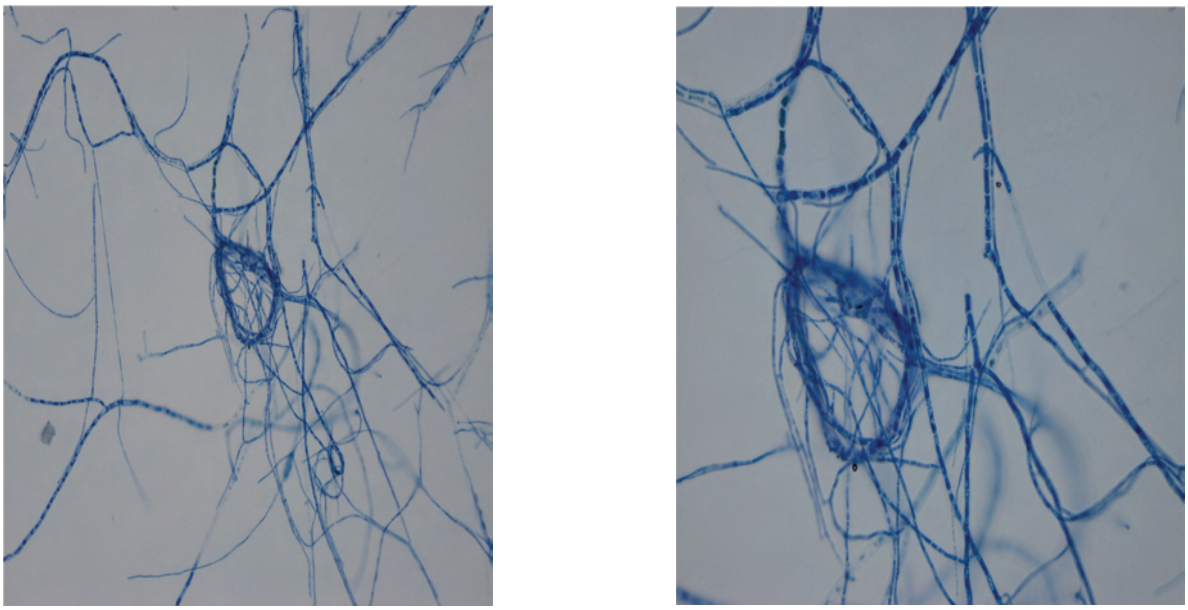
C. Front image after 5 days incubation



D. Reverse image after 5 days

Figure 2. A, B, C, D

The front surface of the Sabouraudes Dextrose Agar showed rapidly growing white cottony fungus within 48 hours, which later on turned into mouse gray color colonies after 5 days incubations (Fig. 2. A, C).). The reverse surface of the Sabouraudes Dextrose Agar had yellow color pigmentation after 5 days incubations (Fig. 2. B, D).



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

Figure 3. Microscopic findings of the fungus on Lacto-Phenol Cotton Blue stain.
Lacto-Phenol Cotton Blue stain for microscopic examination showed many branching, septate hyaline and phaeoid fungal filaments.

Discussion

Colletotrichum is a medically important fungus belonging to the order Melanconiales under the class Coelomycetes and the subdivision Deuteromycotina and has a worldwide distribution, but is found mainly in the tropical and the subtropical regions[7]. The members of the genus *Colletotrichum* are primarily plant pathogens which cause fungal infection such as anthracosis, necrosis, and fruit rot mainly in cereals, grasses, legumes, vegetables, perennial crops. What causes this infection in humans in the world so far reported five species, *Colletotrichum coccodes, C. crassipes, C. dematium, C. gloeosporioides, and C. graminicola*[8-12].

In a retrospective survey of the cases diagnosed with fungal keratitis in a hospital in the United States from 1980 to 2001, 2.8% of the isolated fungus was *Colletotrichum*[13]. And recently, of the five medically important members in the genus *Colletotrichum*, keratitis due to *Colletotrichum graminicola* is rare but was reported in India[14].

In our country, only one case of fungal keratitis caused by *Colletotrichum* Species revealed by sequencing of the D1-D2 domain of 28S rDNA was reported in 2006[6].

The most important morphological features to identify the *Colletotrichum* genus are acervular conidiomata, often with setae with unbranched, thick-walled, pigmented and the appressoria which means thick-walled swellings at the end of a hypha or a germ tube[15].

The *Colletotrichum* species can be misidentified as *Fusarium* species due to the similarity of cylindrical conidia. So, we must to carefully look for the presence or absence of the septation within the conidia and the presence or absence of the appressorias, to distinguish the Colletotrichum secies from the Fusarium species. To distinguish the species level, *Colletotrichum* conidia and appressoria should be used, but, it can be difficult to distinguish since there will be different observation period in some species. So, to overcome the limitation of the identification based on these morphological characteristics, there were reports that you can successfully identify the 5 major *Colletotrichum* secies by ribosomal DNA (rDNA) large-subunit (LSU) of the internal transcribed spacer 1 (ITS1) region and sequence analysis of the D1-D2 domain[16].

We introduce here a case of ophthalmic infections, keratoscleritis due to the *Colletotrichum* species in an immunocompetent farmer with underlying diabetes mellitus and hypertension, initially not suspected by SDA and LPCB stain and revealed by PCR and 18S rDNA sequencing as the

Colletotrichum species.

The rapid identification of casative fungal microorganism and proper treatment can prevent various complications even to blidness, the use of molecular diagnosis method for ophthamic infection can be recommended.

Acknowledgement

"This research was supported by the 2014 scientific promotion program funded by Jeju National University"

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