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Case Report

Roussoella solani causing keratomycosis, with an observed both sexual and asexual morphs

Kiyofumi Mochizuki [a, *], Takashi Nishida [a], Kazuhiro Murata [a], Kyoko Ishida [a, b], Atsuko Sunada [c], Seishi Asari [c], Kiyofumi Ohkusu [d], Kazuaki Tanaka [e]

[a] Department of Ophthalmology, Gifu University, Graduate School of Medicine, Gifu, Japan
[b] Department of Ophthalmology, Toho University, Ohashi Medical Center, Tokyo, Japan
[c] Department of Medical Technology, Osaka University Hospital, Osaka, Japan
[d] Department of Microbiology, Tokyo Medical University, Graduate School of Medicine, Tokyo, Japan
[e] Faculty of Agriculture and Life Science, Hirosaki University, Aomori, Japan

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ABSTRACT

We describe an 82-year-old male farmer who had diabetes mellitus with no history of ocular trauma by soil or plants and who developed a corneal infection due to a fungus. The organism was identified as *Roussoella solani* based on both the morphological characteristics and phylogenetic analysis using LSU and ITS nrDNA sequences. The sexual stage of *R. solani* is described and illustrated for the first time. The patient was treated successfully with a combination of topical and systemic voriconazole and micafungin. This case is the first report of keratomycosis caused by *R. solani*.

1. Introduction

Although keratomycosis is a rare and serious corneal infection, it is common, especially in the tropical and subtropical regions [1,2] and also present in Northern European countries and Japan. The main factor predisposing to the infection is ocular trauma and it is common among agriculture workers. *Fusarium*, *Aspergillus* and *Candida* are the most common pathogens of mycotic keratitis [1,2].

Roussoella spp. are a saprobic fungi found mainly from bamboo and palms [3,4]. *Roussoella* spp. rarely causes human infections, and to the best of our knowledge, *Roussoella* spp. has never been reported to be the cause of any type of ocular infections. We report a first case of keratomycosis, in which *Roussoella solani* was identified as the causative pathogen by both their morphological characteristics and phylogenetic analyses.

2. Case report

An 82-year-old male grape farmer with diabetes mellitus visited our hospital complaining of a foreign body sensation in his right eye. There was no history of ocular trauma caused by soil or plants. Our examination showed that his best-corrected visual acuity (BCVA) was 18/200 in the right eye (OD) and 12/20 in the left eye (OS). Slit-lamp examination revealed multifocal corneal infiltrates with mild conjunctival injection (Fig. 1A). Laboratory tests showed no signs of systemic inflammation. He was diagnosed with keratomycosis because microscopic examination of corneal scrapings revealed fungal filaments. Medical treatment was started with frequent topical voriconazole, amphotericin B, and pimaricin, as well as intravenous voriconazole and oral itraconazole. However, the corneal lesions did not improve and inflammation in the anterior segment increased with a new hypopyon (Fig. 1B and C). One month later, the topical amphotericin B, pimaricin, and oral itraconazole were discontinued based on the results of antifungal sensitivities tests and corneal toxicity, and the treatment was replaced by topical micafungin and intravenous micafungin. A few days after beginning of the new treatment, the corneal infiltration gradually decreased. After 10 weeks, the corneal epithelium and stroma healed leaving a mild corneal scar. Intravenous voriconazole and micafungin were discontinued and the treatment was replaced by oral voriconazole. Then, the patient was discharged. The administration of voriconazole was discontinued 3 months after the discharge, and the antifungal drops were stopped 6 months

* Corresponding author. Gifu University, Graduate School of Medicine, 1-1 Yanagido, Gifu-shi 501-1194, Japan. Fax: +81 58 230 6289.
E-mail address: mochi-gif@umin.ac.jp (K. Mochizuki).

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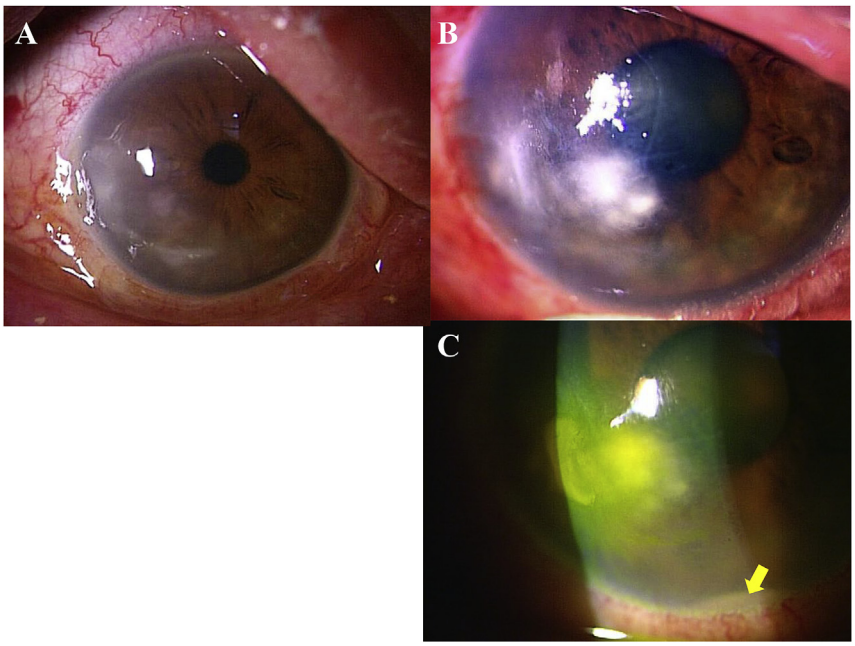

Fig. 1. Findings in a patient with *Roussoella solani* keratitis. A. Appearance of the right eye at the initial examination. B. Four weeks later, increased corneal lesions. C. One month later, anterior segment inflammation with new hypopyon (arrow).

after the discharge. At the six-month follow-up examination, his BCVA was 20/200 OD because of cataract development. No recurrence of the infection has been observed for two years.

The minimal inhibitory concentrations (MIC) to amphotericin B, flucytosine, fluconazole, itraconazole, miconazole, micafungin, voriconazole, and pimaricin were 0.25, >64, >64, 8.0, 4.0, <0.015, 0.12 and 4.0 μg/mL respectively.

3. Materials and methods

Fungal cultures on Sabouraud dextrose agar plate yielded a grayish-white mycelial colony (isolate Gifu-U). Induction of asexual/sexual fructification in culture was attempted by culturing the isolate (Gifu-U) on rice straw agar. After sporulation of the isolate, a single ascospore isolate (KT 3264) and a single conidium isolate (KT 3265) were further obtained to confirm the teleomorph–anamorph connections of this fungus. The micromorphology and growth characteristics of the isolates were studied using the methods described in detail by Tanaka et al. [3]. DNA from mycelia was extracted using the ISOPLANT Kit (Nippon Gene, Japan) according to the manufacturer's instructions. Partial sequences of the large ribosomal subunit (LSU) and those of the internally transcribed spacer (ITS) regions of nrDNA were determined for identification of the three isolates. These regions were amplified by the polymerase chain reaction (PCR) using the primer pairs LR0R–LR7 [5] for LSU and ITS1–ITS4 [6] for ITS. PCR amplification was performed as described by Tanaka et al. (2009). The PCR products were sequenced directly at SolGent (South Korea).

4. Results

4.1. Fungal identification

LSU and ITS sequences were obtained from the three examined isolates. A BLAST search using the LSU sequences of our isolates indicated that this fungus is *R. solani* (KX228312; 774/774 = 100% similarity). The closest hits using the ITS sequences of our fungus was also *R. solani* (KX228261; 554/557 = 99.5% similarity). The sequences were identical among three isolates, suggesting that both asexual and sexual morphs observed in this study belong to the same species. The morphological features of the asexual morph of our isolates were consistent with the original description of *R. solani* which had no information on sexual morph [7]. We identify our isolates as *R. solani* based on molecular and morphological data.

Roussoella solani [8] (Fig. 2)

Sexual morph: Ascomata perithecial, 190–280 μm high, 170–300 μm diam, globose. Ostiolar neck papillate, 90–100 μm long, 60–80 μm diam. Ascomatal wall 12.5–17.5 μm thick at sides. Pseudoparaphyses numerous, branched. Asci cylindrical, fisitunicate, 76.5–85 (–94) × 4.5–5.5 μm, with 8 uniseriate ascospores. Ascospores 7.5–9 × 3.5–4 μm, l/w 2.2–2.5, fusiform to cylindrical, 1-septate, pale brown, with striate ornamentation, without sheath.

Asexual morph: Conidiomata pycnidial, 130–230 μm high, 110–180 μm diam, globose, ostiolate. Conidiomatal wall 7.5–11.5 μm thick at sides. Paraphyses and conidiophores absent. Conidiogenous cells lageniform, 5–10 × 3.5–5 μm, phialidic. Conidia cylindrical to subglobose, 3.9–5.3 × 1.9–2.2 μm, l/w 1.9–2.8, aseptate, hyaline to pale brown, smooth.

Colonies on potato dextrose agar (Difco) 30–45 mm after 20 d at 25 °C in the dark, White to Lavender Grey (125) [9]; Rosy Buff (61) to Greyish Rose (55) pigment produced.

Materials examined: Japan, Gifu, Gifu University, mycelial isolate from diseased eye, Gifu-U = MAFF 245430. Single ascospore isolate from ascomata of Gifu-U, KT 3264 = MAFF 245431. Single conidium isolate from conidiomata of Gifu-U, KT 3265 = MAFF 245432.

Sequences: LC195211 (LSU) and LC195220 (ITS) from Gifu-U; LC195209 (LSU) and LC195218 (ITS) from KT 3264; LC195210 (LSU) and LC195219 (ITS) from KT 3625.