New Filamentous Fungus *Sagenomella chlamydospora* Responsible for a Disseminated Infection in a Dog

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Received 30 September 2002/Returned for modification 27 November 2002/Accepted 2 January 2003

A filamentous fungus that caused a fatal systemic infection in a dog has been identified as the new species *Sagenomella chlamydospora*. When the case was initially reported, the fungus was identified as *Paecilomyces* sp. This study emphasizes how difficult can be the identification of the causative agent of an infection when an uncommon microorganism is involved. This is the first time that this genus has been involved in animal infections, including humans.

Recently García et al. (7) reported and illustrated a case of disseminated mycosis in a dog (a German shepherd cross). The fungus was isolated in the postmortem examination from lesions in numerous organs, i.e., the kidneys, mitral valve, abdominal aorta, and vertebral discs. The diagnosis was difficult because the clinical symptoms were very unspecific. The first signs were multiple discospondylitis of unknown origin and moderate cervical rigidity. A complete blood test, antibacterial serology, and urine analysis showed no abnormalities. However, the animal had a history of depression, anorexia and weight loss, listlessness, and slight spasticity in the walk. The fungal etiology of the infection was recognized when an aspergillosis enzyme-linked immunosorbent assay, using a crude extract of *Aspergillus fumigatus* mycelium as an antigen, was positive. A presumptive diagnosis of systemic aspergillosis was established, and the dog was treated with oral ketoconazole (200 mg/8 h) with clinical improvement, although the enzyme-linked immunosorbent assay for anti-*Aspergillus* remained positive. The dog died suddenly. The postmortem examination of tissue sections from various organs stained with hematoxylin and eosin revealed the presence of abundant branched, septate, hyaline hyphae (see Fig. 6 to 8 of reference 7). A single fungus was isolated from all those examined organs. It was initially identified as *Paecilomyces* sp., and the case was published (7). Later, the case strain was sent to the Faculty of Medicine of the Rovira i Virgili University in Reus, Spain, for specific identification.

The fungus was inoculated on malt extract agar (MEA) (Oxoid, Basingstoke, England) and oat meal agar (30 g of oat flakes, 1 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.5 g of KH_2PO_4 , 15 g of agar, 1,000 ml of tap water), and incubated at 25, 37, and 40°C in the dark. Growth ranges and colony morphology at 25°C were very similar on both media. The colonies reached 11 to 13 mm in diameter after 7 days. They were white to cream colored, cottony at the center, and membranous towards the edge (Fig. 1A). The reverse was colorless, but became pale brown at the

center with age. The vegetative hyphae were hyaline, 1 to 2 μm wide, smooth walled, and usually formed strands from which simple conidiogenous cells (phialides) and chlamydo-spores rose perpendicularly (Fig. 1B and 2A). The phialides were predominantly lateral, hyaline, smooth walled, cylindrical, sometimes centrally swollen, and usually tapered towards the apex, 3 to 15 μm long by 1 to 2.5 μm wide, with a short cylindrical collarette and internal wall thickening at the tip (Fig. 1C and 2B). The conidia were hyaline, smooth walled or slightly rough, pyriform, fusiform, or ellipsoidal, 2.5 to 6.5 μm long by 1.5 to 4 μm wide, and with distinct connectives (narrowed and thickened structures formed by a differentiation of the conidial wall) on both ends and formed coherent chains (Fig. 1C and D and 2C). An abundant production of chlamydo-spores, often more abundant than conidia, was a typical feature of this fungus. The chlamydo-spores were solitary, usually arising on short stalks, hyaline, smooth and thick walled, unicellular, globose, subglobose, or obpyriform, occasionally broadly ellipsoidal, and 5 to 8 μm long by 4 to 7 μm wide (Fig. 1D and 2D).

Colonies on both media grew faster at 37°C than at 25°C and reached a diameter of 19 to 20 mm after 7 days. They were cream colored, with a sparse aerial mycelium, slightly rugose at the center, waxy, and radially folded toward the periphery. Only chlamydo-spores with morphological features similar to the ones described above were observed. The fungus did not grow at 40°C.

Living cultures of the case isolate were deposited in the Faculty of Medicine in Reus (Spain) as FMR 7371, in the CABI Bioscience in Edgware (England) as IMI 387422, and in the Centraalbureau voor Schimmelcultures in Utrecht (The Netherlands) as CBS 109945.

The members of the hyphomycetous genus *Sagenomella* are mainly characterized by their slow growth on MEA, the predominance of simple phialidic conidiogenous cells, often centrally swollen, and their conidia with connectives on both ends, which form coherent chains. *Sagenomella* was reported by Gams (5) to accommodate some new species morphologically similar to the genera *Acremonium*. Additionally, other known *Acremonium* and *Paecilomyces* species (4, 10) were transferred to that genus. Some of these species had been previously included in the genus *Saghamala* by Subramanian (13, 14) on

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FIG. 1. *S. chlamydospora* IMI 387422. (A) Colonies growing on MEA at 25°C. (B to D) Chlamydospores and phialidic conidiogenous cells with conidia in cohering chains.

the basis of how phialidic conidia cohere to each other to form chains. However, after an accurate study of the conidial chain ontogeny of different hyphomycetes, Gams (5) considered *Sagrahamala* a synonym of *Acremonium*. *Sagenomella* currently encompasses 12 species, including the anamorphs of *Sagenoma ryukyuensis* and *Talaromyces ocoitl*, two ascomycetes which belong to the family Trichocomaceae (Eurotiales) (3, 5, 8, 15). However, none of the species of *Sagenomella* already described has the conjunction of morphological features observed in this case strain, and it is therefore described below as a new species. It is so named because of its abundant production of chlamydospores.

***Sagenomella chlamydospora* Gené et Guarro, sp. nov.** Coloniae in vitro albae vel cremeae, gossypinae. Hyphae vegetativae leves, 1 to 2 μm latae. Phialides orthotropicae, plerumque simplices, cylindrico cum apice attenuatum, 3 to 15 by 1 to 2.5 μm . Conidia in catenulis cohaerentibus, hyalina, levia vel leniter aspera, pyriformia, fusiformia vel ellipsoidea, 2.5 to 6.5 by 1.5 to 4 μm . Chlamydosporae copiosae, laterales, hyalinae, aseptatae, plerumquam globosae vel subglobosae, 5 to 8 by 4 to 7 μm .

Holotype. IMI 387422, from disseminated infection in a dog. *Sagenomella alba*, *Sagenomella sagenomatis*, and *Sagenomella sclerotialis* (5, 12) are the morphologically closest species

FIG. 2. *S. chlamydospora* IMI 387422. (A) Chlamydospores and phialidic conidiogenous cells with conidia in cohering chains on a hyphal strand. (B) Phialidic conidiogenous cells on a vegetative hypha. (C) Conidia with connectives in cohering chains. (D) Chlamydospores arising on short stalks from a vegetative hypha.

to *S. chlamydospora*, but they have smaller conidia and no chlamydospores. In addition, *S. alba* does not grow at 37°C, *S. sagematis* can grow above 40°C, and *S. sclerotialis*, although its growth at 37°C is luxuriant, always produces globose sclerotia. The latter structures have never been observed in the new species. The only *Sagenomella* species that develop chlamydospores are *Sagenomella humicola* and *Sagenomella verticillata*, but both form phialides grouped in verticils. The chlamydospores in *S. humicola* are dark brown, and the ones in *S. verticillata* are subhyaline to brown and often disposed in chains. Furthermore, neither *S. humicola* nor *S. verticillata* grows at 37°C.

The initial identification of the case strain as *Paecilomyces* sp. seems logical because some of the morphological features (conidia and chlamydospores) of the most common species of the genus, *Paecilomyces variotii*, are similar to those observed in the present species. Although simple phialides are not typical of *P. variotii*, the phialidic arrangement in some strains of such species can be greatly influenced by the growth conditions. Occasionally, conidiophores can be reduced to simple phialides. The possibility that the case strain is a host-adapted disgonic *P. variotii*, analogous to the host-adapted *Aspergillus fumigatus* often seen in chronic infections, was also considered. *P. variotii* colonies are typically buff or tan colored and very different from those observed in the present fungus. However, it is possible that if the fungal conidiation is sparse because of host adaptation, the normal colony color, which derives from profuse conidiation, might not be detectable. Several cases of disseminated infection by *Paecilomyces* spp. in dogs (7) and other animals (2) have been reported. The recognition of a species different from *P. variotii*, or other fungi of clinical relevance, was confirmed by molecular analysis. The internally transcribed spacer (ITS) regions and 5.8S ribosomal DNA gene of the clinical isolate were sequenced using the ITS-5 and ITS-4 primers (17), and a BLAST sequence homology search was performed in the GenBank database (1). Surprisingly, the

closest sequence belonged to a strain of *Eupenicillium lassenii* (NRRL 5272), another ascomycete of the Trichocomaceae but with a *Penicillium* anamorph that is morphologically completely different from *Sagenomella* spp. However, the similarity between the two sequences was very low (91%). The sequence of the case strain was also compared with the sequences of two strains of *P. variotii* (AF033395 and AF291870) deposited in GenBank, and the similarity values were lower than 86.06%. The sequence of the case strain was deposited in GenBank with the accession number AJ519984.

The in vitro activity of amphotericin B, flucytosine, ketoconazole, itraconazole, ravuconazole, terbinafine, and voriconazole against the case strain was determined by a broth microdilution method (11), mainly following to the guidelines of the National Committee for Clinical Laboratory Standards for molds (9). The test was carried out using RPMI 1640 medium buffered to pH 7 with 0.165 M morpholinepropanesulfonic acid, an inoculum of 10^4 CFU/ml, an incubation temperature of 30°C, an incubation time of 96 h, and an additive drug dilution procedure. MICs were 1 µg of amphotericin B per ml, 0.12 µg of ketoconazole per ml, 0.25 µg of itraconazole per ml, 128 µg of fluconazole per ml, 0.03 µg of terbinafine per ml, 0.03 µg of ravuconazole per ml, 1 µg of voriconazole per ml, and >128 µg of flucytosine per ml. The dog was unsuccessfully treated with ketoconazole, even though its in vitro antifungal activity was good. When the fungal infection was diagnosed, the infection may already have been widely disseminated in the organism, creating an almost certainly irreversible situation (7).

The detection of fungal infections in animals is difficult. It requires expert clinicians with plenty of experience and considerable knowledge of mycology. Most mycoses can be easily confused with other conditions, since the clinical picture of the animal systemic mycoses is very unspecific, making clinical diagnosis very difficult. In dogs, the first signs are usually peripheral nervous signs, mainly lameness, spinal pain, and lethargy, all as consequences of the mold settling in the spinal column (6, 7). The causative agent needs to be promptly diagnosed and identified, usually by a joint microbiological and histopathological study, if the infection is to be correctly treated and resolved. This is also very important because it reduces the risk of the infection spreading to humans (16).

This study emphasizes how complex and difficult it can be to diagnose and identify the etiologic agent of an infection. In animals, this is still more complicated than in humans because there is less experience and the information available on fungal infections is scarce. In the present case, the fungal origin of the infection was recognized only through a cross-reaction by an anti-*Aspergillus* ELISA. The symptoms were unspecific, and the fungal origin was not confirmed until the postmortem examination, when a unique fungus was repeatedly isolated from different organs and their histopathological examination revealed the presence of compatible fungal structures. The identification of the causative agent has also been complex because it is the first time that this species has been found and that the genus *Sagenomella* has been involved in animal infections, including those of humans. Indeed, it is a very rare fungus, characterized by poorly differentiated reproductive structures, which can be easily confused with degenerated better-known fungi, such as species of *Paecilomyces* or *Acremonium*. Molec-

ular analysis was useful in reconsidering the initially wrong mycological diagnosis.

We are indebted to R. Summerbell and W. Gams (Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands) for their comments on identifying the fungus.

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