Recurrent Colonization of Successively Implanted Tracheoesophageal Vocal Prostheses by a Member of the \textit{Fusarium solani} Species Complex


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Tracheoesophageal vocal prostheses (TVP) in laryngectomized patients commonly deteriorate due to overgrowth by yeasts, particularly \textit{Candida} species. We describe the first case of colonization of such devices by a member of the \textit{Fusarium solani} species complex in a patient with a history of glottal carcinoma. Three isolates, from three prostheses, were found morphologically consistent with the traditional picture of \textit{F. solani}. Ribosomal sequence analysis showed that the isolates belonged to a distinct, as yet apparently unnamed phylogenetic species within the \textit{F. solani} species complex. This species, one of two distinct genetic types (genotype 2) traditionally considered part of the plant-pathogenic subtaxon \textit{Fusarium solani} f. sp. radicicola, has not previously been identified as an agent of human or animal disease, although it is closely related to a known etiologic agent of mycetoma, an \textit{Acremonium}-like species recently renamed \textit{Fusarium falciforme}. Sequence and multisatellite M13 polymorphism analysis revealed no distinctions among the case isolates. Production of cyclosporine was detected for all three case isolates.

The presence of microorganisms on medical prostheses implanted in the human body is undesirable for two reasons. First, the prosthesis may represent a local infection focus or act as a reservoir shedding the microorganisms into the circulation. Second, the microorganisms may cause physical deterioration of the device, thus necessitating its replacement. A typical example of the second phenomenon is the failure of tracheoesophageal voice prostheses (TVP) following their colonization by \textit{Candida} spp. (4). These TVP consist of a silicone tube containing a low-pressure one-way valve, which only permits airflow from the trachea into the esophagus. They are implanted in laryngectomized patients to restore their speech. Extensive biofilm formation by implanted in laryngectomized patients to restore their speech

petent hosts and aggressive disseminated infections in neutropenic patients (8).

Generally, in reports of novel and interesting fungal infections, an attempt is made to identify the causal agent at least to the species level. With members of the \textit{F. solani} complex, an unusual situation exists that has long caused problems with true species-level analysis. It has been known for more than 40 years that \textit{F. solani}, as traditionally conceived, was a morphologically defined aggregate taxon that actually consisted of multiple separate species, including several mutually intersterile mating populations of the type universally recognized as well-defined biological species (21). Only very recently, however, has formal nomenclature begun to be established for these species (1). Historically, the reason was that they could not readily be identified by microscopy; at present, these species are distinguishable by molecular analysis, but it is not yet clear how many of them have existing valid names among the large number of \textit{Fusarium} names synonymized with \textit{F. solani} by Wollenweber and Reinking (33) and others. Research on this matter is ongoing and involves both analysis of old collection cultures and attempts to re-collect representative material from plant hosts mentioned in original species descriptions. In the meantime, within this species complex, it is possible to identify clearly at least 40 biological and phylogenetic species (the latter mostly delineated by O'Donnell [21] following the phylogenetic species concepts of O'Donnell et al. (20) that cannot as yet be matched with conventional names. Species identification of medically relevant fusaria is important, at least prospectively, because it may correlate with patterns in epidemiology, pathogenicity, and drug response (15). The present report documents the first case in which a very distinct phylogenetic species known so far only under the anachronistic

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name Fusarium solani f. sp. radicicola genotype 2 has been confirmed as becoming established in a human patient, and specifically, causing a problematic in situ colonization of a medical device. An examination of published sequences from Fusarium solani-like species, the patient received a detailed question-naire asking for information about his possible exposure to grains, hay, etc. However, no definitive indication of obvious exposure to causal agents was found. Since the patient did not have impaired immunity and no signs of infection were ob-served, no additional antymycotic therapy was prescribed. The second prosthesis was removed and replaced on 30 May 2002 and was also found to harbor a filamentous fungus, later confirmed to be an F. solani-like strain. To check for possible carryover, swabs from the oral cavity and the airways and finger and toenail clippings from the patient were screened for the presence of fungi. All swabs were negative for fungal growth. No swabs were taken from the fistula or stoma, since new prostheses are mostly inserted as quickly as possible, after aspiration of blood and saliva. Thus, carryover through contact with a contaminated surface (of the fistula) is highly possible. The third prosthesis was removed and replaced on 3 July 2002; it also contained an F. solani-like strain.

**MATERIALS AND METHODS**

Isolation. As part of a larger epidemiological study, mycological examinations were routinely carried out on removed TVP in the Laboratory for Pharmaceutical Microbiology. The TVP were submerged in 10 ml of phosphate-buffered saline, pH 7 (containing 10 mM potassium phosphate), and vigorously mixed on a vortex type mixer (Labo-Tech, Biel-Benken, Switzerland) to release the adherent biomass. The resulting suspension was filtered over a Nylaflo [diameter, 47 mm; pore size, 0.45 μm] Gelman Sciences, Ann Arbor, Mich.) and subsequently placed on CHROMagar Candida (CHROMagar Co., Paris, France), which was incubated for 48 h at 37°C (4).

Primary identification. Filamentous fungi were cultured on either Sabouraud dextrose agar (SDA; Becton Dickinson [BD], Franklin Lakes, N.J.), potato dextrose agar (PDA; BD), synthetic nutrient-poor agar (SNA) (11), or malt extract agar (containing 20 g of Bacto powered malt extract, 1 g of Bacto peptone, 20 g of glucose, and 20 g of Bacto agar per liter [pH 5.6]) at 20°C in the dark. Microscopic examinations were performed on lactophenol cotton blue and cellulose acetate tape mounts of cultures. To distinguish among Fusarium species with and without opportunistic pathogenic potential, growth at 37°C and cycloheximide resistance (25) were evaluated. To this end, the three strains were incubated on SDA at 37°C for 2 weeks and on SDA supplemented with cycloheximide (0.5 mg/liter) and chloramphenicol (0.05 mg/liter) at 28°C for 3 weeks.

Molecular identification. For sequence-based identification, the three serial isolates analyzed were grown in liquid complete yeast medium. The FastDNA kit (Bio 101, Carlsbad, Calif.) was used to extract DNA according to the manufacturer’s instructions. Amplification was performed using primers V9G and LR5 (7, 30), designed to span the internal transcribed spacer (ITS) region of nuclear rRNA genes, and primers V9D (7) and LR5 (30) for the large-subunit (LSU) rRNA gene region. A PCR System 9700 thermocycler (Perkin-Elmer Applied Biosystems, Foster City, Calif.) was programmed to perform initial denaturation at 94°C for 2 min; 35 cycles of 94°C for 35 s, 55°C for 50 s, and 72°C for 2 min; a final extension at 72°C for 6 min; and chilling to 4°C. The reaction mixtures of 50 μl contained 1 μl of genomic DNA extract, 25 pmol of each primer, 200 μmol of each deoxynucleoside triphosphate (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, United Kingdom), 1 U of Taq polymerase (Taq HT Biotechnology, Ltd., Cambridge, United Kingdom), and 1× standard PCR buffer supplied with the Taq polymerase. After purification of the PCR products with the GFX PCR DNA and gel band purification kit (Amersham Pharmacia Biotech) and verification of final concentrations, PCR products were sequenced by using the BigDye terminator cycle sequencing kit (Applied Biosystems) with primers ITS1 and ITS4 (32) for the ITS region, as well as NLI, NL4 (19), and LR5 (31) for the LSU. Sequencing was carried out on an ABI Prism 3700 instrument (Applied Biosystems). Sequence editing was done in Seqman II software (DNAStar, Madison, Wis.). New and published sequences were aligned in Bionumerics 3.5 (Applied Maths, Sint Martens-Latem, Belgium). For ITS analysis, after the alignments of O’Donnell (21) downloaded from the Treebase website (http://www.treebase.org/treebase/) had been examined, small areas of uncertain alignment were removed prior to phylogenetis analysis; LSU align-ment was without ambiguity. The few remaining gaps in the ITS were treated as a fifth character, and all characters were treated as unordered and of equal weight. Maximum-parsimony analysis was performed in PAUP, version 4.0b10 (28), using the heuristic search option with 1,000 random taxon additions and with tree bisection and reconnection as the branch-swapping algorithm. Boot-strap support values were calculated from 1,000 replicates. Neighbor-joining (NJ) analysis was also performed for the LSU data and was run with the PAUP default “uncorrected ‘p” setting. Comparison was also performed with the Jukes-Cantor and Kimura two-parameter algorithms for NJ.

**Strain typing.** The genetic homogeneity of the three serial case isolates was further investigated by randomly amplified polymorphic DNA (RAPD) analysis.
The M13 primer (5'-GAGGTTGCGGTTCT-3'), a moderately repetitive minisatellite region (22), was used according to the procedures of Gräser et al. (13). Briefly, the PCR mixture used consisted of 1 μl of a DNA sample from each test isolate, 5 μl of 10X PCR buffer from the source noted above, 33.5 μl of ultrapure sterile water (Mill-Q gradient system; Millipore, Brussels, Belgium), 10 μl of a 5 mM mixture of deoxynucleotide triphosphates (Amersham Pharmacia Biotech), 2.5 μl of a 250 μM solution of M13 primer, and 0.4 μl of a 3-U/μl solution of Taq polymerase. After initial denaturation at 94°C for 2 min, 40 cycles consisting of 94°C for 30 s, 50°C for 1 min, and 72°C for 2 min were run, followed by a final extension at 72°C for 7 min and cooling to 4°C. Electrophoretic separation was performed for 15 min at 50 V, followed by 100 V for approximately 3 h until good band separation was obtained.

Cyclosporine production. The three F. solani complex strains obtained from our case patient were also tested for cyclosporine production. F. solani sensu lato (i.e., name used in the traditional broad sense) isolate ATCC 46829 (American Type Culture Collection, Manassas, Va.) and Penicillium italicum ATCC 3200 were used as positive and negative controls, respectively (25). In addition, the patented Quorn strain Fusarium venenatum, deposited as Fusarium graminearum (ATCC 20334), was tested as a negative control within Fusarium. The six strains were grown on two different media to induce cyclosporine production: a modified liquid culture medium (MLCM) (5% sucrose, 0.2% Bacto yeast extract, 0.2% Bacto peptone, and 0.01% Tween 80) and SDA Emmons (SDAE) (5 g of Bacto Casitone, 5 g of Bacto beef extract, 20 g of glucose, 20 g of Bacto agar). Negative controls consisting of noninoculated media were also included in the assay.

For the MLCM, the strains were precultured on PDA (BD) at 25°C for 7 days. A 0.5-cm² agar plug taken from the advancing margin of each culture was used to inoculate 30 ml of MLCM. The inoculated media were subsequently incubated for 4 days at 28°C. After incubation, 30 ml of acetonitrile was added to each culture, followed by a brief mixing. Three grams of ammonium sulfate was added to separate the two layers, and a 5-ml aliquot of the upper layer was evaporated to dryness under nitrogen.

The five strains were also grown on SDAE for 7 days at 28°C. Four agar plugs were cut out of the plate culture on the circumference of a 10-mm-radius circle from the center of the dish (the inoculation point) by using a 7-mm (inner diameter) sterile glass tube. Plugs were aseptically transferred to a 10-ml dryness under nitrogen.

The evaporated extracts originating from the MLCM and SDAE trials were first redissolved in 50 μl of absolute ethanol (Romil Ltd., Waterbeach, Cambridge, United Kingdom) and subsequently diluted in 950 μl of cyclosporine-free human blood. Samples were then analyzed by using the Abbott monoclonal fluorescence polarization immunoassay (FPIA) kit (Abbott Laboratories, Abbott Park, Ill.).

Mycoxin production. Prior to full identification of the isolates, in order to carry out a more complete survey of the toxins the patient might be exposed to, a test was conducted for zearealenone and related compounds by using the culture conditions described by O'Donnell et al. (20), the high-performance liquid chromatographic method described by De Saeger et al. (9), and a procedure for extraction of mycotoxins from fungal cells (S. De Saeger, M. M. De Vos, T. G. Bauters, K. Honraet, H. J. Nelis, and C. Van Peteghem, unpublished data). In addition to the three isolates from the prostheses, two laboratory strains (F. graminearum IHEM 2994 and 2995, obtained from the BCCM/IHEM culture collection as CBS 112099, CBS 112100 and CBS 112101, respectively. They were deposited in the Centraalbureau voor Schim-melcultures (CBS) culture collection as CBS 112099, CBS 112100 and CBS 112101, respectively. More-detailed examination on the standard Fusarium media PDA and SNA revealed a growth rate of 54 mm in 7 days at 21°C. Colonies on PDA, a medium used for macromorphology, showed cottony white surface growth and a dilute yellowish brown to reddish brown colony reverse. On SNA, microconidia 7.5 to 14.8 by 2.1 to 4.7 μm (1 cell) or 16 to 24.9 by 4.1 to 5 μm (2 cells) were produced on mostly elongated monophialides as long as 65 μm. The former, often contiguous integrated into equally thin 1- to 3-cell conidiophores for an overall phialide plus conidiophore length of as much as 106.5 μm. Microconidia (Fig. 3) were scarce and were produced on relatively short monophialides. They were mostly 4 cells long, with bluntly rounded apices and obscurely delineated foot cells, and measured 29 to 38.3 by 4.8 to 6.0 μm. Chlamydospores were frequently observed. These findings allowed a more accurate morphological identification of the isolates as F. solani sensu lato. The strains grew well both on SDA at 37°C and on SDA supplemented with cycloheximide and chloramphenicol at 28°C, characteristics proposed by Sugiura et al. (25) as excluding F. solani complex members unequipped to cause opportunistic infection.


showed numerous microconidia of different shapes in false heads on long monophialides with distinct collarettes. The macroconidia had several transverse septa and indistinct foot cells. No polyblastic conidiogenous cells or sporodochia were observed. The three isolates were preliminarily identified as Fusarium spp. based on characteristics given by de Hoog et al. (8). They were deposited in the Centraalbureau voor Schimmelmicroarten (CBS) culture collection as CBS 112099, CBS 112100 and CBS 112101, respectively.

RESULTS

Primary isolation and identification. The CHROMagar cultures derived from the removed prostheses all revealed cottony structures inconsistent with Candida species. Colonies of all three isolates grew rapidly on SDA, attaining a diameter of more than 5 cm in 7 days. On both SDA and malt extract agar, they were white and woolly in texture, with a pale reverse. The color remained white even after 21 days. Microscopic examination revealed micro- and macroconidia. Tape mounts

The sequences of the case isolates showed numerous microconidia of different shapes in false heads on long monophialides with distinct collarettes. The macroconidia had several transverse septa and indistinct foot cells. No polyblastic conidiogenous cells or sporodochia were observed. The three isolates were preliminarily identified as Fusarium spp. based on characteristics given by de Hoog et al. (8). They were deposited in the Centraalbureau voor Schimmelmicroarten (CBS) culture collection as CBS 112099, CBS 112100 and CBS 112101, respectively.

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Molecular comparison and isolate identification at the phylogenetic species level. The patient isolates showed identical ITS sequences and identical M13 RAPD patterns. The former correspondence, within the F. solani complex, suggests identity at the level of the phylogenetic species (21) or a closely inter-related sibling species complex (1) (note that other Fusarium groups differ markedly in the degree to which ITS sequences are species specific [20]), while the latter suggests high genetic relatedness but, at our current level of knowledge for this
group, does not entail an exact scale, e.g., the species level or the clone level. In order to identify the phylogenetic species within the *F. solani* complex involved in the case, we performed BLAST searches in GenBank with the sequences and made separate alignments for the LSU and ITS regions. Alignments consisted of various members of the *F. solani* complex as defined by Summerbell and Schroers (27) as well as appropriate outgroups. The two ribosomal regions were considered separately, for each locus, there is a different array of potential comparison sequences available in GenBank. Consideration of both loci was needed to clarify species identity to the greatest extent made possible by the published sequences available at the time this paper was written. Isolates involved in previous medical cases, in particular, currently tend to appear as either LSU or ITS sequences, but not as both.

Parsimony analysis of 483 ITS region characters yielded 81 parsimony-informative characters. A single most-parsimonious tree was produced and is shown in Fig. 4A with all bootstrap values exceeding 50% on the relevant branches. The tree had a length of 256 steps, a confidence index (CI) of 0.680, and a retention index (RI) of 0.766. Given the identical ITS sequences found in the case isolates, the more phylogenetically conservative LSU region was sequenced only for a single representative (Fig. 4B). Parsimony analysis of 535 characters yielded just 34 parsimony-informative characters, generating 110 most-parsimonious trees of 82 steps with a CI of 0.634 and an RI of 0.762. A representative tree is shown in Fig. 4B with consensus bootstrap values. The trees differed mainly in the branching order of deep branches involving isolates distantly related to the case isolates. NJ trees as described above were constructed for comparison but differed minimally from the parsimony tree (data not shown).

For convenient reading of Fig. 4, some explanation is required in order to minimize confusion caused by the difficult nomenclature currently in broad use for the *F. solani* complex. Firstly, both GenBank and *Fusarium* systematists follow the International Code of Botanical Nomenclature governing fungal names (14) and give preference to the corresponding teleomorph (sexual-state) name, *Nectria haematococca*, for isolates known to mate. (An alternative teleomorph name, *Haematonectria haematococca*, proposed by Rossman et al. [23], was later formally rejected by O’Donnell [21] because the proposed genus *Haematonectria* was paraphyletic, and the generic name *Neocosmospora* had nomenclatural priority for the overall monophyletic group inclusive of the proposed *Haematonectria* species.) Moreover, the name *N. haematococca* is still, pro tem, used in its traditional, broad “species aggregate” sense; in fact, this “species” is a species complex, with distinct biological species given “mating population” (MP) numbers. Among the nonmating species and isolates of unknown mating status, some have been brought into line with modern nomenclature by being linked with defined phylogenetic species, e.g., *Fusarium culmorum*. The remainder are still, by default, generally called *F. solani* sensu lato, an imperfect solution that nonetheless shows that their affinity is with this species complex rather than with other *Fusarium* clades. By using test plants, some of these “*F. solani*” isolates have been assigned to “forma specialis” categories based on their plant disease associations. Since some of these category names have epidemiological significance, indicating which plants harbor potential human pathogens, they are included in Fig. 4 where not superseded by teleomorph MP designations.

Analysis of both ITS and LSU loci yielded a consistent picture showing that the patient isolates belong to a phylogenetic species encompassed within the broader scope of clade 3 as defined by O’Donnell (21). This species has not as yet been linked to a valid species name. The phylogenetically distinct nature of the species in question was supported by a high ITS bootstrap value (98%). Also included in this species besides the case isolates are isolates from ginseng and konjak (Amorphophallus konjac, a southeast Asian food crop) that were classified by Suga et al. (24) as *F. solani* f. sp. radicicola. This forma specialis, however, was shown by Suga et al. (24) to consist of two genetic clusters: one included the isolates just mentioned, while the other was distantly related and contained mainly isolates from plants in the family Solanaceae, including potato, the type host of *F. solani* f. sp. radicicola. Hereafter we refer to the phylogenetic species containing the solanaceous isolates as *F. solani* f. sp. radicicola genotype 1 and to the group incorporating our case isolates as *F. solani* f. sp. radicicola genotype 2.

**Cyclosporine and mycotoxin production.** The three case isolates and the *F. solani* complex reference isolate ATCC 46829 were shown to produce cyclosporine (Fig. 5). The highest cyclosporine levels were detected in MLCM; they reached 2,263 ng/g of medium for the control strain and 912, 1,254, and 681 ng/g for the first, second, and third case isolates, respectively. Statistical analysis of these data included a log transformation used to map the data to a normal distribution (P value, 0.045 by a homogeneity-of-variances test), a one-way analysis of variance (ANOVA), and post hoc tests (Scheffé statistic). The ANOVA gave a P value of <0.001, and the post hoc tests revealed two homogenous subsets at the 0.05 level. The lower subset included *P. italicum* and the *F. venenatum* Quorn strain. The higher subset included the three clinical isolates and the positive-control strain. Neither of the negative controls (the *P. italicum* strain and the Quorn strain) gave detectable production of cyclosporine on either of the two media.

While both *F. graminearum* strains produced detectable
quantities of zearalenone, the patient isolates gave completely negative results for this and all similar compounds.

DISCUSSION

This is the first report of the occurrence of an opportunistic filamentous fungus on a silicone vocal prosthesis. In general, Candida species and bacteria are the main colonizers of TVP (10) and the main causes of prosthesis failure. Bauters et al. (3) suggested that besides progressively damaging the functionality of TVP, biofilms harboring opportunistic pathogens could act as a reservoir for infection. Fusarium species generally do not cause invasive systemic disease in immunocompetent or mildly immunocompromised patients (26). They are, however, notorious producers of various carcinogenic and immunosuppressive mycotoxins (18) and thus may not be innocuous when growing on devices such as vocal prostheses, which are in contact with the gastrointestinal and respiratory tracts. Members of the F. solani species complex, in particular, have become increasingly well known in recent years as producers of potentially immunosuppressive cyclosporines (25).

In this study, this new, potentially harmful quality of fungal biofilms on prostheses has been evidenced. Besides the known infection risk posed by the fungus if the patient were to become sufficiently immunocompromised due to medical circumstances unrelated to the vocal prosthesis, e.g., during the course of later chemotherapy, the continuous production of hazardous compounds such as mycotoxins or cyclosporine can present an additional risk for the patient. In vitro production of cyclosporine by the case strains was relatively high in proportion to the expected high levels produced by the positive-control isolate used in the assay. It is likely that the localized in vivo production of high levels of cyclosporine, a known immunosuppressant, could aid in the development of a localized or perhaps even a more extended infection. Unfortunately, no information is available that would allow estimation of the amount of fungal mycelium on the prosthesis; therefore, the patient’s exposure level cannot be estimated. It is not clear whether in situ production of cyclosporine posed any risk to the patient. It is in general of considerable concern that a medical device would support a potentially opportunistic fungus producing an immunosuppressant, in addition to potentially irritating antigens and various other substances of unknown effect, such as fungal extracellular enzymes and naphthoquinone mycelial pigments released by shearing and natural hyphal turnover. Moreover, reports show that if patients harboring a member of the F. solani species complex undergo immunosuppressive therapy, their fusaria can become devastatingly invasive and rapidly fatal (2, 12). Clearly, colonization of TVP may not be essentially harmless, as is commonly thought, and requires careful monitoring.

Phylogenetic identification of the TVP strains led to an interesting situation reflective of the transitional period in which fungal systematics now finds itself. The case isolates are the first representatives of their possibly unnamed and relatively poorly known phylogenetic species to be implicated in a medical or veterinary problem. As mentioned above, this species is one of two distinct genetic groups that were broadly classified as F. solani f. sp. radicicola in traditional systematics (24). The patient’s isolates are in a well-defined clade containing mainly isolates from east Asian crop and forest plants such as ginseng, konjak, a Gladiolus sp. cultivated in Japan (with a slightly deviating ITS sequence noted as genotype 2a in Fig. 4A), and (as evidenced only by an identical LSU sequence so far) incense cedar (Cryptomeria japonica). Soybean plants in the United States have been found to yield similar F. solani f. sp. radicicola genotype 2 isolates (e.g., GenBank accession number AF132801 in Fig. 4A) (16); this crop is historically from east Asia and was relatively recently introduced elsewhere in the world, perhaps with its associated microbiota. One isolate phylogenetically and morphologically identical to the case isolates, however, was obtained from nasal mucus of an Austrian of unrecorded residence and travel history (5). The patient in our own case was non-Asian and was not known to have traveled to Asia or to have any extraordinary connection with Asian materials. We conclude that despite the preponderance of Asia-connected isolations so far, it seems likely that F. solani f. sp. radicicola genotype 2 is cosmopolitan in distribution.
Given its isolation from various plant species and decaying plant materials, this species shows an ecological pattern suggesting that it is not a strongly host-specific plant-pathogenic species, unlike many members of *F. solani* sensu lato. It does not appear that any particular plant species can be named as a hazard that vulnerable patients should avoid contact with.

Most other medically important members of the *F. solani* species complex sequenced so far are found at a considerable phylogenetic distance from the present case isolates. For example, the LSU sequences of several opportunistic isolates cluster strongly with that of *N. haematococca* complex MP V, frequently referred to as *Fusarium solani* f. sp. *cucurbitae* race 2 and tentatively identified with the more formally delineated taxon *Fusarium solani* var. *petroliphilum* (27). ATCC 62877, an isolate obtained by Hiemenz et al. (17) from a subcutaneous *Fusarium* infection in a patient wounded by a stingray barb, has an ITS sequence identical to those of isolates representing the core group of *F. solani* f. sp. *radicicola* genotype 1 isolates from the potato and its close solanaceous relative the tomato. Recently, an *F. solani* isolate with an ITS sequence identical to that of ATCC 62877 was recorded as causing foot mycotema in a French trucker subsequent to penetrative injury with a hand cart (34). The human-pathogenic isolates most closely related to the present patient isolates are genetically distinct mycotema isolates long classified as *Acremonium falciforme* but recently transferred and designated *F. falciforme* after phylogenetic membership in the *F. solani* complex was discovered (27).

*F. falciforme* isolates are morphologically distinct: among other differences from our *F. solani* f. sp. *radicicola* genotype 2 patient isolates, they grow slowly and consistently fail to produce macroconidia in culture. Unfortunately, no ITS sequences of *F. falciforme* are available yet. A small but consistent sequence difference in the relatively conservative LSU region (a single A→C transversion), however, tends to corroborate the pheno-
dypic differences, suggesting that *F. falciforme* is a separate species closely related to *F. solani* f. sp. *radicicola* genotype 2.

The importance in general of delineating such phylogenetic species in *Fusarium* infections and bodily surface colonizations lies in the probable connection of correctly recognized phylogenetic species with characteristics such as drug susceptibility and pattern of disease causation (15). For example, it is not known if all or only some of the species in the *F. solani* complex produce cyclosporines: although some of the biological species in this group have been recognized since the 1960s, the individual mycotoxin spectra of these species have not been systematically studied. Nor have most of the recently delineated phylogenetic species been subjected to individual chemical analysis, and it is not known whether all of these species were represented in classical mycotoxin surveys of *F. solani* sensu lato. This deficiency of information impels us to briefly mention the negative result for zearalenone-like compounds we obtained in proactive handling of the case isolates, even though *F. solani* sensu lato is generally stated not to produce compounds in this class.

Characteristics of the fungal “secondary metabolism,” notably production of mycotoxins and mycotoxin-like substances such as cyclosporines, are seen to be closely correlated with accurate species definitions when these are available; without such taxonomic information, interpretation of biological patterns in *Fusarium* is notoriously chaotic (18). It is likely that many pathophysiological and epidemiological characteristics will show similar species-dependent patterns. Much progress is needed in research on the *F. solani* species complex before such characteristics can be correlated with well-delineated and correctly named species. In the meantime, it is recommended that sequences of confirmed case isolates connected to scientific reports be identified if possible and, in any event, that these isolates be deposited in a recognized fungal culture collection (6). In view of the current trend toward sequencing an increasing number of loci, including nonribosomal loci, for species definition (1, 20, 21, 29), it is recommended that current standards in the *Fusarium* group investigated be adhered to whenever molecular identification is performed. In particular, as soon as adequate comparison data become available for newly investigated gene regions shown to be important for precise species recognition, these genes should also be investigated in studies identifying isolates from medical cases.

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