Development of Molecular Methods for Identification of *Schizophyllum commune* from Clinical Samples

WALTER BUZINA,* DORIS LANG-LOIDOLT, HANNES BRAUN,
KURT FREUDENSCHUSS, AND HEINZ STAMMBERGER

ENT University Hospital, Karl-Franzens-University Graz, A 8036 Graz, Austria

In the last 50 years, to our knowledge, only 16 cases of diseases caused by *Schizophyllum commune* in humans have been reported. Within only 6 months, we found four isolates of this basidiomycetous fungus, obtained from patients suffering from chronic sinusitis. The cultures of the isolated fungi showed neither clamp connections nor fruiting bodies (basidiocarps), which are distinctive features for *S. commune*, but fast-growing cottony white mycelium only. This was harvested, and DNA was extracted. The internal transcribed spacer region of the ribosomal DNA (rDNA) was amplified with fungus-specific primers, and the PCR products were sequenced. Two strains of *S. commune*, collected from branches of a European hornbeam (*Carpinus betulus*) and a tree of heaven (*Ailanthus altissima*), respectively; four specimens from the herbarium of the Institute of Botany, Karl-Franzens-University Graz; and two strains from internationally known culture collections (CBS 340.81 [ATCC 44201] and CBS 405.96) were investigated in the same way. The sequence data of all strains were compared and showed homology of over 99% in this 660-bp-long fragment of rDNA. With these results, a map of restriction enzyme cutting sites and a primer set specific for *S. commune* were created for reliable identification of this human pathogenic fungus.

Compared to the great number of ascomycetous fungi which are the cause of many diseases, filamentous basidiomycetes that cause infections are reported rarely in the medical literature. *Schizophyllum commune* Fries 1821 (*Schizophyllaceae*, *Aphyllophorales*) is one of them and has been described elsewhere as a suspected cause of onychomycosis (16), basidioneuromycosis (2), an allergic bronchopulmonary mycosis (12), mucoid impaction of the bronchi (1, 5), a fungus ball in the lung (27), an ulcerative lesion of the hard palate (23), a brain abscess (24), and several cases of both maxillary and allergic fungal sinusitis (4, 6, 14, 18, 25, 28, 29). A review of cases has been published by Kamei et al. (13). This worldwide-distributed shelf fungus is found quite frequently in nature throughout the year, although it mostly appears during the cold season (22). The commonly known split-gill fungus is found on decaying wood, where it appears with sessile fan- or kidney-shaped fruiting bodies. Typical for *S. commune* is the hymenium on the lower side, which consists of longitudinally split gills (lamellae), where masses of basidiospores are produced and released into the air. The fungus is easy to cultivate on most of the media generally used in clinical laboratories. The appearance of fruiting bodies, hyphal clamp connections, and spicules makes identification easy. Nevertheless, monokaryotic clinical isolates lack these unambiguous features, and the mycelium shows up only as a cottony white mass of hyphae without any distinctive marks. In these cases, the fungus is often identified as "Mycelia Sterilia" or misidentified as an ascomycete. In the last few years, many molecular methods, such as PCR, sequencing of segments of the genome, analyses of the restriction fragment length polymorphisms (RFLP), or hybridization of

specific probes, have been described as tools for identification of medically relevant fungi. The highly variable internal transcribed spacer (ITS) region lies in between fragments of ribosomal DNA (rDNA) and is a valuable target for reliable identification of those strains which cannot be identified to the species level by microscopy.

CASE REPORTS

HNO 104. A 60-year-old woman presented with congestion in the right nostril. She had a history of chronic rhinosinusitis with four functional endoscopic sinus surgeries (FESS) done within the last 5 years. Computerized tomography (CT) scanning showed opacification of the ethmoidal and the maxillary sinus. Endoscopy revealed a highly viscous secretion, which was removed. After 2 weeks of antifungal therapy, the patient recovered without complications.

HNO 34. A 51-year-old woman presented with complaints of nasal congestion on the right side. CT scanning showed opacification of the ethmoidal and the sphenoid sinus. Due to fungal hyphae in the mucus, antifungal therapy was given together with an antihistamine. CT scanning after 3 months showed no opacification, and the patient had no complaints.

HNO 62. A 63-year-old woman presented with complaints of nasal congestion on the left side. FESS was done and revealed a massive sinusitis with polyps in the sphenoethmoidal recessus and the sphenoid sinus. Neither antifungal nor corticosteroid treatment was given, and the patient recovered without complications.

HNO 323. A 63-year-old woman presented with difficulty in breathing through the nose, pain, and pressure on the right side of her face. FESS showed sinusitis and polyps in the ethmoidal sinus, which was filled with viscous mucus. The whole sphenoid sinus was filled with fungal concrement, which was removed.

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* Corresponding author. Mailing address: ENT University Hospital, Karl-Franzens-University Graz, Auenbruggerplatz 26-28, A 8036 Graz, Austria. Phone: 43 316 3853606 or 43 676 7800545. Fax: 43 316 3857643. E-mail: walter.buzina@kfunigraz.ac.at.

TABLE 1. List of samples investigated

No.	Name[a]	Kind of sample	Origin[b]	Year	Sequence[d]	GenBank accession no.
1	GZU 79–96	Herbarium	Canada	1992	+/−	AF280755
2	GZU 29–88	Herbarium	Tunisia	1982	+	AF280752
3	GZU 22–91	Herbarium	Dominican Rep.	1929	(+)	AF280751
4	GZU 196–80	Herbarium	Brazil	1979	+	AF280759
5	GZU 42–2000/339	Fresh basidiocarp	Austria	2000	+	AF280753
6	GZU 42–2000/342	Fresh basidiocarp	Germany	2000	+	AF280754
7	HNO 62 = CBS 109298	Clinical isolate	Nose, sinus	2000	+	AF280750
8	HNO 34 = CBS 109297	Clinical isolate	Sphenoid sinus	1999	+	AF280758
9	HNO 323 = CBS 109299	Clinical isolate	Sphenoid sinus	2000	+	AF280757
10	HNO 104 = CBS 109296	Clinical isolate	Nose, sinus	2000	+	AF280756
11	CBS 340.81[c]	Not known	USA	1981	+	AF348142
12	CBS 405.96	Clinical isolate	USA	1996	+	AF350925

[a] GZU samples are from the Herbarium of the Institute of Botany, Karl-Franzens-University Graz. HNO samples are from the ENT-Hospital, Karl-Franzens-University Graz.
[b] Rep., Republic; USA, United States of America.
[c] Strain CBS 340.81 is the same as ATCC 44201.
[d] +, all; +/−, partial; (+), small part of ITS.

MATERIALS AND METHODS

Fungal strains and cultivation. Altogether, 12 isolates of *S. commune* were examined for this study (Table 1). Four strains were isolated from patients suffering from chronic sinusitis: samples HNO 34 and HNO 104 were obtained by flushing both nostrils of the patients with sterile 0.9% NaCl solution as described by Ponikau et al. (21), and samples HNO 62 and HNO 323 were obtained by sinus surgery. The mucous material was treated with mucolytic dithiothreitol to release the fungal elements, and these were sedimented by centrifugation. Thereafter, the samples were incubated at 20 and 30°C, respectively, on Sabouraud's glucose agar (SGA), Czapek Dox agar, and malt extract agar. To induce formation of dikaryotic mycelium, monokaryotic mycelia of the clinical isolates HNO 34 and HNO 62 were inoculated together on one petri dish (SGA) about 3 cm apart and incubated at room temperature in daylight for 1 week. Mycelium of the confluence zone was examined by microscopy. Two samples were isolated from living basidiocarps growing on broken branches of a European hornbeam (*Carpinus betulus*) in a forest in Graz, Austria, and on a dead branch of a tree of heaven (*Ailanthus altissima*) in the Tiergarten in Berlin, Germany, respectively. Four isolates were obtained from desiccated specimens from the herbarium of the Institute of Botany, Karl-Franzens-University Graz, Graz, Austria. These herbarium specimens were up to 71 years old and originated from North and South America and Africa. To compare the samples with known strains of this fungus, two strains (CBS 340.81 [ATCC 44201] and CBS 405.96) from the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands, were examined as well. Cultures of all clinical isolates were deposited at the CBS with the accession no. CBS 109296 to CBS 109299 (Table 1).

DNA isolation. DNA was extracted from fungal cells as described previously (15). In this procedure, cultivated material (clinical samples and reference strains) or fragments of basidiocarps (herbarium and fresh samples) were homogenized under liquid nitrogen, suspended in lysis buffer (1.4% *N*-cetyl-*N,N,N*,-trimethylammonium bromide [CTAB], 1 M NaCl, 7 mM Tris, 30 mM EDTA), and incubated at 65°C for 1 h. Proteins were removed by extraction with chloroform-isoamyl alcohol (24:1), and DNA was precipitated with precipitation buffer (0.5% CTAB, 40 mM NaCl) and pelleted by centrifugation. Afterwards, the pellet was resuspended in 1.2 M NaCl, and the DNA was reprecipitated with isopropanol (60% final concentration) at −20°C, washed with 70% ethanol, air dried, and resuspended in sterile bidistilled water.

PCR. The 5.8S rDNA and the flanking ITS regions (ITS1 and ITS2) were amplified using the primers ITS5 (GGAAGTAAAAGTCGTAACAAGG) and ITS4 (TCCTCCGCTTATTGATATGC) (30). All primers were prepared commercially by Metabion, Planegg-Martinsried, Germany. PCR was carried out in a reaction mixture containing 14.8 μl of sterile bidistilled water, 5.0 μl of buffer (10 mM Tris-HCl, 1.5 mM MgCl$_2$, 50 mM KCl, 0.1% Triton X-100), 5.0 μl of deoxynucleoside triphosphates (10 mM), 1 U of DNA polymerase (DyNAzyme II; Finnzymes Oy, Turku, Finland), 2.5 μl of each primer (10 μM), and 20 μl of fungal DNA (10 to 50 ng/μl). To prevent evaporation of the mixture during amplification, it was overlaid with 2 to 3 drops of mineral oil (Sigma; M3516). One reaction mixture containing water in place of DNA template was used as the contamination control. For PCR in the thermocycler (TC480; PE Biosystems), the following parameters were chosen: 30 cycles of 1 min at 95°C, 1 min at 50°C, and 2 min at 72°C, with a final extension at 72°C for 10 min. The amplification products (1 μl each) were visualized after gel electrophoresis and staining in ethidium bromide under UV light in a transilluminator.

Cycle sequencing. Excess primers and deoxynucleoside triphosphates were removed with chromatography columns (Microspin S-300 HR; Pharmacia). For sequencing the entire ITS region with the enclosed 5.8S rDNA, we used the primers ITS2 (GCTGCGTTCTTTCATCGATGC) and ITS3 (GCATCGATGA AGAACGCAGC) (30) in addition to the primers ITS5 and ITS4 at a 1.6 μM concentration. Sequencing was carried out with the ABI Prism BigDye Terminator Cycle Sequencing Kit (PE Biosystems) according to the manufacturer's recommendations. The parameters for cycle sequencing in the GeneAmp 2400 thermocycler (PE Biosystems) were 18 s of delay at 96°C, followed by 25 cycles of 18 s at 96°C, 5 s at 50°C, and 4 min at 60°C.

Analysis of sequences. Sequence analysis was performed using an automated sequence analyzer (ABI Prism 310; PE Biosystems) in conjunction with the ABI Prism Auto Assembler software (version 1.4.0, 1995; Applied Biosystems Division, PE Biosystems) and aligned using Pileup of the Wisconsin Package software (version 9.0-open VMS, 1993; Genetics Computer Group).

RFLP. For fast and reliable identification of isolates of *S. commune* without the need of sequencing, a map of cutting positions for the restriction enzymes *Eco*RI and *Ava*I was created. Therefore, the sequence data were cut virtually with the program Restriction Enzyme Analysis, which is an online freeware program (http://darwin.bio.geneseo.edu/~yin/WebGene/RE.html). This restriction map was verified by RFLP analysis of the amplified ITS regions. For this, 10 μl of PCR product from medical samples was mixed with 7 μl of bidistilled water and 3 μl of 1:2 enzyme buffer (Boehringer, Mannheim, Germany) and incubated at 37°C for 4 h. Restriction fragments were separated on a 2% agarose gel and visualized as described above.

Species-specific primers. Highly variable regions of both ITS1 and ITS2 were screened for possible priming sites. The selected sequences were checked with the program Primer Designer (version 2.0; Scientific & Educational Software) for their applicability as primers, as were GC content, melting temperature, and the lack of hairpins and possible dimers. These priming sites were also examined for their specificity for *S. commune* by comparing the sequences with entries in gene data banks (http://www2.ebi.ac.uk/fasta3/).

Nucleotide sequence accession numbers. All sequence data were submitted to GenBank (http://www.ncbi.nlm.nih.gov/) and were registered with the accession no. AF280750 through AF280759, AF348142, and AF350925 (Table 1).

RESULTS

All four clinical isolates were cultivated successfully on all media provided (SGA, Czapek Dox agar, and malt extract agar) at 20 and 30°C after 5 to 10 days of incubation. The cultures showed a cottony white mycelium with small knots of compressed hyphae 5 to 10 mm in diameter, described as "haploid fruiting" by Raper and Krongelb (22) (Fig. 1a). Older cultures produced yellowish droplets of exudation on the sur-

FIG. 1. (a) Three-week-old culture of *S. commune* on SGA. (b) Clamp connections (indicated by arrows) on dikaryotic hyphae, derived through mating two monokaryotic isolates (HNO 34 and HNO 62).

face, released a yellow to brown pigment into the medium, and had a pronounced odor. Microscopy showed hyaline hyphae of diverse structures with few spicules. Clamp connections were absent in the cultures of all clinical isolates. To produce dikaryotic mycelium, monokaryotic hyphae of two different strains were inoculated together on one petri dish and incubated at room temperature for 3 weeks. The hyphae fused in the confluence zone and formed clearly visible clamp connections (Fig. 1b). Nevertheless, no basidiocarps were produced.

Gel electrophoresis after PCR of the ITS region showed that DNA of all clinical isolates as well as from both fresh and desiccated basidiocarps had been amplified successfully with the primers ITS5 and ITS4 (data not shown). The lengths of all amplicons were identical and lay in the range of 660 bp. The whole ITS region and the enclosed 5.8S rDNA of the CBS strains, the four clinical isolates, and the two samples from the fresh fruiting bodies, as well as the herbarium specimens GZU 29-88 and GZU 196-80, were sequenced successfully (Table 1). DNA of the other two desiccated samples (GZU 22-91 and GZU 79-96) was too degraded to obtain good sequencing results for the entire region. However, some fragments within the 660-bp region could be sequenced. Alignment of the sequence data showed a similarity of 99.4 to 100% for all four clinical isolates, the samples from the living basidiocarps, the herbarium specimens from Tunisia (GZU 29-88) and Brazil (GZU 196-80), and the CBS strains at a length of 660 nucleotides with a GC content of 46.4% (Fig. 2). The sequence fragments of the desiccated samples from Canada (553 bp, 99.6% homology) and the Dominican Republic (286 bp, 97.2% homology) fitted well into the aligned sequences.

Analysis of the amplified ITS region showed various cutting sites for restriction enzymes. Two of them were chosen and drawn in a restriction map: *Eco*RI (281 and 379 bp) and *Ava*I (391, 125, and 144 bp) (Fig. 3a). Amplicons of all four clinical samples were digested with the chosen restriction enzymes. All

fragments of both restriction enzymes were found to be within the estimated size range, and there was no observable difference between the samples.

Both ITS1 and ITS2 contained segments sufficiently different from comparable regions in the genomes of other fungi to be specific for *S. commune*, qualifying them as targets for species-specific PCR primers or DNA probes. The newly designed primers *scom*1 (GTTGACTACGTCTACCTCAC) and *scom*2r (GTTAGGCTCCAGCAGACCTC) were both 20 nucleotides in length; had GC contents of 50 and 60%, respectively; formed no hairpins or dimers; and were in about the same range of melting temperatures. The primers were checked for their specificity by comparing the sequences with entries in gene data banks, and there was no analogy with any other fungi, except for different strains of *S. commune*. All samples of *S. commune* examined were amplified successfully with the primers *scom*1 and *scom*2r, and the resulting 305-bp amplicon was visualized on conventional agarose gels after electrophoresis (Fig. 4). To examine the reproducibility of the primer set, DNA of more than 20 strains of *S. commune*, collected in the United States and Europe, was extracted and amplified successfully in repeated experiments (data not shown). The specificity of the *scom*1 and *scom*2r primer set was confirmed by the unsuccessful amplification of DNA from a variety of fungi under the same conditions as described above (data not shown). The species examined, which included some established human pathogens as well as strains from internationally known culture collections, were *Trichophyton rubrum*, *Microsporum gypseum* (CBS 161.69), *Candida albicans* (ATCC 90028), *Cryptococcus neoformans* (ATCC 90112), *Alternaria alternata*, *Curvularia pallescens* (CBS 102694), *Aspergillus fumigatus*, *Aspergillus ustus* (CBS 209.92), *Penicillium commune*, *Ustilago maydis*, *Fusarium solani*, *Beauveria bassiana,* and *Pseudallescheria boidii*.

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        1                                                                                                    100
     1  agtaaaagtcgtaacaaggtttccgtaggtgaacctgcggaaggatcattaACGAATCAAACAAGTTCATCTTGTTCTGATCCTGTGCACCTTATGTAGT
     2  ....................................................................................................
     3  ....................................................................................................
     4  ....................................................................................................
     5  ----................................................................................................
     6  ----------------------------------------------------------------------------------------------------
     7  ....................................................................................................
     8  ....................................................................................................
     9  ....................................................................................................
    10  ....................................................................................................
    11  ....................................................................................................
    12  ....................................................................................................

        101                                                                                                  200
     1  TCC_C_AAAGCCTTCACGGGCGGCGGTTGACTACGTCTACCTCACACCTTAAAGTATGTTAACGAATGTAATCATGGTCTTGACAGACCCTAAAAAGTTA
     2  ..._._..............................................................................................
     3  ..._._..............................................................................................
     4  ..._._..................T...........................................................................
     5  ---_._..............................................................................................
     6  ----------------------------------------------------------------------------------------------------
     7  ..._._..............................................................................................
     8  ..._._..............................................................................................
     9  ..._._..............................................................................................
    10  ..._._..............................................................................................
    11  ..T.T...........T...................................................................................
    12  ..._._..............................................................................................

        201                                                                                                  300
     1  ATACAACTTtcgacaacggatctcttggctctcgcatcgatgaagaacgcagcgaaatgcgataagtaatgtgaattgcagaattcagtgaatcatcgaa
     2  ....................................................................................................
     3  ....................................................................................................
     4  ....................................................................................................
     5  ....................................................................................................
     6  -----------------------------------------------------...............................................
     7  ....................................................................................................
     8  ....................................................................................................
     9  ....................................................................................................
    10  ....................................................................................................
    11  ....................................................................................................
    12  ....................................................................................................

        301                                                                                                  400
     1  tctttgaacgcaccttgcgccctttggtattccgaggggcatgcctgtttgagtgtcattAAATACCATCAACCCTCTTTTGACTTCGGTCTCGAGAGTG
     2  ....................................................................................................
     3  ....................................................................................................
     4  ....................................................................................................
     5  ....................................................................................................
     6  .................................................................................................T.G.
     7  ....................................................................................................
     8  ....................................................................................................
     9  ....................................................................................................
    10  ....................................................................................................
    11  ....................................................................................................
    12  ....................................................................................................

        401                                                                                                  500
     1  GCTTGGAAGTGGAGGTCTGCTGGAGCCTAACGGAGCCAGCTCCTCTTAAATGTATTAGCGGATTTCCCTTGCGGGATCGCGTCTCCGATGTGATAATTTC
     2  ....................................................................................................
     3  ....................................................................................................
     4  ....................................................................................................
     5  ..............................................C.....................................................
     6  A...............................................------------------------------------------------....
     7  ....................................................................................................
     8  ....................................................................................................
     9  ....................................................................................................
    10  ....................................................................................................
    11  ..............................................C.....................................................
    12  ....................................................................................................

        501                                                                                                  600
     1  TACGTCGTTGACCATCTCGGGGCTGACCTAGTCAGTTTCAATAGGAGTCTGCTTCTAACCGTCTCTTGACCGAGACTAGCGACTTGTGCGCTAACTTTTG
     2  ....................................................................................................
     3  ....................................................................................................
     4  ........................G.........................................TT................................
     5  ..........................................................................T.........................
     6  -------------------------------------------------------...............T.............................
     7  ....................................................................................................
     8  ....................................................................................................
     9  ....................................................................................................
    10  ...........................................................T.........T..............................
    11  ....................................................................................................
    12  .....................................................................T..............................

        601                                              660
     1  ACTtgacctcaaatcaggtaggactacccgctgaacttaagcatatcaataagcggagga
     2  ...........................................................
     3  ...........................................................
     4  ...........................................................
     5  ...........................................................
     6  ..._.......................................................
     7  ...........................................................
     8  ...........................................................
     9  ...........................................................
    10  ...........................................................
    11  ...........................................................
    12  ...........................................................
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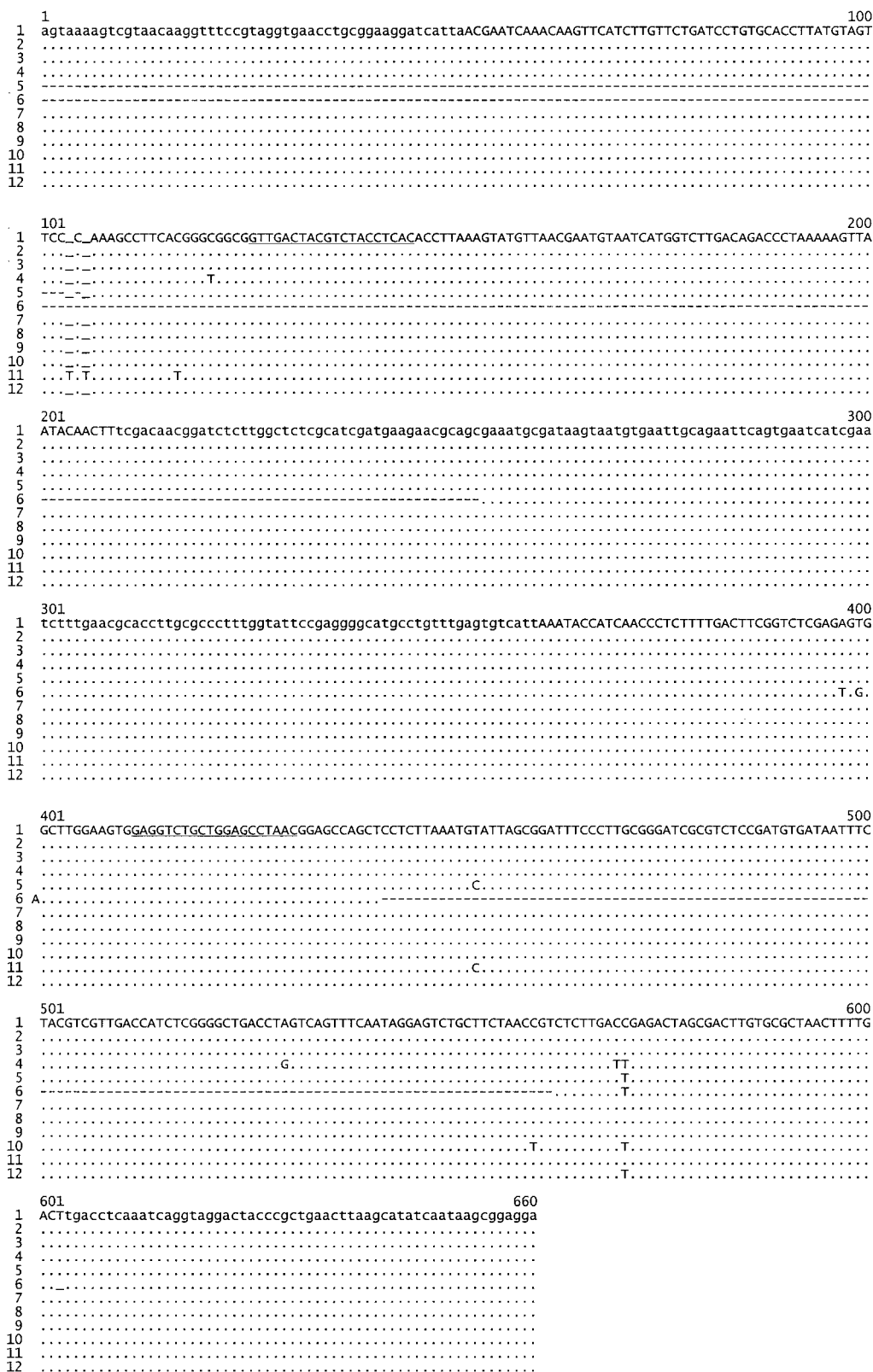
FIG. 2. Sequences of *S. commune* ITS region. ITS1 and ITS2 are in uppercase; small-subunit, 5.8S, and large-subunit rDNA are in lowercase. Priming sites for *scom*1 (positions 126 to 145) and *scom*2r (positions 412 to 431) are underlined. Dots represent similarity with the first sequence, dashes represent unknown characters, and underlined spaces represent gaps introduced for alignment. Sequences: 1, GZU 42-2000/339; 2, GZU 42-2000/342; 3, GZU 29-88; 4, GZU 196-80; 5, GZU 79-96; 6, GZU 22-91; 7, HNO 34; 8, HNO 62; 9, HNO 323; 10, HNO 104; 11, CBS 340.81; 12, CBS 405.96.
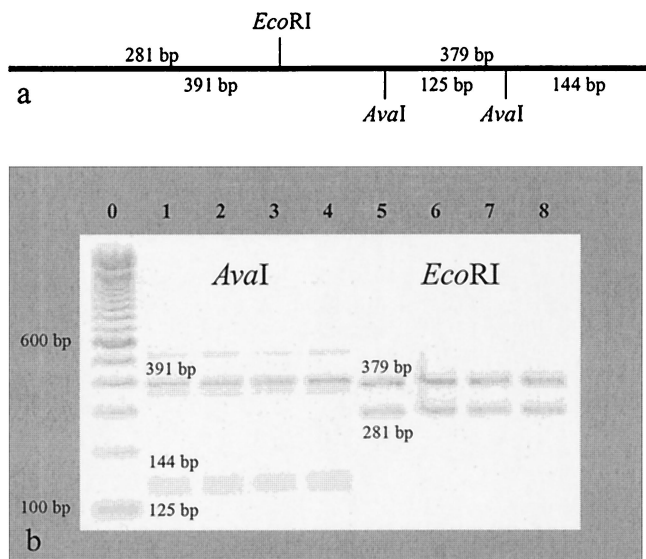
FIG. 3. (a) Restriction map of the ITS region for the restriction enzymes *Eco*RI and *Ava*I. (b) RFLP pattern of the clinical isolates of *S. commune*. Lane 0, 100-bp DNA ladder; lanes 1 and 5, HNO 62; lanes 2 and 6, HNO 34; lanes 3 and 7, HNO 323; lanes 4 and 8, HNO 104.

DISCUSSION

In the last fifty years, there have been only 16 reported cases of diseases caused by *S. commune*, most of them concerning the upper respiratory system. We found four isolates of *S. commune* from patients with sinusitis within only 6 months, suggesting that this fungus is a much more common human pathogen than expected. The period in which *S. commune* was isolated (December to May) correlates well with the main fruiting season of this fungus in central Europe, which is wintertime. All four patients of this study were female, and this corresponds with previous findings that *S. commune* seems to be a gynecotropic agent (13, 24). The age of the patients varied from 51 to 63 years (mean, 59.3 ± 5.7 years). All cultures of the clinical isolates showed neither clamp connections on the hyphae nor fruiting bodies, except for so-called haploid (monokaryotic) fruiting (22). The macroscopic features, such as rapid growth, cottony white surface, and some droplets of yellowish exudation, made an identification based on morphological characteristics impossible. As has been observed previously, *S.*

FIG. 4. Agarose gel showing the amplicons of 12 isolates of *S. commune*, amplified with the species-specific primers *scom*1 and *scom*2r. Lane 0, 100-bp DNA ladder; lane 13, open tube control. The numbering of samples 1 to 12 is the same as in Table 1.

commune often appears as a monokaryotic isolate in clinical samples and is thus unable to form the characteristic clamp connections and fruiting bodies (27). Kamei et al. (13), when reviewing the cases in which this basidiomycete was isolated from clinical samples, found 58% of the isolates to be monokaryotic. It can be assumed, therefore, that many cases of infections caused by *S. commune* were and are misdiagnosed (12). The formation of dikaryotic hyphae through mating experiments using a known strain of *S. commune* mostly leads to clamp connections and spicules (27, 29). Nevertheless, Raper and Krongelb (22) reported successful fruiting in only 80% of mating experiments because of incompatibility between some mating strains. Incubation in daylight, which is normally not possible with clinical laboratory incubators, may support the formation of basidiocarps. However, this procedure may take too much time for identification of the pathogen in most cases.

Comparison of the ITS sequences from fungi isolated from four patients with sinusitis, on the one hand, and with specimens both from desiccated and from living basidiocarps from three continents (North and South America, Africa, and Europe), on the other, showed a very high similarity in the 660-bp-long rDNA fragment investigated. The test strains CBS 340.81 and CBS 405.96 (the latter was isolated by L. Sigler et al. [27] from a patient in California) fitted well into the aligned sequences. This can be seen as evidence for a highly conserved ITS region within the species of *S. commune*, independent of where and when the fungus was found. The samples under investigation were from three continents, and their age ranged from some days to 71 years. Such a high intraspecific conservation, but with sufficient difference with regard to other medically relevant fungi, offers a great tool for identifying this fungus.

Although sequencing the ITS region of the rDNA is the most accurate method to identify monokaryotic nonclamped strains of *S. commune*, it is too costly and time-consuming for the clinical routine. Above all, most clinical laboratories do not have the ability to perform sequence analysis. PCR of the ITS region followed by an analysis of the RFLP can be a valuable tool for a fast and reliable identification of *S. commune*, as is described frequently for other medically important fungi (e.g., see references 3, 10, 11, 17, 19, and 31). Another possibility is to perform a PCR with the newly designed species-specific primers *scom*1 (GTTGACTACGTCTACCTCAC) and *scom*2r (GTTAGGCTCCAGCAGACCTC). This set of primers creates a specific amplicon of 305 bp within the ITS region and can thus also be used in a nested PCR together with the primers ITS5 and ITS4 when only a very small amount of sample is available. No other fungus (or any other organism) in the gene data banks was found to contain these priming sites. Using the set of PCR primers to amplify 13 of the most common pathogenic and nonpathogenic fungi was unsuccessful. Therefore, it can be assumed that positive amplification with this set of primers is highly specific for *S. commune*. Repeated amplifications of more than 30 strains of *S. commune* on different PCR machines showed 100% reproducibility of the primer set *scom*1-*scom*2r (data not shown). The sites of both primers, *scom*1 and *scom*2r, are also convenient as targets for labeled DNA probes. There are various protocols for hybridization and detection of DNA probes to identify other human
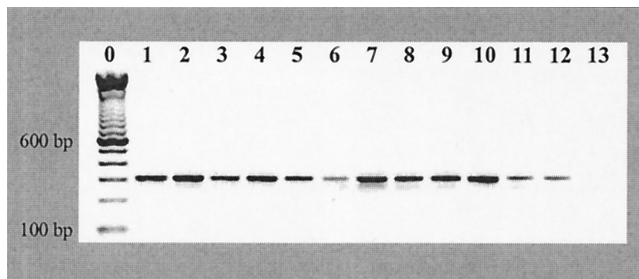
pathogenic fungi for which this primer pair may be applicable (e.g., see references 7, 8, 9, 20, and 26).

Our data suggest that whenever a white, cottony, rapidly growing culture is obtained from clinical samples and fails to show distinct microscopic features for a clear identification, one should think of *S. commune,* and this possibility may be confirmed by one of the above techniques. This may show that the occurrence of *S. commune* as a human pathogenic fungus is much more frequent than assumed previously and will lead to a better understanding of its role in human health.

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