JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2004, p. 622–626 Vol. 42, No. 2
0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.2.622–626.2004

Experience with the MicroSeq D2 Large-Subunit Ribosomal DNA Sequencing Kit for Identification of Filamentous Fungi Encountered in the Clinical Laboratory

Leslie Hall, Sherri Wohlfiel, and Glenn D. Roberts*

Division of Clinical Microbiology, Mayo Clinic, Rochester, Minnesota 55905

Received 19 August 2003/Returned for modification 27 October 2003/Accepted 13 November 2003

Described herein is our experience with the MicroSeq D2 large-subunit rDNA sequencing kit for the identification of filamentous fungi encountered in the mycology laboratory at the Mayo Clinic. A total of 234 filamentous fungi recovered from clinical specimens were used in the evaluation. All were identified by using phenotypic characteristics as observed macroscopically and microscopically on any medium or a combination of media, which included Sabouraud's dextrose, inhibitory mold, cornmeal, Czapek-Dox, potato dextrose, and V8 juice agars; all isolates were sequenced using the MicroSeq D2 large-subunit rDNA sequencing kit. Of the of 234 isolates, 158 were correctly identified to the appropriate genus or genus and species by using nucleic acid sequencing. Sequences for 70 (29.9%) of the isolates (27 genera) were not included in the MicroSeq library. Of the 80 dematiaceous and 154 hyaline fungi sequenced, 65 and 51.2%, respectively, gave results concordant with those determined by phenotypic identification. Nucleic acid sequencing using the MicroSeq D2 large-subunit rDNA sequencing kit offers promise of being an accurate identification system; however, the associated library needs to include more of the clinically important genera and species.

The identification of filamentous fungi in the clinical laboratory can be challenging; success in this endeavor depends on the organism and the experience of the microbiologist or technologist. For workers to become familiar with the fungi commonly seen in the local area is expected since the fungi are normal inhabitants of the environment and may often be found in the respiratory tract secretions of healthy individuals. However, it is important to remember that all are potentially pathogenic in the immunocompromised patient. Nucleic acid probes to identify the dimorphic pathogens *Blastomyces dermatitidis*, *Coccidioides immitis*, and *Histoplasma capsulatum* are commercially available; these are more often used by larger laboratories with substantial experience. Many of the hyaline and dematiaceous fungi do not sporulate, or else they are uncommon enough that phenotypic methods are not always helpful for identification. The Biolog system (Biolog, Hayward, Calif.) offers a commercially available phenotypic array that can identify 618 different filamentous fungi; however, no clinical evaluation has been done.

Newer antifungal agents have a narrower spectrum of activity, and whether identification to the species level will have an effect on therapeutic success is not known. Until this question is resolved, it is necessary for the laboratory to provide the most accurate possible identification of fungi so that the clinical, microbiologic, and treatment outcomes can be determined.

Nucleic acid sequencing appears to provide more objective separation of genera and species than that provided by conventional techniques. Nucleic acid sequencing has already become an important tool that is useful for the identification of aerobic and anaerobic bacteria, mycobacteria, and fungi (including yeasts) (1, 2, 4, 6). Turenne et al. (6) showed that sequencing of the ITS2 region has great potential for the identification of yeasts and several of the filamentous fungi, including some species of *Aspergillus*, *Zygomycetes*, and dermatophytes. A recent review by Iwen et al. (3) also discussed the internal transcribed spacer regions as potential targets to identify fungal pathogens in clinical culture specimens. Ninet et al. (5) demonstrated that the dermatophyte species could easily be identified by using the MicroSeq D2 large-subunit ribosomal DNA (rDNA) fungal sequencing kit and their custom database of sequences derived from well-characterized isolates.

To our knowledge, this is the first report of an experience with the commercially available MicroSeq D2 large-subunit rDNA fungal sequencing kit (Applied Biosystems, Foster City, Calif.) used in a clinical microbiology laboratory to identify filamentous fungi.

MATERIALS AND METHODS

Filamentous fungus isolates. A total of 234 filamentous fungi were used in the evaluation (see Tables 1 to 4); all were recovered from clinical specimens at the Mayo Clinic or were referred by other laboratories to Mayo Medical Laboratories for identification.

Identification of cultures by phenotypic methods. All cultures of filamentous fungi were identified on the basis of a combination of macroscopic and microscopic morphological features. Media used to induce sporulation included Sabouraud's dextrose, inhibitory mold, cornmeal, Czapek-Dox, potato dextrose, and V8 juice agars. Since fungi were not routinely identified to the species level by the clinical laboratory, a determination of how well the library identified the filamentous fungi to species was not made in most instances.

Large-subunit ribosomal DNA sequencing. The MicroSeq D2 large-subunit rDNA fungal sequencing kit is composed of PCR and cycle sequencing modules, identification and analysis software, and a library of fungal nucleic acid sequences.

DNA was extracted from fungal cells by placing a 1.0-μl loopful of organism into a 2.0-ml microcentrifuge tube containing 200 μl of PrepMan Ultra sample

* Corresponding author. Mailing address: Division of Clinical Microbiology, Mayo Clinic, 200 First St. SW, Rochester, MN 55905. Phone: (507) 284-3704. Fax: (507) 284-4272. E-mail: roberts.glenn@mayo.edu.

TABLE 1. Comparison of dematiaceous filamentous fungi identification using conventional methods and nucleic acid sequencing (≤1%)

Conventional phenotypic identification	MicroSeq sequence-based identification (no. of isolates)	No. of isolates	No. of isolates with concordant results[a]	Distance score avg range
Alternaria species	*Alternaria alternata* (4) *Stemphylium/Cochliobolus* (1)[c]	5	4	0
Aureobasidium pullulans	*Aureobasidium pullulans* (1)	1	1	0.62
Bipolaris species	*Bipolaris spicifera* (3)	3	3	0.00
Chaetomium species	*Chaetomium globosum* (4)	4	4	0–0.94
Chalara species	*Chaetomium globosum* (1)	1	0	0.94
Cladosporium species	*Mycosphaerella tassiana* (3)[b] *Exophiala jeanselmei* (1)	4	3	0–0.23
Curvularia species	*Pyrenophora semeniperda* (1)[b]	1	1	0
Epicoccum species	*Phyllosticta maydis* (2)	2	0	0.62
Exophiala jeanselmei	*Exophiala jeanselmei* (1)	1	1	0.31
Exserohilum species	*Setosphaeria monoceras*[b]*-Bipolaris indicia* (2)	2	2	0–0.62
Pestalotia species	*Pestalotia rhododendri* (1)	1	1	0
Phialophora verrucosa	*Phialophora americana* (1)	1	1	0.93
Pithomyces species	*Pithomyces atro-olivaceus* (2)	2	2	0–0.65
Scedosporium prolificans	*Petriella boulangeri* (4)[b]	4	4	0.94
Scedosporium apiospermum	*Pseudallescheria ellipsoidea* (1)[b]	1	1	0
Scytalidium species	*Scytalidium hyalinum* (1) *Phyllosticta maydis* (1)	2	1	0
Sporothrix species	*Hypopichia burtonii* (1)[b]	1	0	0
Sporotrichum pruinosum	*Phanerochaete chrysosporium* (1)[b]	1	1	0.57
Ulocladium species	*Ulocladium chartarum* (1) *Allewia eureka* (1)	2	1	0–0.031

[a] Sequence identification concordant with genus or genus and species.
[b] Teleomorph.
[c] The MicroSeq system could not distinguish between the two genera listed.

TABLE 2. Comparison of dematiaceous filamentous fungi identification using conventional methods and nucleic acid sequencing (≥1%)

Conventional phenotypic identification	MicroSeq sequence-based identification (no. of isolates)	No. of isolates	No. of isolates with concordant results[a]	Distance score avg range
Arthrinium phaeospermum	*Arthrinium phaeospermum* (2)	2	2	2.83–3.13
Aureobasidium pullulans	*Aureobasidium pullulans* (1)	1	1	2.78
Botrytis species	*Gloeophyllum trabeum* (1)	1	0	17.89
Chalara species	*Trichosporon jirovecii* (1)	1	0	21.51
Cladophialophora bantiana	*Exophiala jeanselmei* (1)	1	0	5.28
Cladosporium species	*Ramularia vallisumbrosae* (1)	1	0	1.89
Curvularia species	*Bipolaris hawaiiensis/Cochliobolus* (1)[c]	1	0	2.48
Cyphellophora species	*Phaeococcomyces exophialae* (1)	1	0	7.69
Engyodontium album	*Engyodontium album* (1)	1	1	1.25
Eutypella species	*Chaetomium globosum* (1)	1	0	8.78
Exophiala jeanselmei	*Phaeococcomyces exophilae* (2) *Exophiala jeanselmei* (1)	3	3	2.15–3.08
Nigrospora species	*Nigrospora aryzae* (2)	2	2	1.25
Nodulisporium species	*Nigrospora aryzae* (1)	1	0	9.09
Ochroconis constricta	*Venturia inaequalis* (1)	1	0	13.31
Ochroconis species	*Venturia inaequalis* (1)	1	0	14.60
Phaeococcomyces exophialae	*Phaeococcomyces exophialae* (1)	1	1	7.06
Phialophora species	*Aplanopsis spinosa* (1) *Lecythophora mutabilis* (1)	2	0	2.2–47.68
Phoma species	*Phoma exigua* (1) *Neotestudina rosatii* (1)	2	1	6.21–11.58
Pithomyces species	*Pithomyces atro-olivaceus* (4)	4	4	1.44–1.86
Rhinocladiella species	*Mycogone perniciosa* (1)	1	0	7.23
Scopulariopsis brumptii	*Penicillium rubrum* (1)	1	0	5.28
Scedosporium apiospermum	*Pseudallescheria ellipsoidea* (1)[b]	1	1	1.25
Sporothrix species	*Candida fennica* (1)	1	0	14.35
Sporothrix schenckii	*Ophistoma piceae* (4)[b] *Mycogone perniciosa* (1)	5	4	2.19–7.23
Sporotrichum pruinosum	*Merulius tremellosus* (1)	1	0	6.55
Stachybotrys species	*Myrothecium inundatum* (1)	1	0	5.38
Ulocladium species	*Ulocladium chartarum* (1)	1	1	2.48
Exophiala dermatitidis	*Rhinocladiella aquaspera* (1)	1	0	3.08

[a] Sequence identification concordant with genus or genus and species.
[b] Teleomorph.
[c] The MicroSeq system could not distinguish between the two genera listed.

TABLE 3. Comparison of hyaline filamentous fungi identification using conventional methods and nucleic acid sequencing (≤1%)

Conventional phenotypic identification	MicroSeq sequence-based identification (no. of isolates)	No. of isolates	No. of isolates with concordant results[a]	Distance score avg range
Aspergillus flavus	*Aspergillus ochraceous* (1)	1	1	0.62
Aspergillus fumigatus	*Neosartorya pseudofischeri* (10)	10	0	0.31
Aspergillus glaucus	*Eurotium-Setosphaeria-Bipolaris* (4)	4	0	0
Aspergillus nidulans	*Emericella nidulans* (1)[b]	4	1	0.16–0.55
	Emericella parvathecia (3)			
Aspergillus niger	*Aspergillus niger*/*Aspergillus foetidus* (5)[c]	8	7	0–0.93
	Aspergillus niger/*Aspergillus awamori* (2)[c]			
	Aspergillus species (1)			
Aspergillus species	*Aspergillus candidus* (1)	4	4	0–0.31
	Aspergillus sclerotiorum (1)			
	Aspergillus carneus (1)			
	Emericella parvathecia (1)[b]			
Aspergillus terreus	*Aspergillus carneus* (7)	7	0	0.31
Aspergillus versicolor	*Aspergillus versicolor* (4)	4	4	0–0.93
Fusarium species	*Fusarium solani* (6)	9	9	0–0.62
	Fusarium chlamydosporum (2)			
	Fusarium verticillioides (1)			
Geomyces species	*Geomyces pannorum* (1)	1	1	0
Geotrichum species	*Leptosphaerulina australis* (2)	2		0
Histoplasma capsulatum	*Histoplasma capsulatum* (10)	10	10	0–0.31
Mucor species	*Mucor racemosus* (1)	2	2	0–0.23
	Mucor circinelloides (1)			
Onychocola canadensis	*Filobasidium uniguttulatum* (1)	1	0	0.50
Paecilomyces lilacinus	*Paecilomyces lilacinus* (4)	4	4	0–0.31
Penicillium marneffei	*Penicillium rubrum* (1)	1	0	0.93
Penicillium species	*Penicillium chrysogenum* (2)	6	6	0–0.31
	Penicillium rubrum (2)			
	Penicillium clavigerum (1)			
	Penicillium olsonii (1)			
Rhizopus species	*Rhizopus oryzae* (1)	1	1	0.5
Rhizopus stolonifera	*Rhizopus stolonifera* (1)	1	1	0
Scopulariopsis species	*Scopulariopsis koningii* (4)	4	4	0
Trichoderma species	*Trichoderma inhamatum* (2)	4	4	0–0.94
	Trichoderma koningii (1)			
	Trichoderma harzianum (1)			

[a] Sequence identification concordant with genus or genus and species.
[b] Teleomorph.
[c] The MicroSeq system could not distinguish between the two species listed.

preparation reagent (Applied Biosystems). Tubes were vortexed for 10 to 30 s, followed by heating at 100°C for 10 min in a heat block. Lysates were stored at −20°C if testing was not performed immediately.

The D2 large-subunit rDNA fragment was amplified by adding 25 μl of diluted (1:50) genomic DNA to 25 μl of master mix consisting of forward and reverse primers to the PCR module. Conditions for PCRs were as follows: 95°C for 10 min, 35 cycles each at 95°C for 30 s, 53°C for 30 s, 72°C for 1 min, and a final extension step at 72°C for 10 min.

Ten microliters of amplicon was loaded onto a 2% E-Gel, subjected to electrophoresis, and viewed according to manufacturer's instructions (Invitrogen Life Technologies, Carlsbad, Calif.) to determine whether PCR products were present.

Purification of the PCR product to remove excess primers and nucleotides was performed using shrimp alkaline phosphatase (2.0 U/μl) and Exonuclease I (10.0 U/μl) (USB Corporation, Cleveland, Ohio). The enzymes were activated for 15 min at 37°C, followed by inactivation at 80°C for 15 min.

After removal of dyes with Sephadex 650, cycle sequencing was performed using the sequencing module reagents as follows: 25 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. Labeled amplicon was placed on an ABI 3100 16 capillary genetic analyzer (Applied Biosystems).

Analysis of sequence data. All sequence sample files were assembled, edited, and compared to those in the MicroSeq D2 fungal library, which contained 1,072 entries, including 788 species of filamentous fungi (version 1.4.2, February 2002). A distance score of 0.00% (100% match) to ≤1.00% (99% match) was used as a guide for identification since no cutoff value has yet been determined. The organism choice giving the closest match was considered the most likely correct identification. Organisms having a distance score of more than 1.00% were considered to be unique isolates that were most closely related to the closest database match present in the library; however, sequences for many of the fungi were not included in the D2 library. Sequencing results for the filamentous fungi were available within 24 h (2).

Resolution of discrepant isolates. For instances in which the conventional and sequencing identifications differed, cultures were examined microscopically again for characteristic morphological features. Isolates with distance scores of ≥1.0 were not reidentified.

Nucleotide sequence accession numbers. Our clinical mycology laboratory has constructed a database of additional organisms that is not included in the MicroSeq library and of species that exhibit genetic diversity. These may be found in GenBank and are listed sequentially under accession numbers AY234870 to AY235033.

RESULTS

As shown in Tables 1 to 4, 55.9% (131 of 234) of all filamentous fungi included in this evaluation were correctly identified to the appropriate genus or genus and species (there were several instances where two choices were given) using the MicroSeq D2 library. Note that sequences for 70 (29.9%) of the isolates (from 27 genera) studied were not included in the library.

A total of 80 dematiaceous fungi representing 36 genera and/or different species were sequenced, and 52 (65%) gave results that were concordant with those of conventional phenotypic identification. Of these, 39 had a distance score of ≤1% (99% similarity), and 31 (79.5%) gave concordant re-

TABLE 4. Comparison of hyaline filamentous fungi identification using conventional methods and nucleic acid sequencing (≥1%)

Conventional phenotypic identification	MicroSeq sequence-based identification (no. of isolates)	No. of isolates	No. of isolates with concordant results[a]	Distance score avg range
Absidia species	*Zygorhynchus moelleri* (5) *Mucor flavus* (1)	6	0	24.54–26.7
Apophysomyces elegans	*Zygorhynchus moelleri* (2)	2	0	25.23
Aspergillus clavatus	*Neosartorya pseudofischeri* (1)	1	0	1.56
Aspergillus flavus	*Aspergillus foetidus/Aspergillus niger* (6)[c]	6	0	3.12
Beauveria species	*Beauveria bassiana* (1)	1	1	2.20
Blastomyces dermatitidis	*Histoplasma capsulatum* (8)	8	0	1.55
Chrysosporium species	*Malbranchea* (1) *Malbranchea pulchella* (1) *Gymnoascus reesii* (1)	3	0	7.81–8.39
Coccidioides immitis	*Chrysosporium queenslandicum* (4)	4	0	4.04
Cokeromyces recurvatus	*Cokeromyces recurvatus* (1)	1	1	2.05
Cunninghamella species	*Pichia pastoris* (1)	1	0	46.20
Delacroixa coronata	*Conidiobolus incongruous* (1)[b]	1	1	20.76
Fugomyces cyanescens	*Rhodotorula bacarum* (3)	3	0	5.17
Fusarium species	*Fusarium oxysporum* (1)	1	1	1.25
Geotrichum klebhanii	*Geotrichum candidum*, *citri-aurantii* (2)	2	0	6.39
Blastoschizomyces capitatus	*Geotrichum fragrans* (1)	1	0	4.81
Gonytrichum species	*Chaetomium brasiliensis* (1)	1	0	11.29
Gymnascella citrina	*Penicillium rubrum* (1)	1	0	8.07
Malbranchea species	*Malbranchea pulchella* (2)	2	2	3.41–8.36
Mucor species	*Mucor hiemalis* (1) *Mucor circinelloides* (1) *Aplanopsis spinosa* (1)	3	2	1.76–48.44
Myriodontium species	*Arachnotheca albicans* (1)	1	0	7.48
Oidiodendron species	*Oidiodendron tenuissimum* (1)	1	1	2.80
Paecilomyces lilacinus	*Paecilomyces lilacinus* (1)	1	1	4.40
Paecilomyces species	*Paecilomyces puntonii* (2) *Paecilomyces variotii* (1)	3	3	1.86–3.73
Penicillium marneffei	*Penicillium rubrum* (1)	1	0	6.68
Penicillium species	*Penicillium rubrum* (3) *Penicillium solitum* (1)	4	4	1.24–1.55
Rhizomucor miehei	*Rhizomucor miehei* (1)	1	1	3.50
Rhizopus species	*Aplanopsis spinosa* (1)	1	0	47.68
Sepedonium species	*Mycogone perniciosa* (1)	1	0	4.08
Spegazzinia species	*Pithomyces atro-olivaceus* (1)	1	0	6.83
Syncephalastrum racemosum	*Syncephalastrum racemosum* (1)	1	1	2.17
Syncephalastrum species	*Syncephalastrum* (1)	1	1	5.02
Trichothecium roseum	*Stilbella albocitrina* (1)	1	0	2.20–2.52

[a] Sequence identification concordant with genus or genus and species.
[b] Telemorph.
[c] The MicroSeq system could not distinguish between the two species listed.

sults. The remaining 41 had a distance score of ≥1%, and 21 (51.2%) gave concordant results (Table 2). Sequences for 12 (15%) of the dematiaceous fungi studied (11 genera) were not included in the MicroSeq library. Dematiaceous fungi with a distance score of ≤1% were placed into the correct genus using nucleic sequencing with few exceptions, including *Chalara* (1), *Epicoccum* (2), and *Sporothrix* (1) species. However, 14 isolates (11 genera) with a distance score of ≥1% gave discordant results, and 13 (92.9%) were not included in the MicroSeq library.

A total of 154 hyaline fungi representing 45 genera and/or different species were sequenced, and 88 (57.1%) had a distance score of ≤1%; 59 (67.0%) gave results concordant with those of conventional phenotypic identification (Table 3). The remaining six isolates had a distance score of ≥1%, and 20 (30.3%) gave concordant results (Table 4). Sequences for 58 (37.7%) of the hyaline fungi studied (16 genera) were not included in the MicroSeq library.

Isolates of *Aspergillus niger*, *Aspergillus versicolor*, *Fusarium* species, *Mucor* species, *Paecilomyces lilacinus*, *Penicillium* species, *Rhizopus* species, *Scopulariopsis* species, and *Trichoderma* species were correctly characterized to the genus level when the distance score was ≤1%. Isolates of *Aspergillus fumigatus* (10), *Aspergillus terreus* (7), *Blastomyces dermatitidis* (8), *Coccidioides immitis* (4), *Onychocola canadensis* (1), and *Penicillium marneffei* (1) gave discordant results since they were not included in the MicroSeq library. *Arthrinium phaeospermum*, *Aureobasidium pullulans*, *Beauveria* species, *Cokeromyces recurvatus*, *Engyodontium album*, *Exophiala jeanselmei*, *Nigrospora* species, *Pithomyces* species, *Malbranchea* species, *Mucor* species, *Paecilomyces* species, and *Penicillium* species were placed into the appropriate genus despite having distance scores of ≥1%. Of the 40 hyaline fungi (16 genera) with a distance score of ≥1% that gave discordant results, 38 (82.6%) were not included in the MicroSeq library.

DISCUSSION

The Clinical Microbiology Laboratories of the Mayo Clinic use nucleic acid sequencing to identify selected isolates of

mycobacteria, yeasts, and aerobic and anaerobic bacteria. The Clinical Mycology Laboratory has the D2 rDNA fungal identification system component available for use. As described previously, the accompanying library of fungal sequences contains 788 entries that represent 205 genera of filamentous fungi, including the type cultures for most.

The filamentous fungi included in this evaluation were representative of those seen routinely in clinical microbiology. The organisms sent to the Mayo Clinic mycology laboratory were usually those that could not be readily identified by the referring laboratories. We sought to determine how well nucleic acid sequencing would perform in terms of the identification of these organisms.

Overall nucleic acid sequencing identified 67.5% of the filamentous fungi to the correct genus or genus and species levels. No standard cutoff point is available for interpreting the distance score; however, most of the species with concordant phenotypic scores of <1% identification had a score of less than 1.00%. Future studies will determine if this value is valid, but what is more likely is that some genetic diversity within some species will be observed, and the distance scores will have a range.

As has been seen in other areas of clinical microbiology, molecular methods have provided more accurate identification of organisms and, in some instances, changes in taxonomy. Additional work will be needed in order to determine which gene or combination of genes is needed for complete separation of genera and species. Some organisms may require sequencing of more than one target before a definitive identification can be made.

Nucleic acid sequencing will perhaps be of the greatest benefit to the laboratory for the identification of organisms that are not commonly seen. The MicroSeq D2 library is not inclusive of all clinically important fungi and should be expanded so as to include them. However, the library's flexibility is what allows each laboratory to construct a custom database; this ability will make the system even more useful and complete.

The MicroSeq D2 large-subunit rDNA sequencing kit appears to be accurate and useful for the identification of filamentous fungi—even those that are relatively uncommon—that are seen in the clinical laboratory. However, the library does not include some of the common aspergilli, i.e., *Blastomyces dermatitidis* and *Coccidioides immitis* and other environmental flora that cause disease in immunocompromised patients. Nucleic acid sequencing identification of dimorphic pathogens is not critical since they may be identified by the use of nucleic acid probes. Sequencing can best be used for the identification of organisms that cannot be fully identified by their microscopic morphological features, particularly those that do not sporulate within 48 h of subculture. A cost analysis comparing both phenotypic and nucleic acid sequencing for identification showed that no difference existed in the charges. How often nucleic acid sequencing will be used by the clinical laboratory must be determined. However, a shortened turnaround time is important to the clinician and, ultimately, to the patient.

ACKNOWLEDGMENTS

We express our gratitude to David T. Lynch for his technical expertise and to Laura Onken for her outstanding secretarial skills. All of the technologists in the clinical mycology and sequencing laboratories contributed much to the success of this study; for this, we express our sincere appreciation. This work could not have been done without them.

REFERENCES

1. **Chen, Y., J. D. Eisner, M. M. Kattar, S. L. Rassoulian-Barrett, K. Lafe, U. Bui, A. P. Limaye, and B. T. Cookson.** 2001. Polymorphic internal transcribed spacer region 1 DNA sequences identify medically important yeasts. J. Clin. Microbiol. **39:**4042–4051.
2. **Hall, L., S. Wohlfiel, and G. D. Roberts.** 2003. Experience with the MicroSeq D2 large-subunit ribosomal DNA sequencing kit for the identification of commonly encountered clinically important yeast species. J. Clin. Microbiol. **41:**5099–5102.
3. **Iwen, P. C., S. H. Hinrichs, and M. E. Rupp.** 2002. Utilization of the internal transcribed spacer regions as molecular targets to detect and identify human fungal pathogens. Med. Mycol. **40:**87–109.
4. **Kurtzman, C. P., and C. J. Robnett.** 1997. Identification of clinically important ascomycetous yeasts based on nucleotide divergence in the 5′ end of the large-subunit (26S) ribosomal DNA gene. J. Clin. Microbiol. **35:**1216–1223.
5. **Ninet, B., J. Isabell, O. Bontems, B. Lechenne, O. Jousson, R. Panizzon, D. Lew, and M. Monod.** 2003. Identification of dermatophyte species by 28S ribosomal DNA sequencing with a commercial kit. J. Clin. Microbiol. **41:**826–830.
6. **Turenne, C., S. E. Sanche, D. J. Hoban, J. A. Karlowsky, and A. M. Kabani.** 1999. Rapid identification of fungi by using the ITS2 genetic region and an automated fluorescent capillary electrophoresis system. J. Clin. Microbiol. **37:**1846–1851.