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In Vitro Susceptibility Testing and DNA Typing of *Saccharomyces cerevisiae* Clinical Isolates

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***Saccharomyces* spp. are widely distributed in nature and may colonize the normal human gastrointestinal tract. Although *Saccharomyces cerevisiae* isolates have been previously considered nonpathogenic, they appear to be increasingly associated with infections in immunocompromised or otherwise debilitated patients. The antifungal susceptibility and epidemiology of *S. cerevisiae* are poorly defined at present. A series of 76 isolates (mostly stool surveillance and throat swab isolates) from 70 bone marrow transplant patients hospitalized at two different medical centers were characterized by antifungal susceptibility testing and restriction endonuclease analysis of chromosomal DNA. For DNA typing, digestion with *Not*I followed by pulsed-field gel electrophoresis was applied. Typing results revealed 62 distinct DNA types among the 76 clinical isolates. Despite this genomic diversity, clusters of identical isolates were identified among different patients hospitalized concurrently in the same unit, indicating possible nosocomial transmission. The MICs of amphotericin B, 5-fluorocytosine, fluconazole, and itraconazole were determined by a broth microdilution method, as recommended by the National Committee for Clinical Laboratory Standards. The MICs at which 90% of the strains were inhibited were as follows: amphotericin B, 1.0 μg/ml; 5-fluorocytosine, 0.25 μg/ml; fluconazole, 8.0 μg/ml; and itraconazole, 1.0 μg/ml. The relative resistance of *S. cerevisiae* to fluconazole and itraconazole may promote the emergence of this species as a pathogen among immunosuppressed patients.**

The ascomycetous yeast *Saccharomyces cerevisiae* shows a widespread distribution, constituting part of the normal flora of various plants and also being detected in soil (7). Because of its extensive use in the brewing and baking industries, *S. cerevisiae* has been designated the brewer's and baker's yeast. Exposure to this organism is apparently widespread and occurs mostly through food. It occasionally constitutes part of the normal flora of the gastrointestinal tract of humans and has also been detected in the oropharynx and urine (7). Despite widespread exposure and occasional colonization, invasive disease with *S. cerevisiae* has been very rarely reported in the past and *S. cerevisiae* was not considered a pathogen.

The widespread use of immunosuppressive regimens, broad-spectrum antimicrobial chemotherapy, and permanent intravascular catheters has resulted in an increased incidence of disseminated fungal disease. Fungi previously considered contaminants or colonizers have emerged as pathogens (1, 6). There is increasing evidence from the literature that *S. cerevisiae* is associated with invasive disease in this setting. In AIDS patients, transplant recipients, and debilitated patients with malignancies, *S. cerevisiae* has been isolated from the blood or other sterile sites, with or without other microorganisms, and has been associated with severe clinical disease (2, 3, 5, 10, 11, 14, 16). Although the pathogenic potential of *S. cerevisiae* has been questioned, virulence traits, such as the ability to grow in supraoptimal temperatures, formation of pseudohyphae, and increased pathogenicity in mouse infection models, have been associated with clinical isolates but not laboratory strains (4, 8). It appears that host immunosuppression, intrinsic strain virulence, and the concomitant presence of other microorganisms all may contribute to development of disease. In the immunocompromised host the isolation of these fungi from a sterile source in association with clinical disease should be considered significant and treated aggressively with antifungal agents (2, 3, 10).

At present, there is no established treatment for infections with *S. cerevisiae*. Very few reports in the literature have evaluated the in vitro susceptibilities of *S. cerevisiae* clinical isolates (15, 17). Patients have been treated empirically, mainly with amphotericin B, with or without flucytosine, or with other antifungal agents. Therapy has not always been successful (2, 3, 18), but response to treatment may be difficult to assess in debilitated hosts with multiorgan failure. Finally, little is known about the epidemiology of colonization and subsequent infection in immunosuppressed patients.

The objective of this study was to investigate the molecular epidemiology and in vitro susceptibility patterns of *S. cerevisiae* clinical isolates. A large collection of fungi isolated from surveillance cultures of bone marrow transplant patients was used to establish a molecular DNA typing method and to assess in vitro susceptibility to amphotericin B, 5-fluorocytosine (5FC), fluconazole, and itraconazole, according to National Committee for Clinical Laboratory Standards methods.

MATERIALS AND METHODS

Patients and organisms. A total of 76 *S. cerevisiae* isolates were examined retrospectively. They were obtained from 70 patients in two hospitals, 56 isolates from the University of Iowa (medical center A) and 20 from the University of Utah (medical center B). All patients were bone marrow transplant recipients (children or adults). Isolates from the University of Iowa were obtained from patients hospitalized over a period of 8 years (1988 to 1996), while isolates from the University of Utah were obtained from patients hospitalized in the bone marrow transplant unit during 1995. The isolates were obtained primarily from stool surveillance and throat swab cultures; single isolates were obtained from urine, wound, dialysis fluid, peritoneal fluid, and tissue biopsy cultures. Multiple isolates were obtained from three patients (two, three, and four isolates per patient). All isolates were stored in sterile distilled water at ambient temperature. Isolates from patients included in this study were retrieved from the organism bank at the University of Iowa and were reidentified to the species level by the Vitek system.

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Molecular typing and DNA preparation. Ten colonies of each *S. cerevisiae* isolate from 48-h cultures on Sabouraud dextrose agar plates (Remel, Lenexa, Kans.) were incubated overnight at 37°C in 10 ml of YEPD broth (yeast extract, 10 g/liter; peptone, 20 g/liter; and D-glucose, 20 g/liter). The cells were packed by centrifugation (1,000 × *g* for 15 min), washed in sterile distilled water, and introduced into preweighed Eppendorf tubes. The pellets were suspended in a volume of 50 mM sodium EDTA (pH 8.0) (in microliters) equal to the weight of the cells (in milligrams). A 50-μl sample of this suspension was mixed with 150 μl of 50 mM sodium EDTA, 40 μl of Lyticase (L5263; Sigma Chemical, St. Louis, Mo.), and 240 μl of 2% agarose (SeaPlaque GTG; FMC BioProducts, Rockland, Maine) that was previously melted and kept liquid at 56°C. Samples of the above mixture were placed in forms (Bio-Rad, Hercules, Calif.) that were incubated for 2 h at 37°C. The inserts were removed from the forms; placed in 2 ml of a buffer of 10 mM Tris-HCl (pH 7.5), 400 mM EDTA, 1% Sarkosyl, and 0.75 mg of proteinase K (P4914; Sigma) per ml; and incubated overnight at 50°C. The inserts were washed six times in 50 mM sodium EDTA buffer and stored at 5°C until use.

Restriction endonuclease analysis of genomic DNA (REAG). On the basis of preliminary experiments with different restriction endonucleases, *Not*I was selected as the most appropriate enzyme for the study of *S. cerevisiae* genetic analysis. Agarose inserts containing chromosome-sized DNA were prepared as described above and were placed into 100 mM Tris-HCl (pH 8.0) containing 5 mM magnesium chloride. After two washes with this buffer, the agarose inserts were digested overnight with 20 U of *Not*I (New England Biolabs, Beverly, Mass.) as recommended by the manufacturer. Electrophoresis was performed with the CHEF-DRII pulsed-field gel electrophoresis system (Bio-Rad) in 1.0% agarose gel (SeaKem GTG; FMC Bioproducts)–0.5× Tris-borate-EDTA buffer (pH 8.5) at 13°C and 200 V. The pulse interval was ramped from 20 to 100 s over 24 h. The 48.5-kb bacteriophage lambda DNA ladders (Bio-Rad) were included in each gel as molecular size standards. After electrophoresis, the gels were stained with ethidium bromide, illuminated with UV light, and photographed. Two observers, blinded to the origin of the isolates and the results obtained by the other observer, examined the photographs of the ethidium bromide-stained gels to detect similarities and differences in banding patterns. All bands had to match exactly to classify isolates as identical; any difference in a major or minor band was considered important. Using these criteria, interobserver agreement was 100%.

Antifungal susceptibility testing. Broth microdilution susceptibility testing was performed according to National Committee for Clinical Laboratory Standards proposed standard guidelines using the spectrophotometric method of inoculum preparation, an inoculum concentration of 0.5×10^3 to 2.5×10^3 cells per ml, and RPMI 1640 medium buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) buffer (9). Antifungal agents were obtained from their respective manufacturers and included amphotericin B, 5FC, fluconazole, and itraconazole. Yeast inocula (100 μl) were added to wells of microdilution trays containing 100 μl of drug solution (2× final concentration). The trays were incubated in air at 35°C and were inspected at 48 h of incubation. The MIC endpoints were read visually and were defined for 5FC, fluconazole, and itraconazole as the lowest concentration in which a prominent decrease in turbidity (80% reduction) was observed and for amphotericin B as the lowest concentration producing complete inhibition of growth (9). Drug-free and yeast-free controls were included, and quality control was ensured by testing *Candida parapsilosis* (ATCC 22019) and *Candida krusei* (ATCC 6258) as recommended (9).

RESULTS

Genomic DNA typing. Restriction endonuclease analysis using *Not*I followed by pulsed-field gel electrophoresis generated a large number of DNA fragments and identified 62 distinct REAG types, 1 through 62 (Table 1 and Fig. 1). REAG type 2 encompassed four isolates; REAG types 4, 15, and 49 each encompassed three isolates; and REAG types 13, 44, 51, 54, and 61 were each represented by two isolates. The remaining 53 isolates represented 53 unique REAG types. In only one instance was a DNA type shared by patients from different medical centers (type 44; one patient from each hospital).

Three patients had more than one *S. cerevisiae* isolate over time. From the first, four isolates were obtained; two were identical DNA types, and two were different. For another patient with two isolates, the analysis showed that they were identical. Finally, in another patient with three *S. cerevisiae* isolates, typing demonstrated that all three were different DNA types. The dates on which the cultures were obtained were available for this patient. The time interval between the first and second cultures was 1 month, while that between the second and the third was 11 months.

TABLE 1. Distribution of DNA types among clinical isolates of *S. cerevisiae* from two medical centers as determined by REAG with *Not*I

Medical center	DNA type	No. of isolates	No. of patients
A	1	1	1
	2	4	4
	3	1	1
	4	3	3
	5	1	1
	6	1	1
	7	1	1
	8	1	1
	9	1	1
	10	1	1
	11	1	1
	12	1	1
	13	2	2
	14	1	1
	15	3	3
	16	1	1
	17	1	1
	18	1	1
	19	1	1
	20	1	1
	21	1	1
	22	1	1
	23	1	1
	24	1	1
	25	1	1
	26	1	1
	27	1	1
	28	1	1
	29	1	1
	30	1	1
	31	1	1
	32	1	1
	33	1	1
	34	1	1
	35	1	1
	36	1	1
	37	1	1
	38	1	1
	39	1	1
	40	1	1
	41	1	1
	42	1	1
	43	1	1
	44	1	1
	45	1	1
	46	1	1
	47	1	1
	48	1	1
B	49	3	3
	50	1	1
	44	1	1
	51	2	2
	52	1	1
	53	1	1
	54	2	2
	55	1	1
	56	1	1
	57	1	1
	58	1	1
	59	1	1
	60	1	1
	61	2	2
	62	1	1

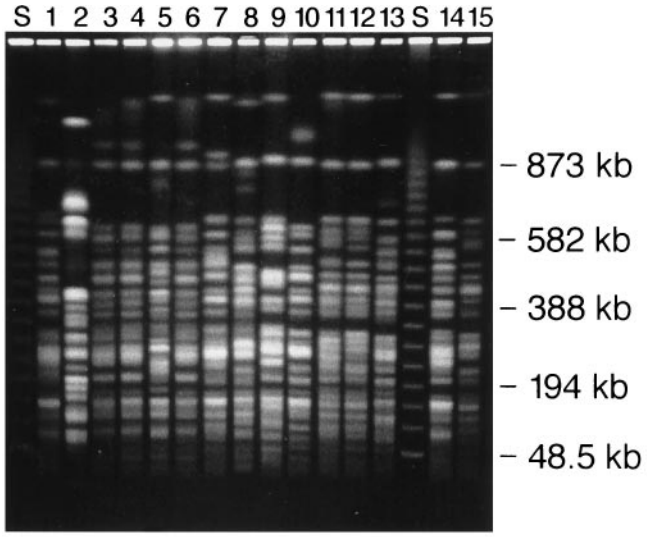

FIG. 1. Representative REAG types of *S. cerevisiae* obtained by using *Not*I followed by pulsed-field gel electrophoresis. Lanes: 1, REAG type 60; 2, REAG type 1; 3, 4, and 6, REAG type 2; 5, REAG type 3; 7, REAG type 4; 8, REAG type 5; 9, REAG type 6; 10, REAG type 7; 11 and 12, REAG type 49; 13, REAG type 50; 14, REAG type 44; 15, REAG type 59; and S, lambda phage DNA concatemers as molecular size standards (in kilobases).

In several instances the same DNA type of *S. cerevisiae* was obtained from epidemiologically related patients (Table 1). DNA types 2 (four patients), 4 (three patients), 13 (two patients), and 15 (three patients) represented four clusters of identical DNA types among patients hospitalized within a 1- to 3-month interval in the bone marrow transplant unit of medical center A. Likewise, DNA types 49 (three patients), 51 (two patients), 54 (two patients), and 61 (two patients) represented four clusters of identical DNA types among epidemiologically related patients hospitalized in medical center B. Clustering of isolates with the same DNA type among geographically and temporally related patients within a given institution indicates possible nosocomial transmission within that institution.

Antifungal susceptibility testing. Table 2 shows the in vitro susceptibilities of 74 *S. cerevisiae* isolates to amphotericin B, 5FC, fluconazole, and itraconazole. Results were not obtained for two strains because of poor growth in the test medium. Elevated MICs were observed for both fluconazole (MIC at which 90% of strains were inhibited, 8.0 μg/ml) and itraconazole (MIC at which 90% of strains were inhibited, 1.0 μg/ml). In general, isolates requiring high itraconazole MICs also required elevated fluconazole MICs (data not shown). The MICs of amphotericin B and 5FC were consistent with previous values reported for this organism (17). Overall, the antifungal susceptibility profile was not useful in distinguishing one DNA type from another.

DISCUSSION

The results of this study establish the usefulness of DNA-based typing methods for strain delineation of *S. cerevisiae*. Digestion with the restriction endonuclease *Not*I followed by pulsed-field gel electrophoresis appears to be an excellent approach for the DNA typing of these fungi. Considerable strain diversity was demonstrated among *S. cerevisiae* isolates colonizing the gastrointestinal tract of patients undergoing bone marrow transplantation.

Despite the large number of strains identified, DNA typing was still able to identify isolates with the same REAG profile among different patients. The fact that nine distinct DNA type clusters were detected and the respective cultures for more than half of the isolates were obtained concurrently from patients hospitalized in the same unit indicates possible nosocomial transmission of *S. cerevisiae*. The finding of possible nosocomial transmission in the two centers included in this study suggests that exogenous acquisition, most probably via the hands of patient care providers, should be considered in efforts to control transmission of this organisms in units with immunosuppressed patients.

In general, each patient was colonized with her or his own distinct DNA type. *S. cerevisiae* was isolated only once from the majority of patients despite weekly surveillance cultures during hospitalization for bone marrow transplantation. This may reflect transient colonization with this fungus. For the few patients with multiple isolates, DNA typing showed that patients may be colonized with either the same or different strains over time. These results are consistent with those of Sobel et al., who reported karyotyping of 17 *S. cerevisiae* isolates from seven patients presenting with recurrent vaginitis and demonstrated nine distinct electrophoretic patterns. None of the patients shared strains of *S. cerevisiae* (15).

The antifungal susceptibility profile of *S. cerevisiae* is consistent with that reported by previous investigators (15, 17). The majority of the isolates were inhibited by clinically achievable concentrations of the antifungal agents tested in serum. Elevated MICs were observed for both fluconazole and itraconazole (Table 2). Likewise, both Sobel et al. (15) and Tiballi et al. (17) demonstrated decreased susceptibility to fluconazole among clinical isolates of *S. cerevisiae* obtained from patients with vaginal infections (15) or oropharyngeal colonization (17). These results indicate that similar to *C. krusei* and *Candida glabrata* (12), *S. cerevisiae* may be intrinsically less susceptible to fluconazole and other azoles. This decreased susceptibility to azoles can result in the emergence of *S. cerevisiae* clinically in patients receiving these agents for prophylaxis or therapy. In AIDS patients with oral thrush, colonization with *C. glabrata* and *S. cerevisiae* has been shown to increase after treatment with fluconazole or clotrimazole (13).

In conclusion, we have established an appropriate DNA typing method for *S. cerevisiae* and documented the genetic diversity of a large number of isolates from two medical institutions. Although *S. cerevisiae* may be considered an endogenous pathogen, the potential for cross contamination in the hospital environment is documented in this study. *S. cerevisiae* appears to be susceptible to both amphotericin B and 5FC, as indicated by in vitro susceptibility testing; however, the decreased susceptibility to fluconazole and itraconazole may promote the emergence of this fungus as a pathogen among immunosuppressed individuals.

TABLE 2. In vitro susceptibility of 74 *S. cerevisiae* isolates to four antifungal agents as determined by the National Committee for Clinical Laboratory Standards microdilution broth method

Antifungal agent	MIC (μg/ml)[a]		
	Range	50%	90%
Amphotericin B	0.12–2	1	1
5FC	0.25–32	0.25	0.25
Fluconazole	0.12–16	2	8
Itraconazole	0.015–1	0.5	1

[a] 50% and 90%, MIC at which 50 and 90% of the isolates tested were inhibited.

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