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Evaluation of Pulsed-Field Gel Electrophoresis as a Typing System for *Candida rugosa*: Comparison of Karyotype and Restriction Fragment Length Polymorphisms

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Nosocomial infections with *Candida* species have emerged as an increasingly important cause of morbidity and mortality in intensive care units. Ten *Candida rugosa* isolates from a previously documented cluster of *C. rugosa* infections in one hospital (nine burn unit isolates and one isolate from another hospital ward) and eight *C. rugosa* isolates recovered in a referral fungus testing laboratory (comparison isolates) from distinct geographic areas were investigated by molecular techniques. Isolates were from multiple anatomic sites. Pulsed-field gel electrophoresis (PFGE) of whole-cell DNA was performed with the 18 *C. rugosa* isolates as a marker of strain identity. The PFGE karyotypes of the *C. rugosa* isolates were demonstrated from four to seven chromosome bands. Karyotyping revealed the same PFGE pattern for the nine outbreak isolates from the burn unit, confirming clonal strain transmission. The isolate from the other hospital ward had a distinct karyotype. Distinct PFGE karyotype patterns were demonstrated for the eight comparison isolates. Restriction fragment length polymorphisms (RFLP) generated from whole-cell DNA digested with *Sfi*I demonstrated the same RFLP pattern among outbreak isolates. Among comparison isolates, karyotyping distinguished some isolates that were indistinguishable by RFLP patterns. Karyotyping by PFGE appears to be the most useful molecular typing tool for discrimination among strains of *C. rugosa* and will be a useful marker for evaluating the epidemiology of future *C. rugosa* infections.

Nosocomial infections with *Candida* species are recognized as a significant cause of morbidity and mortality in both seriously ill immunocompetent and immunocompromised patients (1, 6, 12, 16, 25). Infections with non-*C. albicans Candida* species appear to be increasing (8, 12, 13, 25).

Candida rugosa was originally isolated from human feces in 1917 by Anderson and was called *Mycoderma rugosa* (23). It has subsequently been isolated from clinical samples from humans, and it has been reported as a human pathogen in several cases (2, 5, 18, 21). Some of the reported cases involved an immunocompromised patient with acute myelocytic leukemia who had disseminated infection with cutaneous lesions that yielded *C. rugosa* (21), a patient with alcoholic cirrhosis and an intravenous catheter-associated fungemia due to *C. rugosa* (18), a child with *C. rugosa* central venous catheter-related infection (2), and a cluster of *C. rugosa* infections recently reported in a burn intensive care unit (5).

Recent studies using molecular DNA typing methods have suggested the occurrence of cross-transmission of nosocomial *Candida* species from environmental and human sources (3, 12–14, 17). Pulsed-field gel electrophoresis (PFGE) allows a good resolution and reproducible separation of *Candida* chromosomes. PFGE has been applied successfully to many *Candida* species, including *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. lusitaniae*, and *C. glabrata* (4, 6, 7, 9–11, 19, 24). To our knowledge, the molecular typing of *C. rugosa* has not previously been reported. We performed PFGE on the *C. rugosa* isolates from the burn unit outbreak and other *C. rugosa* isolates from a referral fungus testing laboratory to investigate the molecular typing characteristics of *C. rugosa.*

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MATERIALS AND METHODS

Isolates. Eighteen *C. rugosa* isolates were evaluated. Table 1 shows the sources and sites of these isolates. Eight isolates from three patients were recovered from a previously reported cluster of *C. rugosa* infections in a burn unit (5). One environmental isolate from the glucose monitor was also recovered in the burn unit. One isolate recovered from a patient in the same hospital but not in the burn unit was also studied. Eight comparison isolates corresponding to patients at six geographically distinct institutions were recovered from a referral fungus testing laboratory. These clinical isolates were isolated from multiple anatomic sites and at separate times over a period of 5 years (Table 1). All specimens were identified in the Fungus Testing Laboratory directed by one of the authors (M.G.R.). Identification was performed by negative germ tube formation, the yeast API 20C method, and the Vitek yeast identification system (biomerieux Vitek Inc., Hazelwood, Mo.). Yeast cells were initially isolated on Sabouraud dextrose agar (BBL, Cockeysville, Md.), stored in sterile water, and frozen at −70°C until analysis.

Preparation of DNA. For the isolation of whole-cell DNA, yeast cells were grown on Sabouraud agar plates for 48 h at 30°C. Yeast colonies were resuspended in 2 ml of 75 mM NaCl–25 mM EDTA (pH 8.0) to a turbidity of approximately 5.0 McFarland and pelleted at 230 × *g* for 10 min. The cells were then suspended in 2 ml of the NaCl-EDTA solution. Low-melting-point 1.5% agarose (Boehringer Mannheim, Indianapolis, Ind.) was prepared in 125 mM EDTA (pH 7.5) and placed into the cell suspension at 37°C. One hundred microliters of 2,000 U of Zymolyase 20T (ICN Biomedicals, Costa Mesa, Calif.) per ml was then added to the suspension of cells. The cell-agarose-Zymolyase suspension was pipetted into a plug mold and refrigerated for 1 h at 4°C. The solidified plugs were removed and placed in 5 ml of 0.5 mM EDTA (pH 9.0)–7.5% β-mercaptoethanol and were incubated overnight in a 37°C water bath with gentle shaking. After 12 h (overnight), the buffer was removed and the plugs were washed once with 5 ml of 50 mM EDTA (pH 7.5). The wash solution was replaced with 5 ml of ESP solution (0.5 M EDTA [pH 9.0 to 9.5], 1% sarcosine [Sigma Chemical Co., St. Louis, Mo.], 50 μg of proteinase K [Sigma] per ml). The

TABLE 1. Outbreak and comparison isolates of *C. rugosa*

Isolate no. (type of isolate[a])	Patient no.	Source	State where isolated
1 (O)	*[b]	Glucose monitor	Calif.
2 (O)	1	Blood	Calif.
3 (O)	1	Wound	Calif.
4 (O)	1	Urine	Calif.
5 (O)	1	Blood	Calif.
6 (O)	2	Wound	Calif.
7 (O)	3	Blood	Calif.
8 (O)	3	Wound	Calif.
9 (O)	3	Joint fluid	Calif.
10 (O)	4	Humeral fracture	Calif.
11 (C)	5	Blood	Iowa
12 (C)	6	Urine	N.Y.
13 (C)	7	Lower back	Tex.
14 (C)	8	Subclavian catheter	Ky.
15 (C)	9	Blood	N.Y.
16 (C)	10	Blood	Calif.
17 (C)	11	Blood	Calif.
18 (C)	12	Blood	Calif.

[a] O, outbreak isolate; C, comparison isolate.
[b] *, environmental culture.

plugs were incubated overnight in a water bath at 50°C with gentle shaking. After 12 h (overnight), the plugs were transferred to 0.5 M EDTA (pH 9.0) and stored at 4°C until electrophoresis.

PFGE. One-third of the plug was loaded into the wells of a 1% agarose gel. The gel was then placed into the electrophoresis chamber of a CHEF DR II (Bio-Rad) apparatus with a running buffer of 0.089 M Tris–0.089 M borate–0.0025 M EDTA diluted in distilled water to a 0.5× concentration. A constant temperature of 14°C was provided by using a chilled water bath and pump. The electrophoretic conditions that gave the best separation of the chromosomes were pulse intervals of 130 s at 140 V for 20 h, then 300 s at 150 V for 14 h, and then 300 s at 110 V for 22 h. *Saccharomyces cerevisiae* chromosomal DNA expressed in kilobases was used as a size standard.

Restriction fragment length polymorphism (RFLP). Whole-cell DNA prepared as described above was digested with *Sfi*I (Boehringer Mannheim) according to the recommendations of the manufacturer. The electrophoretic condition that gave the best separation was pulse intervals of 1 to 38 s at 185 V for 17 h. The gels were stained with ethidium bromide and photographed under UV illumination. Differences among isolates were determined by visual comparison of the DNA patterns. Isolates were considered distinct by karyotype if patterns differed by one band. Isolates were considered distinct by PFGE digestion pattern if patterns differed by more than three bands (22).

RESULTS

The PFGE karyotype of the outbreak isolates of *C. rugosa* was composed of four chromosome bands ranging in size from 950 to >2,500 kb (Fig. 1). The *C. rugosa* karyotype demonstrated the same electrophoretic karyotype patterns for the outbreak isolates (patients and environmental) from the burn unit (Fig. 1). The isolate from the other ward of the hospital had a distinct karyotype. RFLP typing showed the same result with patterns obtained by *Sfi*I digestion of whole-cell DNA (Fig. 2).

The PFGE karyotype of six of the comparison isolates was composed of five chromosome bands; one karyotype contained six chromosome bands, and one contained seven chromosome bands (Fig. 3). These karyotypes were compared with the outbreak isolate karyotype containing four chromosome bands. Each comparison isolate had a distinct PFGE karyotype pattern. Some RFLP patterns of comparison isolates did not distinguish some strains distinguishable by PGFE karyotyping (Fig. 4). Isolates with indistinguishable RFLP patterns but distinct karyotypes were from geographically distinct areas.

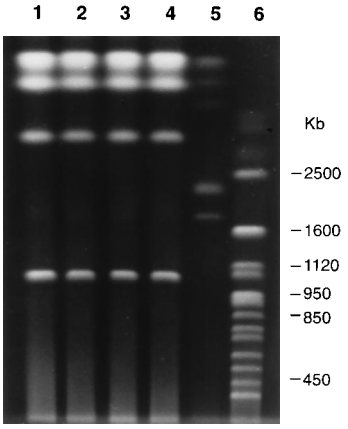

FIG. 1. Electrophoretic karyotypes of *C. rugosa* isolates. Karyotypes of representative outbreak isolates from three patients in a burn unit and from an environmental culture (glucose monitor) in the burn unit are shown in lanes 1 to 4. The distinct karyotype of an isolate from a patient on another ward of the hospital is shown in lane 5. An *S. cerevisiae* chromosome size standard is shown in lane 6.

DISCUSSION

For an assessment of the epidemiology and source of cross-transmission of nosocomial *Candida* species from environmental and human sources, it is necessary to develop reliable and discriminatory typing procedures (3, 6, 14, 19). Several epidemiologic studies have demonstrated the efficacy of PFGE as a means of strain delineation for *C. albicans* and other *Candida* species (4, 7, 9–11, 15, 20, 24).

This investigation represents, to our knowledge, the first application of a typing system for isolates of *C. rugosa*. We investigated the molecular typing characteristics of *C. rugosa*, performing PFGE on the nine isolates from the cluster of *C. rugosa* infections in a burn unit, one comparison isolate from another ward of that hospital, and the eight comparison isolates obtained from a referral fungus testing laboratory. The *C. rugosa* isolates from the burn unit cluster share the same PFGE karyotype pattern, which differs from the karyotype patterns of the comparison isolates. The same results were seen for the RFLP patterns obtained by *Sfi*I digestion of whole-cell DNA of the outbreak isolates. This result suggests that clonal dissem-

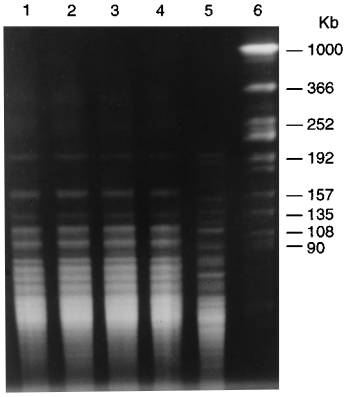

FIG. 2. PFGE of *C. rugosa* DNA digested with *Sfi*I. The RFLP patterns of representative outbreak isolates from three patients and an environmental isolate in a burn unit are shown in lanes 1 to 4. The distinct RFLP pattern of an epidemiologically unrelated patient isolate is shown in lane 5. An *Escherichia coli* MG 1655 size standard is shown in lane 6.

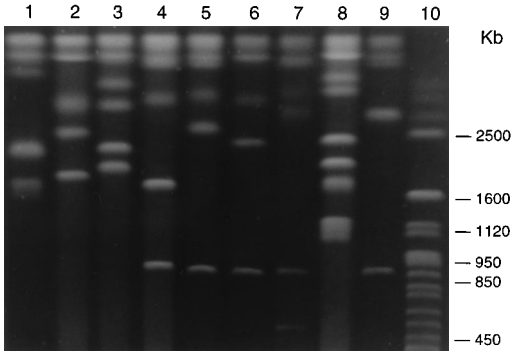

FIG. 3. Electrophoretic karyotypes of *C. rugosa* isolates. Karyotypes of comparison isolates are shown in lanes 1 to 8; the karyotypes are distinct. The karyotype of the outbreak strain is shown in lane 9. An *S. cerevisiae* chromosome size standard is shown in lane 10.

ination of a single strain was responsible for the cluster of *C. rugosa* infections in the burn unit.

PFGE karyotyping showed distinct patterns for *C. rugosa* isolates from distinct geographic areas. Somewhat surprisingly, *Sfi*I RFLP patterns did not distinguish some isolates that were distinguishable by karyotype. This result indicates that some genomic fragments generated by restriction enzyme digestion are very similar even though the karyotypes, and thus the strains, are distinct. Previous studies have suggested the need to correlate karyotypes as well as RFLP types in the molecular typing of *Candida* species (4, 14). In some instances, RFLP typing has been more helpful than karyotyping in distinguishing isolates. Our study indicates that the correlation of karyotyping and RFLP results is needed and that for *C. rugosa*, the karyotype may be more useful for determining strain identity. PFGE typing is a useful molecular typing tool for the discrimination of strains of *C. rugosa* and will be a useful marker for monitoring future *C. rugosa* infections in the hospital.

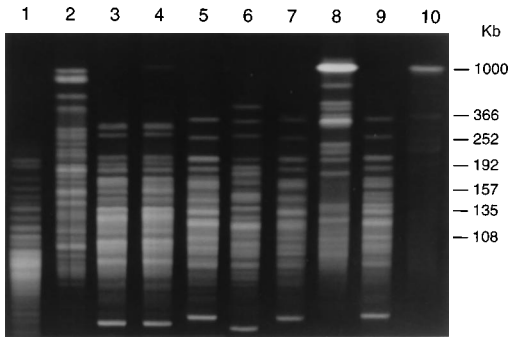

FIG. 4. PFGE of *C. rugosa* digested with *Sfi*I. The RFLP patterns of comparison isolates are shown in lanes 1 to 8. The RFLP pattern of the outbreak strain is shown in lane 9. A common RFLP pattern is seen in lanes 3 and 4; another common pattern is seen in lanes 5, 7, and 9. An *E. coli* MG 1655 size standard is shown in lane 10.

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