JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1987, p. 675–679
0095-1137/87/040675-05$02.00/0

Vol. 25, No. 4

Application of DNA Typing Methods to Epidemiology and Taxonomy of *Candida* Species

STEWART SCHERER[1] AND DAVID A. STEVENS[2]*

Department of Microbiology, University of Minnesota Medical School, Minneapolis, Minnesota 55455,[1] and Department of Medicine, Santa Clara Valley Medical Center and Institute for Medical Research, and Stanford University Medical School, San Jose, California 95128[2]

Received 26 August 1986/Accepted 25 November 1986

Methods are described for extraction of DNA from the yeast form of *Candida* spp., followed by digestion and electrophoresis of DNA fragments. The resulting gel patterns (>100 bands) were used to type *Candida* isolates. Four intense bands identified, three of which are present in each isolate (6 to 7, 3.7 or 4.2, and 2.5 to 3 kilobases), appear to be DNA encoding the rRNA. The methods proved to be both simple and reproducible. The patterns were shown to be stable through several hundred doublings from multiple single colonies. A survey of isolates showed that, on the basis of similarity of gel patterns, several *Candida* species could be sorted into mutually exclusive groups, and subgroups could be created. Analyses of this survey suggested the possible epidemiologic and taxonomic applications of these methods. DNA typing methods appear to offer important potential advantages over phenotyping methods. The methods provide a base for further epidemiologic studies and for further development of techniques, such as the use of cloned probes for studies of DNA homology.

Candida species are ubiquitous human pathogens, causing localized, invasive or disseminated disease in normal or immunocompromised hosts, promoted by such common factors as invasive procedures, catheters, immunosuppressive therapy, malignancy, immaturity, immunodeficiency, granulocytopenia, broad-spectrum antimicrobial agents, intravenous drug abuse, etc. Candidiasis is found both within the hospital environment and outside it. Various methods have been attempted to type *Candida* isolates, with the objective of developing an epidemiologic tool. Such a tool could be used to track *Candida* spp. within a host, between hosts, or between host and inanimate objects (or vice versa), or to associate particular strains with various anatomic or geographic sites, particular disease entities, or particular host characteristics. These methods have relied on such phenotypic characteristics as streak morphology (1), resistance to various chemicals (18), mixtures of resistance patterns and biochemical tests (10), enzyme profiles (3), and susceptibility to toxins (14). Problems encountered have included reproducibility, cumbersome test methods, and susceptibility of result to a variety of variables, as presented in detail in the Discussion.

We report a method which relies on DNA extraction, digestion of the *Candida* genome with a restriction endonuclease, and electrophoresis under standard conditions, resulting in DNA of various restriction lengths in gel producing unique patterns analogous to "fingerprints." Analogous approaches have proven successful in using plasmid, viral, or bacterial DNA patterns as an epidemiologic tool. The use of DNA polymorphisms in epidemiology offers several possible advantages. In principle, one may find an arbitrarily large number of polymorphisms with a single method, Second, highly polymorphic loci may exist, such that many possibilities can be scored rather than a single positive-negative result. Such methods would be in contrast to phenotypic characteristics, which may not reflect genetic relatedness (for example, genes for dissacharide utilization may be found in many different loci in the genetic map of *Saccharomyces cerevisiae* [2]), whereas for the application of a tool to the uses described one is interested in the identity of strains (therefore, their genomes) rather than identity of phenotype. We describe here our methods and our initial results with these methods. We find these methods to have desirable characteristics in terms of reproducibility, ease of performance, many polymorphisms noted, and distinction between species. They provide a base for further epidemiologic studies and further development of technique and may prove to be applicable to a variety of fungi.

MATERIALS AND METHODS

Isolates. *Candida* isolates were identified by standard methods described previously (4, 12, 16) and stored on agar slants under sterile distilled water at 4°C. Alternatively, 1.5 ml of a stationary-phase culture was mixed with 0.1 ml of dimethyl sulfoxide and frozen at −70°C.

Cells were grown in yeast nitrogen base or YPD (1 g of yeast extract, 2 g of Bacto-Peptone, and 2 g of glucose per 100 ml of sterile distilled water) broth as indicated (all reagents from Difco Laboratories, Detroit, Mich.).

Preparation of DNA. A modification of procedures previously applied to *S. cerevisiae* was used (6). Five-milliliter tubes of YPD broth were inoculated with a loopful of stored cells and cultured at 37°C overnight. Packed cells from 1.5 ml of medium were prepared by centrifugation, mixed with 1 ml of 1 M sorbitol, and recentrifuged. The packed cells were suspended in 1 ml of a solution (pH 7.5) of 1 M sorbitol plus 50 mM potassium phosphate buffer with 0.1% (vol/vol) 2-mercaptoethanol and 0.2 mg of Zymolase 100T (Miles Laboratories, Inc., Naperville, Ill.) per ml. This was incubated for 30 min at 30°C. The resultant spheroplasts were packed by centrifugation and suspended in 0.5 ml of 50 mM sodium EDTA buffer (pH 8.5) with 2 mg of sodium dodecyl sulfate per ml to which was added 0.003 ml of diethyl pyrocarbonate. This was mixed and then incubated at 70°C for 30 min. A 50-μl portion of 5 M potassium acetate was added, followed by mixing and then incubation at 0°C for 30 min. The mixture was clarified by centrifugation, and the supernatant was decanted into 1 ml of ethanol and mixed.

* Corresponding author.

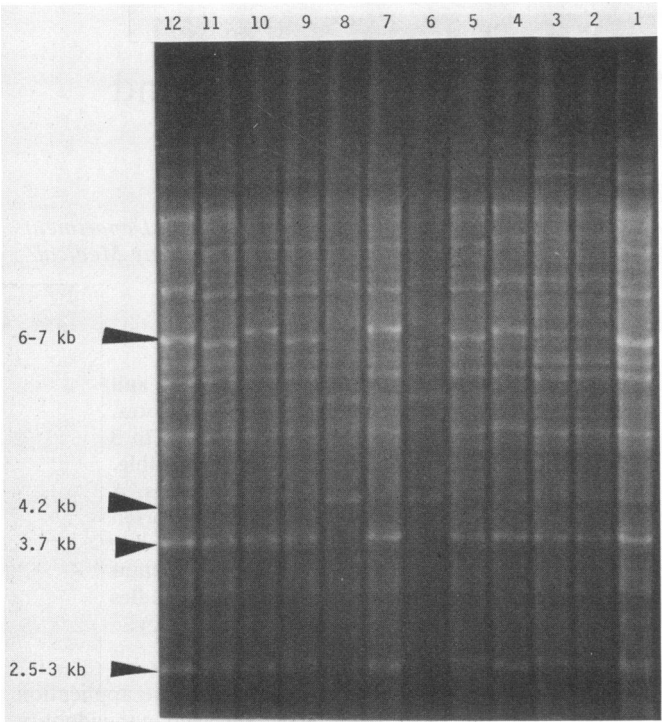

FIG. 1. Reliable differentiation of *C. albicans* and examples of intensely staining bands (arrows) seen in all *C. albicans* isolates. The gels are *Eco*RI endonuclease digests of DNA of isolates 610 (lanes 1 and 9, numbering from right to left), Sh8 (lanes 2 and 3), Sh27 (lanes 4 and 12), 603 (lanes 5 and 11), and B23578 (lanes 6 and 8). Isolates 603 and 610 are indistinguishable by these methods.

This was then clarified by centrifugation and the supernatant was discarded. To the precipitate was added several volumes of 70% ethanol, after which the ethanol was removed. The precipitate was allowed to dry and then suspended in 0.1 ml of 10 mM Tris chloride buffer (pH 7.5)–1 mM EDTA (TE solution) containing 10 μg of RNase A (Worthington Diagnostics Freehold, N.J.; previously treated by boiling for 1 min in TE solution at a concentration of 1 mg/ml) per ml. A 200-μl amount of 2-propyl alcohol was added, the mixture was clarified by centrifugation, and the supernatant was decanted. An excess volume of ethanol was added to the precipitate, and the ethanol was then removed. The precipitate was allowed to dry and then suspended in 0.05 ml of TE solution. Such DNA preparation is sufficient for preparing five restriction digests.

Restriction digests. A 10-μl portion of the DNA sample prepared as described was mixed with an equal volume of double-strength buffer (100 mM Tris chloride, pH 7.5, 20 mM $MgCl_2$, 200 mM NaCl). A 0.1-μl amount of *Eco*RI nuclease restriction enzyme (a kind gift from Paul Modrich, Duke University) containing 500 U was added (1 U completely digests 1 μg of phage lambda DNA in 60 min), and the mixture was incubated at 37°C. We found empirically that 5 min was sufficient to result in complete digestion. Digestion was stopped by heating the mixture to 70°C.

Gel electrophoresis. Gels of 0.8% agarose with a Tris-acetate-EDTA buffer system previously described (5) were used. Electrophoresis was performed at 1 to 2 V/cm overnight. Gel photography used 300-nm transillumination through an orange filter and Polaroid 667 or 55 film.

RESULTS

Initial results with DNA methods. A random sample of six *Candida albicans* clinical isolates subjected to the DNA extraction, digestion, and electrophoresis methods described above revealed sharp gel patterns, with >100 identifiable bands varying in intensity. Preliminary data (not shown) showed that the largest number of distinct bands were seen when *Eco*RI was the restriction enzyme used versus *Hin*dIII or *Bam*HI. With *Eco*RI there were distinct patterns for each of the six isolates. Moreover, every isolate demonstrated three intensely staining bands (Fig. 1), the intense staining indicating multiple copies of each of these three fragments in *C. albicans* DNA.

The sizes of these three bands were determined by electrophoresis of the *C. albicans* DNA digests in lanes adjacent to mixtures of *S. cerevisiae* and lambda phage DNA fragments of known length (Fig. 2). The largest and the smallest of these three were each determined to be variable in size from *C. albicans* isolate to isolate. Continuing studies with larger numbers of *C. albicans* isolates have revealed the largest of these three fragments to be 6 to 7 kilobases (kb) in

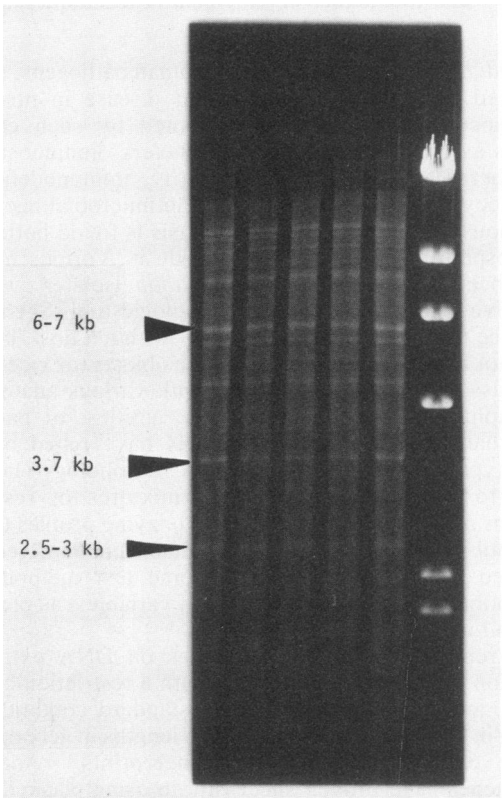

FIG. 2. Gel electrophoresis of DNA of *C. albicans* isolates after digestion with restriction endonuclease *Eco*RI, adjacent to classical sizing markers from digest of lambda phage DNA with endonuclease *Hin*dIII (5). The intensely staining bands are marked with arrows and are fragments of 6 to 7, 3.7, and 2.5 to 3 kb in length. The same bands are marked in Fig. 1, as well as a fragment of 4.2-kb length seen in a minority of isolates instead of the 3.7-kb fragment. The bands marked have been shown to be homologous with *S. cerevisiae* rDNA. Lanes are, right to left, lambda DNA digest and then *C. albicans* isolates 366, 85-B-14, 85-B-2, 85-B-3, and 85-B-5. These isolates are indistinguishable by this method. 366 is a multiply passaged laboratory isolate, and the others are fresh clinical isolates. 85-B-3 and 85-B-14 are isolates from different sites in the same patient.

length and the smallest to be 2.5 to 3 kb. A band intermediate in size between these two (3.7 kb) has been constant in size in most isolates. One of the six initial isolates, and a minority of isolates subsequently studied, did not show this intensely staining intermediate band, but instead showed another intermediate band of 4.2 kb in length. DNA-DNA hybridization studies with the electrophoresed *C. albicans* DNA digests and a radiolabeled cloned probe of *S. cerevisiae* DNA encoding for rRNA (rDNA) have shown that the 6- to 7-, 2.5- to 3-, 3.7-, and 4.2-kb intensely staining bands are homologous with the probe (13). Homology appeared to be greatest with the latter two bands. We thus assume that these three bands are *C. albicans* rDNA, with two polymorphic (6 to 7 and 2.5 to 3 kb) fragments and one dimorphic (3.7 or 4.2 kb) fragment. Other intense bands are likely to be mitochondrial DNA (20).

Reproducibility of staining patterns. That the staining patterns were reproducible in repeated runs was established in two ways. First, six isolates were subcultured on each of two agar slants, and the 12 slants were randomly assigned numbers. Subcultures of each of the 12 slants were then processed separately by the procedures described above. The resulting patterns (Fig. 1) were interpreted by one of us without knowledge of the code, and the isolates were correctly paired. The reader can confirm this by examination of Fig. 1, particularly if the figure is examined first, without reference to the legend.

Second, a small number of isolates previously tested were included with subsequent batches of isolates for testing, coded so that the interpreter of the gel patterns was unaware of the number or designation of previously tested isolates. During this testing we established that, since recognition of separate gel patterns (analogous to interpretation of fingerprint patterns) required examination of both the position of the rDNA bands described and the presence or absence of the remaining, less intense bands and areas of low staining, adjacent lanes are required in some instances as a final test of indentity and nonidentity. In these studies, the same isolates stored frozen, at 4°C under distilled water, or at room temperature on agar slants were used as stocks. We thus also demonstrated stability of individual pattern under various storage conditions.

Stability of DNA gel patterns. The preceding studies show two desirable qualities of a method which might be used for epidemiologic typing, ease of preparation and reproducibility. To be useful for epidemiologic purposes, we thought the gel patterns should be stable through multiple generation and growth cycles of the organism, so that an organism could be traced through its multiplication during carriage by a host or in the environment or its multiplication during passage from site to site within a host or within the environment (or between hosts or host-environment interchanges).

To test this, the following experiment was performed. An isolate of *C. albicans*, Sh27, was streaked to single colonies on agar. A single colony was suspended in 2 ml of sterile distilled water, and the liquid was mixed. From this, 0.1 ml was transferred to each of five 2-ml tubes of yeast nitrogen base broth labeled A through E. These tubes were incubated for 24 h at 35°C, and then 1 drop of turbid growth from each was added to five tubes of 2 ml of fresh broth. This process was repeated through 25 24-h and 5 72-h alternating cycles of growth. At the conclusion we had five tubes which contained *C. albicans* that had undergone approximately 420 generations (estimated from spectrophotometric determination of doubling time for this isolate under these incubation conditions) in parallel, but descended from a single colony. Liquid from each of these five tubes and from the original distilled water suspension (held at 4°C) of the initial colony were streaked to yield single colonies. Two colonies were picked from each to make 12 subcultures. These were then processed by using the DNA methods described above, and the gel patterns were examined. The subcultures produced indistinguishable patterns. We concluded that these patterns are stable through several hundred doublings of the organism. This experiment was repeated in similar fashion starting with three other isolates, with identical conclusions.

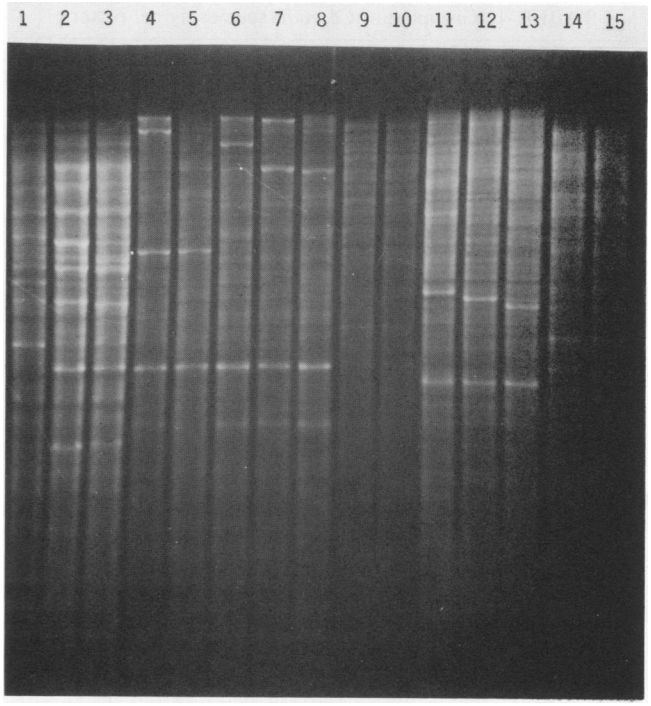

FIG. 3. Examples of *Candida* isolates used to develop typing scheme (Table 1). Lanes (left to right) are digests of the following: 1 to 3, *C. albicans* isolates B23578, Sh27, and Sh8; 4 to 8, *C. tropicalis* isolates 84-30, 85-43, 82-15, 85-27, and 85-30; 9 and 10, *C. parapsilosis* 85-11; 11 and 12, *C. parapsilosis* 82-33 and 85-15; 14 and 15, *C. parapsilosis* 83-60 and 85-3. (Digest in lane 13 is from an isolate not included in the present study, as standard identification methods used were unable to resolve whether this was *C. parapsilosis* or *C. lusitaniae*; by DNA typing methods this would appear to be a *C. parapsilosis* of group V.)

Initial survey of *C. albicans* and other *Candida* species. Prior to more formal epidemiologic applications of these methods, it was of interest to us to compare more isolates of *C. albicans* with each other and with those of other *Candida* species. For this study, 36 isolates were selected: 17 *C. albicans*, 10 *C. tropicalis*, 7 *C. parapsilosis*, 1 *C. krusei*, and 1 *C. pseudotropicalis*. These 36 were randomly assorted and coded with letters. The subcultures were then processed by the procedures described above, and the resultant gels were interpreted without knowledge of the composition of the group of 36.

The resultant patterns (Fig. 3) were interpreted in the following way. Seven groups were created, containing 17, 10, 1, 1, 2, 2, and 3 isolates, respectively. The first of these was tentatively called "*C. albicans*" on the basis of similarity to the patterns described in the preceding experiments. On decoding, this group contained all *C. albicans* included and no other species. The remaining six groups contained, respectively, all 10 *C. tropicalis*, 1 *C. krusei*, and 1 *C.*

TABLE 1. Grouping of *Candida* species by gel pattern

Group/*Candida* sp.	Subgroup	Isolate(s)
I. *C. albicans*[a]	1	610, 603, 85-B-9
	2	366, 85-B-14, 85-B-2, 85-B-3, 85-B-5, 85-B-15
	3	Sh8
	4	H12
	5	85-B-12
	6	140/1
	7	85-B-8
	8	Sh27
	9	85-B-11
	10	B23578
II. *C. tropicalis*	1	das-1, 85-30, 84-5
	2	85-12, 82-15, 82-60
	3	84-93, 85-27
	4	84-30
	5	85-43
III. *C. krusei*	1	81-B-5
IV. *C. pseudotropicalis*	1	SA
V. *C. parapsilosis*	1	85-15
	2	82-33
VI. *C. parapsilosis*	1	81-B-4
	2	85-11
VII. *C. parapsilosis*	1	85-3, 83-60, 82-43

[a] Some of these isolates have been reported previously with respect to other properties, including virulence, serotype, and corticosteroid-binding protein characteristics (7), and have been deposited on request with the American Type Culture Collection. The corresponding ATCC numbers are: H12, 56879; Sh8, 56881; Sh27, 56882; 140/1, 56883; 366, 56884; 603, 56885; 610, 56886.

pseudotropicalis and, finally, three groups which each contained *C. parapsilosis* (2, 2, and 3 members each) (Table 1). No group created on the basis of this "blind" interpretation of the patterns contained more than one species. Thus was demonstrated the potential of these methods to distinguish *Candida* species taxonomically.

The five groups which contained more than one member could be further subdivided. Two subgroups of three and six *C. albicans* isolates each showed identical patterns; we suggest that these may represent the most common *C. albicans* DNA types in clinical specimens (examples are shown in Fig. 1 to 3). These two subgroups each contained both fresh clinical and old laboratory-passaged isolates, further suggesting that gel patterns are stable to prolonged storage as well as multiple passage of isolates. Of possible interest is that the 17 *C. albicans* isolates contained 4 whose source was known to be urine; all 4 (from different patients) were found in the subgroup of 6 identical isolates. One of the other two isolates in this same subgroup was a stool isolate from the same female patient who provided a urine isolate (the only such pair in this pilot study). This suggests that some genotypes may be found to be particularly associated with some body sites and possibly that patients may carry the same genotype preferentially at more than one body site. Evidence for the former (16) and the latter (9, 10, 15, 18) possibilities has been presented by using phenotyping methods.

The remaining eight *C. albicans* isolates were distinguishable from each other and from the preceding two groups of nine, resulting in a total of 10 subgroups. Only one isolate showed the 4.2-kb intermediate band of high intensity, the other 16 containing the 3.7-kb band. As indicated previously, some repeat runs were necessary to pair isolates in adjacent lanes when there were questions of identity or nonidentity. *C. albicans* subgroup 1 with three members included two isolates known to be discordant for several phenotypic characteristics, including virulence in vivo (7), proteinase production, salt tolerance, and safranine resistance (12), but concordant for six other phenotypic properties. The latter three of the four discordant characteristics mentioned have been used in a phenotypic typing scheme (12); this suggests that such typing schemes will give results that are not identical to classifications based on genome analysis by DNA restriction enzymes. The two subgroups with more than one member each both included isolates from different hospitals, suggesting that some genotypes may be widely distributed. Ten isolates from the same hospital were distributed into six different subgroups, indicating that isolates from the same hospital are polymorphic.

The group of 10 *C. tropicalis* isolates could be broken into five subgroups; three of these contained 2 to 3 isolates which were indistinguishable from each other. Finally, one of the three groups which each contained only *C. parapsilosis* contained three indistinguishable isolates, but the other two groups each contained two isolates that could be further distinguished from each other. These subgroupings further suggest that (i) there are several different DNA patterns of *C. albicans*, *C. tropicalis*, and *C. parapsilosis* (and probably other species) in clinical specimens and (ii) the same epidemiologic applications proposed previously for *C. albicans* may also apply to other *Candida* species. These data also suggest that isolates presently identified as *C. parapsilosis* are more genotypically heterogeneous than *C. albicans* or *C. tropicalis* isolates.

DISCUSSION

In a review of typing methods for *Candida* species, Warnock listed three desirable criteria of a typing method: that it differentiate a sufficient number of strains, that it give clear and reproducible results, and that it not be too difficult to perform (17).

The initial method described, streak morphology (1), gave unstable results on storage of specimens, was unable to clearly differentiate *C. albicans* from *C. tropicalis*, and was never applied outside the originating laboratory. The two phenotyping methods most widely studied are those which rely on resistance to chemicals (8, 9, 18) or on a combination of resistance and biochemical attributes (10–12, 15). The former method requires inclusion of 10 reference strains with the unknowns, titration of all with multiple plates with various concentrations, and then selection of the plate which shows the standard pattern of inhibition for the reference strains to read the results of the unknowns. Inconsistencies between runs may be due to susceptibility of the methods to fungal inoculum size, as well as to the small changes in reagent concentration which are apparent. Variability has been shown to be 8% (5 of 66 isolates) between a pair of runs (8). The latter method, it appears, imposes a sharp dividing line on a continuous distribution of properties among *C. albicans* strains. This method is sensitive to inoculum size, temperature, small imprecisions in medium preparation, and drying of the plates. Variability under standardized conditions was 5 to 10% in three runs for seven of the nine tests involved.

The methods we have described are currently being used in studies of correlation with phenotypes and in epidemiologic investigations of carriage in individuals, transmission between sex partners, and transmission within a hospital, and the features of ease and reproducibility described have been corroborated in these studies. We have referred already to the possible uses in taxonomy.

The methods, moreover, lend themselves to further development. For example, the current procedures appear to rely on one highly polymorphic locus, the rDNA, and other repeated DNA segments (presumably mitochondrial DNA) to discriminate between strains. DNA fragments can be cloned in a vector, amplified, and radiolabeled to produce a probe, which can then be examined for its hybridization to areas of the gel pattern. Using a DNA probe homologous to dispersed repeated sequences would permit many loci to be examined simultaneously. The use of such probes, which may detect differences in DNA sequence occurring within or adjacent to the regions homologous to the probe, would allow even more stringent tests to identity or nonidentity. Single-copy sequences could also be examined. In addition, DNA polymorphisms, caused by transposable elements and other mechanisms, often arise at rates that can be measured in the laboratory. This should make it possible to estimate the number of generations that separate strains of interest. When such methods fail to reveal a difference, one would then be able to state a limit for how long two seemingly identical strains have been apart.

Clones could be selected which have sequences unique to a particular *Candida* species. Use of such probes could allow, without the need for electrophoresis, identification of digests of colonies (e.g., from clinical cultures) as to species by dot-blotting methods. Preliminary studies in development of probes have indicated the possibility of further distinctions of identity and nonidentity and of cloning DNA fragments unique to *C. albicans*, as will be reported (S. Scherer and D. A. Stevens, submitted for publication).

ACKNOWLEDGMENTS

We thank Stanley Falkow for his interest in this collaboration and Alon Perlman for technical assistance with parts of these studies.

This work was supported in part by Public Health Service grant 1-R01-AI-23850-01 from the National Institutes of Health.

LITERATURE CITED

1. **Brown-Thomsen, J.** 1968. Variability in *Candida albicans* (Robin) Berkhout. I. Studies on morphology and biochemical activity. Hereditas **60**:355–397.
2. **Carlson, M., B. C. Osmond, and D. Botstein.** 1980. SUC genes of yeast: a dispersed multigene family. Cold Spring Harbor Symp. Quant. Biol. **45**:799–804.
3. **Casal Roman, M., and M. J. Linares Sicilia.** 1983. Preliminary investigation of *Candida albicans* biovars. J. Clin. Microbiol. **18**:430–431.
4. **Cooper, B. H., J. B. Johnson, and E. S. Thaxton.** 1981. Clinical evaluation of the Uni-Yeast-Tek system for rapid presumptive identification of medically important yeasts. J. Clin. Microbiol. **8**:349–355.
5. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
6. **Davis, R. W., M. Thomas, J. Cameron, T. St. John, S. Scherer, and R. Padgett.** 1980. Rapid DNA isolations suitable for enzymatic and hybridization analysis. Methods Enzymol. **65**:404–411.
7. **Loose, D. S., D. A. Stevens, D. J. Schurman, and D. Feldman.** 1983. Distribution of a corticosteroid-binding protein in *Candida* and other fungal genera. J. Gen. Microbiol. **129**:2379–2385.
8. **McCreight, M. C., and D. W. Warnock.** 1982. Enhanced differentiation of isolates of *Candida albicans* using a modified resistogram method. Mykosen **25**:589–598.
9. **Meinhof, W.** 1982. Demonstration of typical features of individual *Candida albicans* strains as a means of studying sources of infection. Chemotherapy (Basel) **28**(Suppl. 1):51–55.
10. **Odds, F. C., and A. B. Abbott.** 1980. A simple system for the presumptive identification of *Candida albicans* and differentiation of strains within the species. Sabouraudia **18**:301–317.
11. **Odds, F. C., and A. B. Abbott.** 1983. Modification and extension of tests for differentiation of Candida species and strains. Sabouraudia **21**:79–81.
12. **Odds, F. C., A. B. Abbott, R. L. Stiller, H. J. Scholer, A. Polak, and D. A. Stevens.** 1983. Analysis of *Candida albicans* phenotypes from different geographical and anatomical sources. J. Clin. Microbiol **18**:849–857.
13. **Philippsen, P., M. Thomas, R. A. Kramer, and R. W. Davis.** 1978. Unique arrangement of coding sequences of 5S, 5.8S, 18S and 25S ribosomal RNA in *Saccharomyces cerevisiae* as determined by r-loop and hybridization analysis. J. Mol. Biol. **123**:387–404.
14. **Polonelli, L., C. Archibusacci, M. Sestito, and G. Morace.** 1983. Killer system: a simple method for differentiating *Candida albicans* strains. J. Clin. Microbiol. **17**:774–780.
15. **Skorepora, M., and H. Hauck.** 1985. Differentiation of *Candida albicans* biotypes by the method of Odds and Abbott. Mykosen **28**:323–331.
16. **Stiller, R. L., J. E. Bennett, H. J. Scholer, M. Wall, A. Polak, and D. A. Stevens.** 1982. Susceptibility to 5-fluorocytosine and prevalence of serotype in 402 *Candida albicans* isolates from the United States. Antimicrob. Agents Chemother. **22**:482–487.
17. **Warnock, D. W.** 1984. Typing of *Candida albicans*. J. Hosp. Infect. **5**:244–252.
18. **Warnock, D. W., D. C. E. Speller, J. K. Day, and A. J. Farrell.** 1979. Resistogram method for differentiation of strains of *Candida albicans*. J. Appl. Bacteriol. **46**:571–578.
19. **Williamson, M. I., L. P. Samaranayake, and T. W. MacFarlane.** 1986. Biotypes of oral *Candida albicans* and *Candida tropicalis* isolates. J. Med. Vet. Mycol. **24**:81–84.
20. **Wills, J. W., B. A. Lasker, K. Sirotkin, and W. S. Riggsby.** 1984. Repetitive DNA of *Candida albicans*: nuclear and mitochondrial components. J. Bacteriol. **157**:918–924.