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Application of a Numerical Index of Discriminatory Power to a Comparison of Four Physiochemical Typing Methods for *Candida albicans*

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Four typing methods for the strain differentiation of *Candida albicans* (morphotyping, resistotyping, extracellular enzyme production, and carbon source assimilation reactions) were compared on a single population of 100 strains. An index of discriminatory power was used to facilitate this comparison. Extracellular enzyme production had poor discriminatory power, and carbon source assimilation reactions were poorly reproducible. Morphotyping and resistotyping had acceptable reproducibility and discrimination. The use of resistotyping and morphotyping in parallel enhanced discrimination without an unacceptable decrease in reproducibility, although other combinations did not enhance discrimination because reproducibility was impaired.

Despite the variety of typing methods for *Candida albicans* described in recent years (6, 11, 13, 14, 16, 20), none has particularly good discrimination and some suffer from poor reproducibility (10). Furthermore, the choice of the most appropriate method is not obvious, since different authors have used different populations on which to validate their methods. Even using the same killer strains, two sets of workers (5, 18) found rather different discriminations when methods were applied to different populations. There is, therefore, a need to compare existing typing methods on the same population.

The poor discrimination of available typing methods may be due to deficiencies in the methods themselves; alternatively, it may be that strains of *C. albicans* isolated from clinical material are highly homogeneous. If the reason for the poor discrimination is the former, then there are two possible approaches to increasing discrimination. The first is to attempt development of a new typing method that is highly discriminatory, although the diversity of typing methods already described for *C. albicans* would suggest that this approach would be fruitless. The other approach is to use more than one typing method, either in parallel or in a hierarchical system.

In this paper four typing methods, resistotyping, morphotyping, and biotyping by extracellular enzyme production and by carbon source assimilation reactions, are compared on the same population of 100 strains. In particular, the discrimination of each method is compared with those of others, alone and in combination.

MATERIALS AND METHODS

Strains. The discriminatory powers of the four typing methods were calculated on data from 100 unrelated strains of *C. albicans* from a variety of anatomical and geographical sites within the British Isles (Table 1). All strains produced true hyphae on corn meal agar, and 98 produced chlamydospores. Two were chlamydospore negative but were identified as *C. albicans* by the Mycological Reference Laboratory (Central Public Health Laboratory, London) on the basis of fermentation and assimilation reactions.

Carbon source assimilation reactions. The carbon source assimilation reactions of the 100 strains were tested by using the API 50CH kit (API-Bio Merieux [United Kingdom] Ltd., Basingstoke, England). This kit detects assimilation of glycerol, erythritol, D-arabinose, L-arabinose, ribose, D-xylose, L-xylose, adonitol, β-methylxyloside, galactose, glucose, fructose, mannose, sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, α-methylmannoside, α-methylglucoside, *N*-acetylglucosamine, amygdalin, arbutin, esculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentibiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate, and 5-ketogluconate. Additional carbon sources from the ATB 32GN kit (API-Bio Merieux) were itaconate, suberate, malonate, acetate, DL-lactate, L-alanine, 3-hydroxybenzoate, L-serine, propionate, caprate, valerate, citrate, histidine, 4-hydroxybenzoate, and L-proline. These kits consist of strips containing several wells or cupules, each of which contains a different carbon source. The test procedure used was that recommended in the kit instructions. Briefly, a suspension in distilled water, equivalent to a McFarland standard no. 2, was made from an overnight culture on YM agar (no. 0712-01-8; Difco Laboratories, Detroit, Mich.), and 5 drops from a Pasteur pipette were added to the 6 ml of inoculating medium. The cupules were filled to capacity, and 135 μl was added to each well. The strips were incubated in a moist atmosphere for 48 h at 37°C, and the presence or absence of growth was recorded.

Extracellular enzyme production. Extracellular enzyme production was detected by means of the API ZYM system as previously described (6). This kit detects the presence of alkaline phosphatase, esterase (C_4), lipase esterase (C_8), lipase (C_{14}), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase.

Resistotyping. The resistotyping set used was based on inhibitors described by previous authors (13–16, 23). The set

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TABLE 1. Origin of 100 strains of *C. albicans*

Strain no.	Site of isolation	Origin
1–19	Feces	Cardiff
20, 21, 23–34	Vaginal swabs	Cardiff
22, 38, 47, 48	Vaginal swabs	Southampton
35–37, 39, 40	Vaginal swabs	Birmingham
41–46	Vaginal swabs	Manchester
49	Urine culture	Cork, Ireland
50	Urine culture	Burton on Trent
51–57	Urine cultures	Cardiff
58	Pustule	Luton
59–67	Skin lesions	Cardiff
68	Throat swab	Luton
69–77	Oral lesions	Dental school, Cardiff
78	Blood culture	Burton on Trent
79, 81, 82, 84, 87–92, 94, 95, 96	Blood cultures	London
80	Postmortem kidney	Cardiff
83	Abdominal cavity	Cork, Ireland
85	Cerebrospinal fluid	Oxford
86	Blood culture	Oxford
93	Blood culture	Southampton
97 (NCPF[a] 3153)		207 (serotype A) (8)
98 (NCPF 3118)		Tongue of patient with chronic glossitis England 1944, serotype A
99 (NCPF 3179)		ATCC 10231 (British Pharmacopoeia, 1986)
100 (NCPF 3108)		Sucrose-negative variant from lung, England, 1957

[a] NCPF, National Collection of Pathogenic Fungi, Central Public Health Laboratory, London.

included sodium selenite (no. S-1382; Sigma Chemical Co., St. Louis, Mo.), orthoboric acid (AnalaR, no. 10058; BDH Chemicals Ltd., Poole, England), cetrimide sold as mixed alkyl trimethyl ammonium bromide (no. M-7635; Sigma), malachite green (no. 34045; BDH), copper (II) sulfate pentahydrate (AnalaR, no. 10091; BDH), benzalkonium chloride (no. B-1383; Sigma), chlorhexidine diacetate hydrate (no. 23,386-2; Aldrich Chemical Co. Ltd., Gillingham, Dorset, England), mercurochrome (no. 29177; BDH), sodium arsenate heptahydrate (AnalaR, no. 10237; BDH), flucytosine (no. Ro 2-9915; Roche Products Ltd., Welwyn Garden City, England), sodium chloride (no. S-9625; Sigma), and MacConkey agar no. 3 (no. CM115; Oxoid Ltd., Basingstoke, England). Table 2 shows the concentrations of the stock solutions. Two plates per inhibitor were prepared by melting 80 ml of previously autoclaved YM agar in a boiling water bath, cooling to 56°C, and adding either 4.0 or 3.6 ml of stock solution per bottle. Four plates were then poured from each bottle, stored at 4°C, and used the next day after drying for 20 min.

Strains were prepared for resistotyping by serial subculture on YM agar twice at 37°C and once at 28°C. The final subculture at 28°C was from a light suspension of the organism in water. Finally, suspensions of the test isolates were made in distilled water to lie between McFarland standards 5 and 6. The fungal suspensions were then inoculated onto the agar plates from a multiwell block with a 21-pin multipoint inoculator. All plates were incubated for 44 h at 37°C except for the cetrimide plates, which were incubated at 26°C. The amount of growth of each strain was

TABLE 2. Chemical inhibitors in the resistotyping set for *C. albicans*

Inhibitor chemical	Concn of stock solutions (g/100 ml)	Reactions of control strain:				
		RC6	RC7	RC8	RC9	RC10
Sodium selenite	1.3	+	±	−	±	−
Boric acid	2.5	+	±	+	−	−
Cetrimide	0.3	−	+	+	+	−
Malachite green	0.0005	+	−	±	+	+
Cupric sulfate	2.5	±	+	−	−	−
Benzalkonium chloride	0.1	+	+	+	+	−
Chlorhexidine	0.25	+	+	−	+	+
Mercurochrome	0.5	+	−	−	−	−
Sodium arsenate	5.0	−	+	−	−	−
Flucytosine[a]	0.075	−	−	−	−	−
Sodium chloride	7.5 g in 80 ml	+	+	+	±	±
MacConkey agar		−	−	+	−	−

[a] Flucytosine resistance was routinely found.

assessed and coded as either positive (good growth), equivocal, or negative (poor or no growth). The final result for each inhibitor was considered resistant when there was good growth on both concentrations, susceptible when there was no growth on either or only scanty growth on one plate, and equivocal otherwise. On the salt and MacConkey agars the final code reflected the degree of growth on the single plate. Susceptibility results are coded with capital letters, and equivocal results are coded by lowercase letters. Control strains were RC6, RC7, RC8, RC9, and RC10 (12); their reactions are shown in Table 2.

Morphotyping. The morphotyping method used was that of Phongpaichit et al. (17). However, to enable easier statistical analysis, the coding system was reduced to just six fringe types and two colonial surface types (9). The six fringe types were no fringe, discontinuous fringe, wide fine fringe, wide coarse fringe, narrow fine fringe, and narrow coarse fringe. The two surface states were smooth and featured.

Reproducibility. The test reproducibility is the percentage of times that it is the same or indistinguishable on repeat testing. Tests that had a probability of less than 0.05 of being greater than 90% reproducible were excluded from further analysis. This was calculated from the binary standard deviation (2). The variance of a binary distribution is given by the equation $s^2 = p(1 - p)/N$, where p is the proportion positive and N is the total number. The two-tailed 90% confidence intervals are then given by the equation $c = p \pm 1.645s$. Thus, when the upper value of c was less than 90% that test was excluded from further analysis. For 100 repeats a test was excluded if reproducibility was less than 84% and for 15 repeats if less than 73%.

The reproducibilities of morphotyping and resistotyping were calculated by repeat testing of all 100 strains. The reproducibilities of carbon source assimilation reactions and extracellular enzyme production were calculated from repeat testing of just 15 strains chosen at random. All repeat testing was carried out between 1 and 3 months after the initial tests. Between testing all strains were subcultured at least twice to check that the test results were stable to subculture.

Discriminating power. The discriminating index (D) used here is derived from that of Hunter and Gaston (10). This is the probability that two randomly chosen strains, sampled consecutively, would be distinguished by the test. For data from a single test or from a combination of tests this is given by the equation

$$D = 1 - \frac{1}{N/(N-1)} \sum_{j=1}^{N} a_j$$

where a_j is the number of strains in the population that are indistinguishable from the jth strain and N is the number of strains in the population. This equation is a generalization of the original index; for situations in which all strains can be grouped into mutually exclusive groups, it gives the same value.

RESULTS

Reproducibility. The reproducibility rates of the 12 resistotyping tests were as follows: 100% for selenite, borate, mercurochrome, arsenate, flucytosine, and MacConkey agar; 99% for copper sulfate; 98% for sodium chloride; 95% for malachite green; 93% for cetrimide; 92% for benzalkonium chloride; and 90% for chlorhexadine. The in vitro reproducibility of resistotyping as a whole varied depending on the number of test differences required to distinguish among strains. For a single test difference the reproducibility was 77%, for two test differences it was 93%, for three test differences it was 97%, and for four test differences it was 100%.

For morphotyping, the reproducibility of the colonial surface features was only 82%, so this character was excluded from further analysis. The reproducibility of the colonial fringe characteristics was 89%.

The carbon source assimilation reactions were all 100% reproducible, except for the following: sorbose, starch, salicin, mannose, α-methyl-D-mannoside, L-arabinose, xylitol, and L-serine (93%); melezitose and amygdalin (87%); and adonitol and 5-ketogluconate (80%). Esculin assimilation was excluded, because it was extremely difficult to read. The reproducibilities of carbon source assimilation reactions as a whole were 53% taking one test difference, 60% for two test differences, 80% for three test differences, and 93% for four test differences.

Of the tests based on extracellular enzyme production, cystine arylamidase was difficult to code. This was reflected in a reproducibility of only 53%, leading to its exclusion. Of the other tests, only one, naphthol-AS-BI-phosphohydrolase, differed on just one occasion. Thus, the reproducibility of this test and the method as a whole was 93%.

The reproducibilities of resistotyping and morphotyping combined were 67% for one test difference, 92% for two test differences, and 97% for three major test differences. For all methods combined these were 40% for one, 47% for two, 80% for three, 93% for four, and 100% for five major differences (Tables 3 and 4).

Discrimination. Of the 94 tests other than morphotyping used to examine this population, 46 showed variation. The tests showing variation in the number of positive, negative, and equivocal reactions are shown in Tables 5 and 6, together with the discriminatory index for each test. The distributions of the six fringe groups were 23 with no fringe, 2 with discontinuous fringe, 6 with wide fine fringe, 3 with wide coarse fringe, 57 with narrow fine fringe, and 9 with narrow coarse fringe. This gave a discriminatory index of 0.615.

The discriminatory indices for the four typing methods are shown in Table 3. The use of extracellular enzyme production alone for typing shows very low discrimination. The discriminatory power of morphotyping varied depending on the coding system used; the reduced code described here gave poor discrimination. Resistotyping and carbon source assimilation reactions gave excellent discrimination based on one test difference, although discrimination fell markedly when more than one test difference was required to distinguish strains.

TABLE 3. Discriminating powers and reproducibities of four typing methods for increasing levels of dissimilarity

Dissimilarity level	D (% reproducibility)			
	Assimilation	API ZYM	Morphotype	Resistotype
1	0.891 (53)	0.308 (93)	0.615 (89)	0.903 (77)
2	0.607 (60)	0.068 (100)	0.000	0.694 (93)
3	0.331 (80)	0.003		0.438 (97)
4	0.174 (93)	0.000		0.221 (100)
5	0.109			0.094
6	0.077			0.032
7	0.064			0.005
8	0.057			0.000

The discriminatory powers of some of the possible combinations of methods are shown in Table 4. The combination of morphotyping and resistotyping led to an acceptable increase in discrimination over either alone. The other combinations shown here also appeared to increase discrimination, such that for all four methods combined the discrimination was extremely good (D = 0.996 for one test difference). However, the reproducibilities of these combined methods tended to be somewhat poorer; that greater numbers of differences were required to reliably discriminate among strains, and discrimination fell.

DISCUSSION

The index of discriminatory power simplified the comparison of the typing methods in this study. The original discriminatory index, based on probability theory, was an improvement over the semisubjective assessment of discrimination usually undertaken (10). However, this index required that all strains be placed in mutually exclusive groups. Although this may be practicable for some typing methods, such as serotyping, it is impossible for typing methods that require more than one test difference to distinguish strains or those that give some equivocal results. The index described here overcomes this problem by comparing each strain with all others in the population. Thus, any degree of dissimilarity can be taken as the distinguishing level. Furthermore, the method assumes no prior grouping of strains. If strains can be placed into mutually exclusive

TABLE 4. Discriminating powers and reproducibilities of some combined typing methods for increasing levels of dissimilarity

Dissimilarity level	D (% reproducibility)		
	Resistotype, morphotype	Resistotype, morphotype, assimilation	Resistotype, morphotype, assimilation, API Zym
1	0.957 (67)	0.993 (40)	0.996 (40)
2	0.814 (92)	0.957 (53)	0.970 (47)
3	0.593 (97)	0.861 (80)	0.899 (80)
4	0.362 (100)	0.718 (93)	0.774 (93)
5	0.178	0.552 (100)	0.617 (100)
6	0.071	0.391	0.454
7	0.024	0.268	0.317
8	0.003	0.182	0.220

TABLE 5. Carbohydrate assimilation reactions showing variation and their discriminating powers[a]

Test	No. with assimilation reaction:		D
	Positive	Negative	
API 50CH			
Glycerol	1	99	0.020
L-Arabinose	1	99	0.020
D-Xylose	97	3	0.059
Adonitol	87	13	0.228
L-Sorbose	11	89	0.198
Rhamnose	1	99	0.020
Sorbitol	99	1	0.020
α-Methyl-D-mannoside	3	97	0.059
α-Methyl-D-glucoside	97	3	0.059
N-Acetylglucosamine	99	1	0.020
Arbutin	4	96	0.078
Salicin	1	99	0.020
Maltose	98	2	0.040
Melibiose	1	99	0.020
Trehalose	94	6	0.114
Starch	3	97	0.059
Glycogen	1	99	0.020
Xylitol	97	3	0.059
D-Turanose	97	3	0.059
D-Tagatose	1	99	0.020
D-Arabitol	96	4	0.078
Gluconate	1	99	0.020
2-Ketogluconate	99	1	0.020
5-Ketogluconate	4	96	0.078
ATB 32GN			
Acetate	97	3	0.059
DL-Lactate	93	7	0.132
L-Alanine	98	2	0.040
L-Serine	85	15	0.258
Valerate	81	19	0.311
Citrate	13	87	0.228
L-Proline	99	1	0.020

[a] Galactose, D-glucose, D-fructose, D-mannose, mannitol, and sucrose gave positive reactions for all strains. Erythritol, D-arabinose, ribose, L-xylose, β-methylxyloside, dulcitol, inositol, amygdaline, cellobiose, lactose, inulin, melezitose, D-raffinose, *p*-gentiobiose, D-lyxose, D-fucose, L-fucose, L-arabitol, itaconate, suberate, malonate, 3-hydroxybenzoate, propionate, caproate, histidine, 3-hydroxybutyrate, and *p*-4-hydroxybenzoate gave negative reactions for all strains.

TABLE 6. Other tests showing variation and their discriminating power[a]

Test	No. with the following reaction:			D
	Positive	Negative	Equivocal	
Extracellular enzymes				
Valine arylamidase	97	3	0	0.059
Naphthol-AS-BI-phosphohydrolase	97	3	0	0.059
β-Glucosidase	6	94	0	0.114
N-Acetyl-β-glucosaminidase	92	8	0	0.149
Resistotyping				
Selenite	98	1	1	0.020
Boric acid	51	31	18	0.319
Cetrimide	45	41	14	0.373
Malachite green	42	16	42	0.135
Copper sulfate	12	66	22	0.160
Benzalkonium chloride	62	27	11	0.338
Chlorhexidine	54	30	16	0.327
Arsenate	21	59	20	0.250
Flucytosine	19	62	19	0.238
Salt	73	14	13	0.206
MacConkey agar	1	99	0	0.020

[a] Alkaline phosphatase, esterase (C_4), esterase lipase (C_8), lipase (C_{14}), leucine arylamidase, acid phosphatase, and α-glucosidase were positive for all strains. Trypsin, chymotrypsin, α-galactosidase, β-galactosidse, β-glucuronidase, α-mannosidase, and α-fucosidase were negative for all strains. All strains were susceptible to mercurochrome.

groups, then this and the original equation give identical values.

All four methods analyzed here suffer from disappointing discriminatory power when compared with bacterial typing methods, although they are equivalent to the other methods for *C. albicans* (10). API ZYM gave the poorest discrimination of all the methods (0.308); previous authors' results are only marginally better (0.547 [6] and 0.471 [24]). Since the method is also not 100% reproducible, we suggest that the API ZYM system has little value for strain differentiation of *C. albicans*.

The carbon source assimilation reactions did give acceptable discrimination (0.891) for the combined API 50CH and ATB 32GN. This compares favorably with the only other reported typing method based on assimilation reactions of 0.431 with the API 20C system (25). These kits need little skill to perform or read, and results are available 48 h after inoculation. However, reproducibility was poor, as might have been predicted from previous work (22), and must limit their value as a typing method.

The discriminatory power for morphotyping is an underestimate, since all observable features were not analyzed. With the limited code described above, the discrimination, although much better than that of the API ZYM system, was still poor. A problem with the method is the time for incubation, although this would compare favorably with time taken to refer strains to reference laboratories. Nevertheless, morphotyping used alone is of value, particularly in laboratories where *C. albicans* would be typed infrequently. It requires no special equipment and is eminently suited to laboratories that do not have access to advanced technology. Furthermore, morphotyping has the major benefit of being the only typing method for *C. albicans* that indicates virulence (9).

Resistotyping has the best combination of discrimination and reproducibility of the four methods examined. The method of coding used in this study removed the need for titrations and reduced the amount of medium required. Although in vitro reproducibility was only 77% for one test difference, this increased to 93% when two test differences were required. However, this method is labor intensive and demands a high degree of skill. Resistotyping is not a suitable method unless laboratory technicians have regular and frequent experience in its use. For laboratories that regularly type *C. albicans*, it is probably the best of the methods examined here.

Among the combined methods, resistotyping and morphotyping, used together with two major differences to distinguish strains, gave improved discrimination without a marked deterioration in reproducibility. None of the other combinations improved the discrimination over these two methods; the decrease in reproducibility was such that greater numbers of differences were required to reliably discriminate among strains.

Certain combinations of typing methods have already been used to characterize populations of *C. albicans*. Burnie et al. (3) described a combination of biotyping, resistotyping, and serotyping to characterize strains from a presumed

outbreak on an intensive care unit. Lehmann et al. (12) used an extended panel of killer strains in combination with susceptibility to flucytosine, sodium arsenate, and cupric sulfate. Williamson et al. (25) used a combination of API ZYM, API 20C, and resistance to boric acid. However, none of these authors reported comprehensive studies to determine the reproducibility of such combined methods, nor were they able to quantify any increase in discrimination obtained. This is the first study of combined typing methods for any organism that has used such an objective index of discrimination.

The four methods examined in this study suffered from relatively poor reproducibility. This may indicate problems with the method, although another explanation may be related to the phenomenon of phenotypic switching (21). Phenotypic switching was first described in studies of colonial morphology but can affect a wide variety of physiological characteristics (1). The poor reproducibility in these studies could also be explained by phenotypic switching, particularly since the strains were subcultured between testing. Indeed, recent work on antigenic variation in *C. albicans* (4, 7, 19) must cast doubt on the validity of a further typing method, immunoblotting (11), as an epidemiological tool.

It has already been noted that two different groups of researchers found markedly different discriminatory power by using the same typing method to analyze two different populations. In this paper we present one of the first studies comparing the discriminations and reproducibilities of different *C. albicans* typing methods on a single population. We suggest that there is a need for an international standard population of strains such that more meaningful comparisons of typing methods can be made.

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LITERATURE CITED

1. **Anderson, J. M., and D. R. Soll.** 1987. The unique phenotype of opaque cells in the "white-opaque transition" in *Candida albicans*. J. Bacteriol. **169**:5579–5588.
2. **Armitage, P., and G. Berry.** 1987. Sampling, p. 78–92. Statistical methods in medical research, 2nd ed. Blackwell Scientific Publications, Oxford.
3. **Burnie, J. P., F. C. Odds, W. Lee, C. Webster, and J. D. Williams.** 1985. Outbreak of systemic *Candida albicans* in intensive care unit caused by cross infection. Br. Med. J. **290**:746–748.
4. **Calderone, R., and E. Wadsworth.** 1987. Characterization with crossed immunoelectrophoresis of some antigens differentiating a virulent *Candida albicans* from its derived avirulent strain (42552). Proc. Soc. Exp. Biol. Med. **185**:325–334.
5. **Caprilli, F., G. Prignano, C. Latella, and S. Tavarozzi.** 1985. Amplification of the killer system for differentiation of *Candida albicans* strains. Mykosen **28**:569–573.
6. **Casal, M., and M. J. Linares.** 1983. Preliminary investigation of *Candida albicans* biovars. J. Clin. Microbiol. **18**:430–431.
7. **Chaffin, W. L., J. Skudlarek, and K. J. Morrow.** 1988. Variable expression of a surface determinant during proliferation of *Candida albicans*. Infect. Immun. **56**:302–309.
8. **Hasenclever, H. F., and W. O. Mitchell.** 1961. Antigenic studies of *Candida*. I. Observation of two antigenic groups in *Candida albicans*. J. Bacteriol. **82**:570–573.
9. **Hunter, P. R., C. A. M. Fraser, and D. W. R. Mackenzie.** 1989. Morphotype markers of virulence in human candidal infections. J. Med. Microbiol. **28**:85–91.
10. **Hunter, P. R., and M. A. Gaston.** 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J. Clin. Microbiol. **26**:2465–2466.
11. **Lee, W., J. Burnie, and R. Matthews.** 1986. Fingerprinting *C. albicans*. J. Immunol. Methods **93**:177–182.
12. **Lehmann, P. F., L. E. Cowan, R. M. Jones, and W. J. Ferencak III.** 1987. Use of killer fungi and anti fungal chemicals in characterisation of yeast species and biotypes. Trans. Br. Mycol. Soc. **88**:199–206.
13. **McCreight, M. C., and D. W. Warnock.** 1982. Enhanced differentiation of isolates of *Candida albicans* using a modified resistogram method. Mykosen **25**:589–598.
14. **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.
15. **Odds, F. C., and A. B. Abbott.** 1983. Modification and extension of tests for differentiation of *Candida* species and strains. Sabouraudia **21**:79–81.
16. **Phelps, M., G. A. J. Ayliffe, and J. R. Babb.** 1986. An outbreak of candidiasis in a special care baby unit: the use of a resistogram typing method. J. Hosp. Infect. **7**:13–20.
17. **Phongpaichit, S., D. W. R. Mackenzie, and C. Fraser.** 1987. Strain differentiation of *Candida albicans* by morphotyping. Epidemiol. Infect. **99**:421–428.
18. **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.
19. **Poulain, D., G. Tronchin, A. Vernes, R. Popeye, and J. Biguet.** 1983. Antigenic variation of *Candida albicans in vivo* and *in vitro*—relationships between P antigens and serotypes. Sabouraudia **21**:99–112.
20. **Scherer, S., and D. A. Stevens.** 1987. Application of DNA typing methods to epidemiology and taxonomy of *Candida* species. J. Clin. Microbiol. **25**:675–679.
21. **Slutsky, B., J. Buffo, and D. R. Soll.** 1985. High-frequency switching of colony morphology in *Candida albicans*. Science **230**:666–669.
22. **Syverson, R. E.** 1981. Variable assimilation of carbon compounds by *Candida albicans*. J. Clin. Microbiol. **13**:163–166.
23. **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.
24. **Williamson, M. I., L. P. Samaranake, and T. W. MacFarlane.** 1986. Biotypes of *Candida albicans* and *Candida tropicalis* isolates. J. Med. Vet. Mycol. **24**:81–84.
25. **Williamson, M. I., L. P. Samaranake, and T. W. MacFarlane.** 1987. A new simple method for biotyping *Candida albicans*. Microbios **51**:159–167.