Morphotype markers of virulence in human candidal infections

P. R. HUNTER, CHERRY A. M. FRASER and D. W. R. MACKENZIE*

*Division of Hospital Infection and *Mycological Reference Laboratory, Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT*

Summary. A study of the morphotypes of 446 strains of *Candida albicans*, isolated from a variety of clinical specimens, is reported. The method was based on a morphotyping scheme that has recently been described, but not all of the potential characters were used in this analysis. By this limited code, 50 different morphotypes were distinguished, the largest group comprising 23% of the population. The simplicity and good discrimination of the method make it a useful typing scheme for *C. albicans*. Discontinuous colonial fringes were associated with strains from oral sites and deep infections. Significantly, 67% of strains from fatal infections were of the discontinuous fringe type, compared to only 11% of strains from other infections. Further associations between morphotype and anatomical source included narrow-coarse fringes in genitourinary isolates.

Introduction

Several typing schemes have been developed in recent years for the sub-species differentiation of *Candida albicans*. These include biotyping (Odds and Abbott, 1980), resistotyping (McCreight and Warnock, 1982), sensitivity to “killer yeasts” (Polonelli *et al.*, 1983), extracellular enzyme production (Roman and Sicilia, 1983), immunoblotting (Lee *et al.*, 1986) and DNA fingerprinting (Scherer and Stevens, 1987). So far none of these methods has been shown to differentiate between strains of high and low virulence.

A new typing scheme for *C. albicans*, which is based on colonial morphology (morphotyping), has recently been described (Phongpaichit *et al.*, 1987). We undertook this study of a collection of clinical isolates to determine the variety and distribution of morphotypes of *C. albicans* and also to determine whether the method is able to detect strains of proven virulence.

Materials and methods

Candida strains

The 446 strains were received directly from laboratories following a request in the Communicable Disease Report in addition to those referred to the Mycological Reference Laboratory, Colindale, London. Strains that had been

submitted as part of another study were excluded from analysis so as not to bias the population of strains in favour of those laboratories taking part in such studies. Multiple isolates from the same patient were also excluded for similar reasons. The strains examined were grouped according to site of isolation as genitourinary and faecal (184 isolates), skin and wound (69), oral cavity and respiratory tract (118), and deep sites including blood culture, CSF and biopsy specimens (75). A further eight strains were excluded from the study because they could not be easily classified into one of the above groups. The geographical location of the laboratories submitting strains and the clinical source of those strains are shown in table I.

Morphotyping

Isolates were sub-cultured on to blood-agar plates and incubated overnight at 37°C, to ensure absence of bacterial contamination. Identification was by germ-tube formation in serum. One colony, chosen at random, was further sub-cultured on to Bacto YM Agar (0712-01-8 Difco, Detroit, MI, USA), from which a suspension in distilled water was made for inoculation on to morphotyping agar as described by Phongpaichit *et al.*, (1987).

After incubation for 10 days, each strain was examined and assigned a morphotype code (Phongpaichit *et al.*, 1987) of two pairs of three numbers, with an occasional additional number being added in parenthesis. To simplify analysis, a limited code was used in this study. Fringe characters were recorded in full, but only the topography of streak surface features was analysed. The abbreviated code is shown in table II and examples of

Table I. Geographical sources of strains isolated from various sites

Source	Number of strains isolated from				
	Genitourinary tract [n = 184]	Skin [n = 69]	Oral cavity [n = 118]	Deep [n = 75]	Total [n = 446]
London and S.E. England (i = 27)	63	39	47	58	207
S.W. England (i = 1)	1	0	1	0	2
Midlands (i = 6)	13	1	2	6	22
N.W. England (i = 7)	42	5	23	5	75
N.E. England (i = 4)	55	3	13	1	72
Wales (i = 2)	9	21	31	2	63
Overseas (i = 3)	1	0	1	3	5

i = number of referring institutions in each region.

Table II. Coding of morphological features

Feature	Code	Description
<i>Fringe</i>		
Distribution	0	Absent
	1	Discontinuous; < 20% of margin
	2	Discontinuous; 20–50% of margin
	3	Discontinuous; 60–90% of margin
	5	Continuous at periphery only or strands conspicuously fan shaped
	7	Continuous; filamentous outgrowths parallel
Width	0	Absent
	2	≤ 2 mm
	3	3–5 mm
	5	≥ 6 mm
Texture	0	Absent
	1	Very coarse
	2	Coarse
	3	Intermediate
	4	Fine
<i>Streak surface</i>		
Topography	0	Smooth
	1	Nodular
	2	Pitted
	4	Crateriform
	5	Crateriform plus wrinkles or folds
	6	Wrinkles or folds
	8	Hairy

initial digit 1, 2, 3, or 5 as discontinuous fringes, 753/4 as wide-fine fringes, 751/2 as wide-coarse, 723/4 and 733/4 as narrow-fine and 721/2 and 731/2 as narrow coarse. For surface features, those with a smooth surface were classed as featureless and all others as featured.

Results

The distribution of morphotypes amongst strains isolated from various sites is shown in table III. With the abbreviated code of this study, 50 different morphotypes were identified. The commonest morphotype codes were 000 0 (23%), 754 0 (16%) and 734 0 (7%).

To determine whether morphotypes varied between geographical regions within the United Kingdom, strains from the South of England and the Midlands were compared with strains from Wales and the North of England (table IV). As strains from deep infections were predominantly from the South, they were not included in the analysis of geographical variation. There was no significant difference in the clinical source of strains between the two regions, although there was a difference in the distribution of fringe characteristics ($\chi^2 = 18.1$, 5df, $p < 0.01$). This was mainly due to a predominance of strains with narrow-fine fringes in the South of England. It is of particular note that there was no difference in the proportion of strains with discontinuous fringes between the two regions.

There was a significant difference in the distri-

strain morphotypes are shown in the figure. For statistical analysis, the morphotype code was reduced to just six fringe states and two surface states, as shown in table IV. Those coded 000 were classed as no fringe, those with

Figure. Some examples of *C. albicans* morphotypes, coded with short code used in this study: (A)=000 0; (B)=754 6; (C)=722 0; (D)=534 4; (E)=332 0; (F)=123 0.

bution of fringe characteristics between the three categories of superficial infection ($\chi^2 = 41.7$, 10df, $p < 0.001$) as shown in table V. The main differences were the preponderance of discontinuous fringes among oral isolates and of narrow-coarse fringes among genitourinary + faecal isolates. There was a significant difference between strains from superficial and those from deep infections ($\chi^2 = 29.0$, 5df $p < 0.01$). This was mainly due to the frequency of discontinuous fringes in deep infections, although deep infections were also less likely to show wide-fine fringes. Furthermore, in those strains from known fatal infections, 67% were of the discontinuous fringe type compared to only 11% from other deep infections ($p = 0.0001$, Fisher's exact test), and none was of the wide-fine fringe type. Therefore, a deep infection caused by a discontinuous fringed

strain was associated with a significantly increased risk of death when compared with a deep infection caused by another fringe pattern. This association of discontinuous fringe type with deep infections held even when strains from the South East were analysed alone ($p = 0.0004$), and was not due to any particular outbreak as all but two were from separate institutions. There were no significant differences found for surface features.

Discussion

There must be some concern about the reproducibility of a typing scheme based on colonial morphology, as this is a feature reported to undergo phenotypic variation (Slutsky *et al.*, 1985). Indeed,

Table III. Distribution of morphotypes of *C. albicans* between strains isolated from various sites

Morphotype code	Number of strains isolated from				
	Genitourinary tract [n = 184]	Skin [n = 69]	Oral cavity [n = 118]	Deep [n = 75]	Total [n = 446]
000 0	45	18	20	19	102
000 4	0	0	1	0	1
000 6	3	0	0	0	3
123 0	0	0	1	0	1
124 0	0	0	0	1	1
223 6	1	0	2	0	3
322 0	0	0	0	1	1
323 0	1	0	0	3	4
324 0	0	0	0	2	2
331 4	0	0	0	1	1
332 0	0	0	3	6	9
532 0	0	1	1	1	3
532 5	0	1	0	0	1
533 0	0	0	2	0	2
534 0	0	0	1	0	1
551 0	0	0	3	0	3
721 0	4	2	1	2	9
722 0	9	1	6	2	18
722 4	1	0	0	1	2
722 6	0	0	1	0	1
723 0	1	0	0	1	2
723 6	1	0	0	0	1
724 0	6	1	3	3	13
724 4	0	0	1	1	2
724 6	2	1	1	0	4
731 0	2	1	0	3	6
732 0	20	0	2	2	24
732 2	0	0	0	2	2
732 4	1	0	0	0	1
732 5	1	0	0	1	2
732 6	5	1	0	2	8
733 0	5	2	5	3	15
733 4	0	1	0	0	1
733 5	3	1	1	0	5
733 6	5	0	2	2	9
734 0	8	6	13	4	31
734 4	3	5	0	0	8
734 5	0	0	1	0	1
734 6	1	2	3	0	6
752 0	8	0	7	3	18
752 2	3	0	0	0	3
752 6	0	0	1	0	1
753 0	3	5	3	1	12
753 5	1	0	0	0	1
754 0	33	11	21	7	72
754 1	0	0	1	0	1
754 2	0	1	0	0	1
754 4	0	2	0	1	3
754 5	1	2	2	0	5
754 6	7	4	9	0	20

Table IV. Distribution of morphotypes between two regions

Morphotypic characters	Number (%) of isolates from	
	South England and Midlands [n = 167]	North England and Wales [n = 202]
<i>Fringe characters</i>		
No fringe	40 (24)	46 (23)
Discontinuous	6 (4)	11 (5)
Complete		
wide-fine	40 (24)	66 (33)
wide-coarse	11 (7)	8 (4)
narrow-fine	52 (31)	32 (16)
narrow-coarse	18 (11)	39 (19)
<i>Streak surface</i>		
featureless	127 (76)	156 (77)
featured	40 (24)	46 (23)

Brown-Thomsen (1968) found poor reproducibility with his typing scheme, which was also based on colonial morphology, as he noted a shift from smooth to rough colonial forms. However, phenotypic switching mainly affects the colony surface. Brown-Thomsen (1968) based his typing scheme mainly on these potentially unstable surface features. It is not yet known if phenotypic switching will affect morphotyping results, particularly as the morphotyping streak is seeded by many thousand colony forming units. The in-vitro reproducibility over 2 years for this morphotyping scheme, relying

mainly on fringe features, is 84% for those giving an identical morphotype, and 96% for those giving a morphotype differing by one or less adjacent characters (Phongpaichit *et al.*, 1987). In a further study (Hunter and Fraser, unpublished data) of 15 cases of candidal vaginitis, in which five colonies from each of the original cultures were morphotyped, we found that there was 100% in-vivo reproducibility.

The discrimination of this method, using the reduced code, is very good compared with some other methods developed for *C. albicans*. In this

Table V. Distribution of morphotypic characters of *C. albicans* between strains isolated from various sites

Character	Number (%) of isolates from				
	Superficial infections			Deep infections	
	Genitourinary tract [n = 184]	Skin [n = 69]	Oral cavity [n = 118]	Fatal [n = 12]	Non-fatal [n = 63]
<i>Fringe characters</i>					
No fringe	48 (26)	18 (26)	21 (18)	2 (17)	17 (27)
Discontinuous	2 (1)	2 (3)	13 (11)	8 (67)	7 (11)
Complete					
wide-fine	45 (24)	25 (36)	36 (30)	0	9 (14)
wide-coarse	11 (6)	0	8 (7)	0	3 (5)
narrow-fine	35 (19)	19 (28)	30 (25)	1 (8)	13 (21)
narrow-coarse	43 (23)	5 (7)	10 (9)	1 (8)	15 (24)
<i>Streak surface</i>					
Featureless	145 (79)	48 (70)	92 (88)	10 (83)	54 (86)
Featured	39 (21)	21 (30)	26 (22)	2 (17)	9 (14)

() = %; n = number of isolates.

study, 50 different morphotypes were described, the largest group accounting for only 23% of all strains. The corresponding figures obtained with other methods are: 10 types for DNA fingerprinting with the most frequent type representing 35% of strains (Scherer and Stevens, 1987); 16 types and 54% for immunoblotting (Lee *et al.*, 1986); four types and 64% for enzyme biotyping (Roman and Sicilia, 1983).

The combination of simplicity with good discrimination make morphotyping an ideal typing method for first-line use. Strains of the same morphotype from epidemiologically related cases can be assumed to be related, until proven otherwise.

Another potential use of any typing scheme is as an indicator of virulence. Whilst some bacterial typing schemes do identify strains of increased virulence (Turk, 1982; Jann and Jann, 1985) this has not been found to be the case for current typing schemes for *C. albicans* (Odds *et al.*, 1983a, b; Hunter and Fraser, 1987). It has been suggested that smooth fringeless colonies (000 0 or A1 by Brown-Thomsen's method) are the wild type (Brown-Thomsen, 1968) and so could be the most virulent. If this is the case then one should expect to see increased numbers of this morphotype in strains isolated from blood cultures. No such association was observed in this study. It has been shown previously that overheating of the media during preparation abolishes fringe formation (Phongpaichit *et al.*, 1987), which may explain the large number of A1 strains previously reported.

Perhaps the most striking finding in this study was the strong association between morphotyping and anatomical source of the isolate. This was particularly marked for the association between discontinuous fringe formation and fatal deep infections. The mechanism for this association is unknown at present, though we have formulated three possible explanations. Firstly, it may be that some surface phenomenon is responsible for both virulence and morphotype. Indeed, those bacterial typing schemes most closely associated with virulence, type on the basis of surface structures (Turk, 1982; Jann and Jann, 1985). The most comprehensively studied virulence factor for *C. albicans* is adherence to epithelial cells (Barrett-Bee *et al.*, 1985; Ghannoum and Elteen, 1986; Bibel *et al.*,

1987). Adherence of *C. albicans* has been shown to be due to molecules present on the cell surface (Ghannoum *et al.*, 1986; Critchley and Douglas, 1987). Whether these molecules have any effect on morphotype is also unknown, though the effect of surface structures on bacterial colonial morphology and virulence is well known (Munoz and Bergman, 1977; Sereny, 1977; Morse and Brooks, 1985). Another possibility is that it is related to anti-fungal therapy; however, as several of the strains we examined were sampled from patients before the start of chemotherapy, this can be excluded. A third possibility could be related to phenotypic switching which has been described in vaginal isolates (Soll *et al.*, 1987). *C. albicans* can exist in either a mycelial or a yeast form, and presumably discontinuous fringe formation occurs because only a small proportion of the cells on the streak margin are growing in the mycelial form. Full virulence may depend on the cell being in different growth forms at different stages of the infection. Clearly, a population of invading cells which are switching between the two forms are likely to be more virulent than cells committed to one or other form.

The other associations of morphotype with anatomical source are also difficult to explain. The increased number of discontinuous fringed strains from oral sites may reflect the variety of the ecological environment within the oral cavity. The variety of niches may favour those strains that can readily adapt from one niche to another. Whether there is any direct relationship between oral strains and strains from deep infections is unclear. Narrow-coarse fringe formation in genitourinary isolates may indicate that these strains adhere more firmly to the epithelial cells lining the genitourinary tract.

Whatever the mechanism of the association between virulence and fringe formation, morphotyping may offer a useful in-vitro indicator of virulence for *C. albicans*. This has obvious value in assessing the clinical significance and prognostic implications of blood-culture isolates of *C. albicans*. Furthermore, detailed study of the mechanism of this phenomenon may well enable the development of more rapid methods for detection of such virulent strains and may also lead to novel therapeutic approaches based on the inhibition of such growth forms.

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