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Strain differentiation of *Candida albicans* by morphotyping

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SUMMARY

Strains of *Candida albicans* can be differentiated by the morphological features of streak colonies developed on malt agar. A morphotyping system is proposed, where numerical codes are assigned primarily on the basis of the nature and extent of marginal fringing and the surface topography of the streak colony. The system allows ready differentiation to be made of morphotypes, requires no specialized equipment or expertise and provides a simple and reproducible means for epidemiological studies of candida and candidosis.

INTRODUCTION

Only a limited number of attempts have been made to develop reliable means for characterizing pathogenic fungi below the species level. This has restricted the number and scope of epidemiological studies which can be made of the mycoses and their causal agents. Almost all studies reported to date have been confined to methods for strain differentiation of *Candida albicans*. These included serotyping (Hasenclever & Mitchell, 1961; Stallybrass, 1964), resistogram patterns (Warnock *et al.* 1979, Odds & Abbott, 1980), differential susceptibility to 'killer' toxins (Polonelli *et al.* 1983), patterns of enzymes (Roman & Sicilia, 1983) and SDS-PAGE 'fingerprinting' (Lee, Burnie & Matthews, 1986).

The applicability of resistogram methods to epidemiological studies of candidosis was first demonstrated by Warnock *et al.* in 1979, and subsequently by others (McCreight & Warnock, 1982; Odds *et al.* 1980; O'Connor & Sobel, 1986).

One system which has been in use in our laboratories for several years for differentiating strains of *C. albicans* is morphotyping. This was developed from the original observation made by Brown-Thomsen in Denmark (1968) that different strains of *C. albicans* produced widely varying morphologies when streaked on malt agar. In this report, basic methodology and the recommended coding system for morphotyping are described. Applications of the morphotyping system to epidemiological studies will be made the subject of subsequent reports.

MATERIALS AND METHODS

Isolates

Clinical isolates are streak-purified: their identity is confirmed by orthodox mycological procedures. Stock cultures of individual isolates can be maintained on

slopes of glucose peptone (GP) agar at 4 °C, and subcultured at intervals of 2 months, without any appreciable change in morphotype.

Medium

Six per cent malt extract agar (Oxoid), with the addition of 2% Difco special agar is distributed in 18–20 ml quantities, after autoclaving at 121 °C for 15 min, into 9 cm petri dishes. Plates can be stored at 4 °C for up to 8 weeks, but are best used within 7 days of preparation.

Inocula

Yeast cells from 24 h cultures on 2% malt agar at 30 °C are suspended in sterile distilled water in a 5 ml screw cap glass bottle to give a distinct turbidity, corresponding to 10^7–10^8 cells per ml. Dilution experiments showed that morphotype coding was not critically related to concentration of cells in the inoculum.

Inoculation

Plates are inoculated in duplicate from the cell suspension with a single diametric streak. Colonial appearance can be affected by minor irregularities of inoculation. These tend to be pronounced when the streak is made with a straight needle or loop, and best results are obtained with a sterile swab, or by drawing sterile capillary tubes containing the test suspension lightly across the agar surface without scratching.

Incubation

Cultures were incubated at 30 °C, or at room temperature, in the dark, for 10 days.

Establishment of morphotype

After 10 days of incubation, visual comparison alone shows marked differences between streaks prepared from different isolates. In the coding system described below, numerals are assigned sequentially to the principal morphological features of the individual streak. Primary, secondary and ancillary features are recognized. Most streaks have a central portion *c.* 5–0 mm wide (*streak*) which may or may not have a surrounding margin, generally consisting of conspicuous filamentous outgrowths (*fringe*) on or below the agar surface.

Experience has shown that features associated with the fringe are more conspicuous, more consistent and more readily coded than those associated with the streak. Distinction is therefore made between primary (fringe) and secondary (streak) features.

Primary features

The first digit of the code refers to the proportion of the margin which is fringed with mycelial growth. When continuous, a distinction is made between strands which are tufted or fan-shaped, or uniformly parallel. The other digits are assigned on the basis of their width and degree of coarseness.

Secondary features

The surface topography of the streak is coded according to its predominant characteristic, viz. smooth, nodular, pitted, cratered (with or without wrinkles and folds), wrinkles or folds, or hairy. The other digits are assigned according to quality (coarse/broad, fine/narrow or absent) and the depth and abundance of the pits, craters, wrinkles or folds or hairs.

Ancillary features

These can be numerous and varied and for any single isolate may be produced regularly and be very distinctive. These features, although distinctive, may also be uncommon and in order to keep the coding system simple are relegated to ancillary status, being included in the morphotype code only when present and pronounced. They are cited within parentheses after the code for primary and secondary features. Ancillary features include the presence of a smooth and mottled surface layer of confluent or discontinuous growth overlying the fringe, presence of lenticular or wedge-shaped zones (non-mycelial) in the streak margins and the presence of localized tufts or continuous bands of aerial filaments (hairs) on the surface of the streak or margin. Only the more common ancillary features are listed in the basic morphotyping schedule.

Morphotyping schedule

Morphological features of the streak are assigned a numerical code according to the features shown in Table 1.

In coding an isolate numerical values are assigned in sequence to give a six-digit code. These values have been chosen to allow for interpolation of assessments between those listed in the schedule. The code is simply a convenient way to express the morphological features of an isolate numerically and to provide fixed reference points against which a wider range of morphological expression can be compared. In distinguishing between different isolates, no significance is attached to variations in code ratings or primary and secondary features of fewer than two of the arbitrary units. For example, an isolate coded as 000-100 is not significantly different from one designated as 100-000, but is regarded as a different strain to isolates coded as 222-000 or 000-426. Representative morphotypes and their numerical codes are shown in Figs 1 and 2. Ancillary features, where prominent, are described within parentheses. Only a limited number are presented. They illustrate the range of morphological variation and the application of the morphotype coding system.

DISCUSSION

Reproducibility of the basic method is good, both for expression and stability of individual features. Re-examination of 50 test isolates after 2·5 years gave identical readings for 42 (84%) of the streaks, only 2 of the remaining 8 differing by a code rating of 2 or more.

Several attempts have been made to introduce a completely synthetic medium, but so far without success. Some of the features described in this morphotyping

Table 1. *Morphological features of the streak*

Feature	Code	Description
Primary features (fringe)		
Distribution	0	Absent
	1	Discontinuous; involving up to 20% of margin
	2	Discontinuous; involving 20–50% of margin
	3	Discontinuous; involving 60–90% of margin
	5	Continuous at periphery only, or strands conspicuously fan-shaped
	7	Continuous; filamentous outgrowths uniformly parallel
Width	0	Absent
	2	2 mm or less
	3	3–5 mm
	5	Greater than 6 mm
Texture	0	Absent
	1	Very coarse
	2	Coarse
	3	Intermediate
	4	Fine
Secondary features (streak surface)		
Topography	0	Smooth
	1	Nodular
	2	Pitted
	4	Crateriform
	5	Crateriform plus wrinkles or folds
	6	Wrinkles or folds
	8	Hairy
Quality	0	Absent
	2	Coarse or broad
	3	Intermediate
	4	Fine or narrow
Depth and abundance	0	Absent
	2	Shallow and sparse
	4	Shallow and abundant
	6	Deep and sparse
	8	Deep and abundant
Ancillary features		
	(1)	Smooth or discontinuous layer of yeast cells overlying fringe
	(3)	Margin between streak and fringe not sharply defined
	(4)	Lenticular zones at colony margin
	(5)	Wedge-shaped zones at colony margin
	(7)	Hairs over streak surface
	(8)	Hairs over streak and fringe surface
	(9)	Hairs over fringe surface

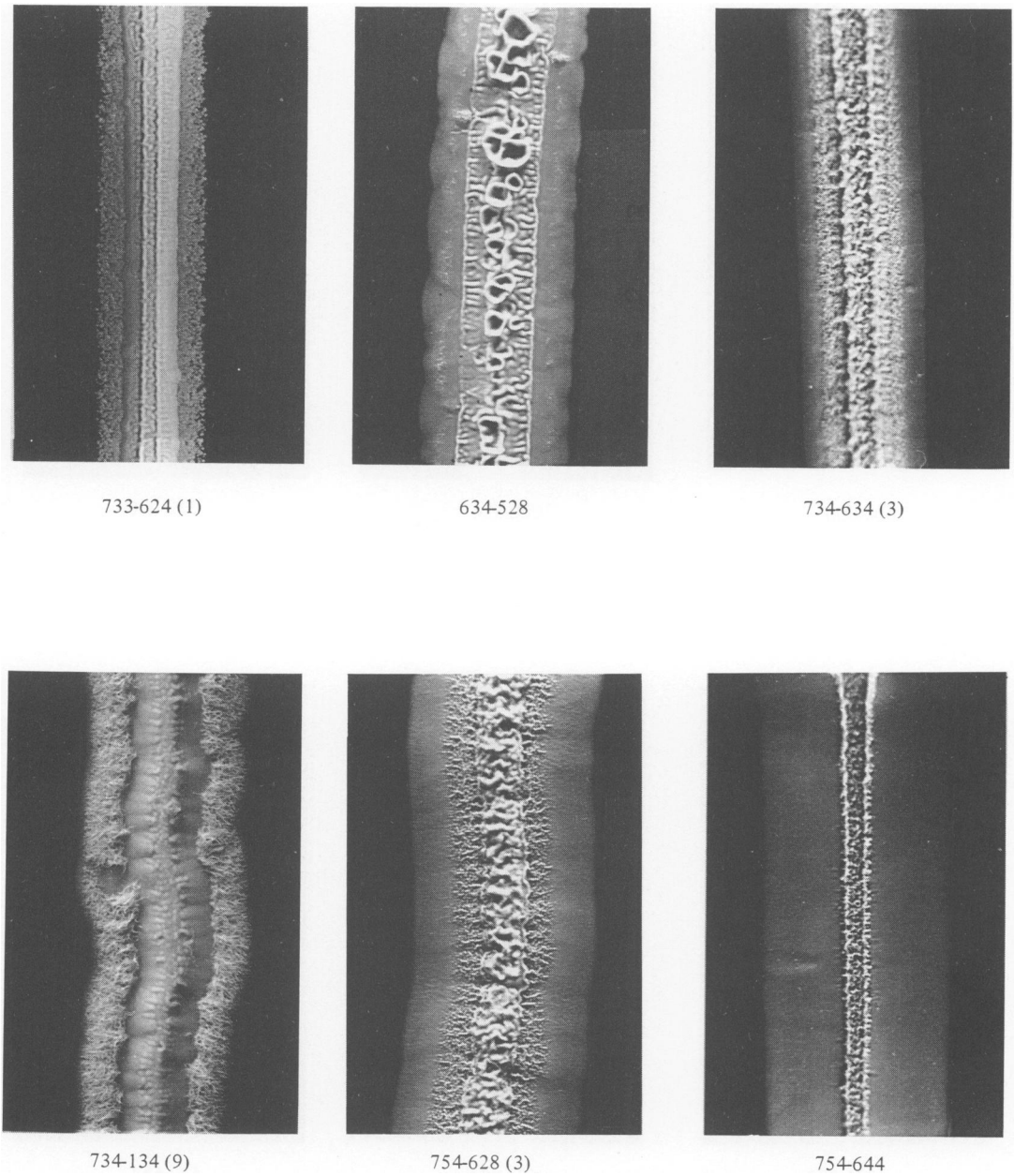

Fig. 1. Morphotypes of *Candida albicans*. Streaks from six different isolates with assigned codes.

scheme are discernible on glucose peptone agar, but are much less obvious. Similarly, replacement of the Oxoid medium with other products has not been successful.

The features revealed by close examination of individual streaks are both varied and distinctive. They are also affected by subtle alterations in the medium or conditions of incubation. Overheating of the medium during its preparation is to

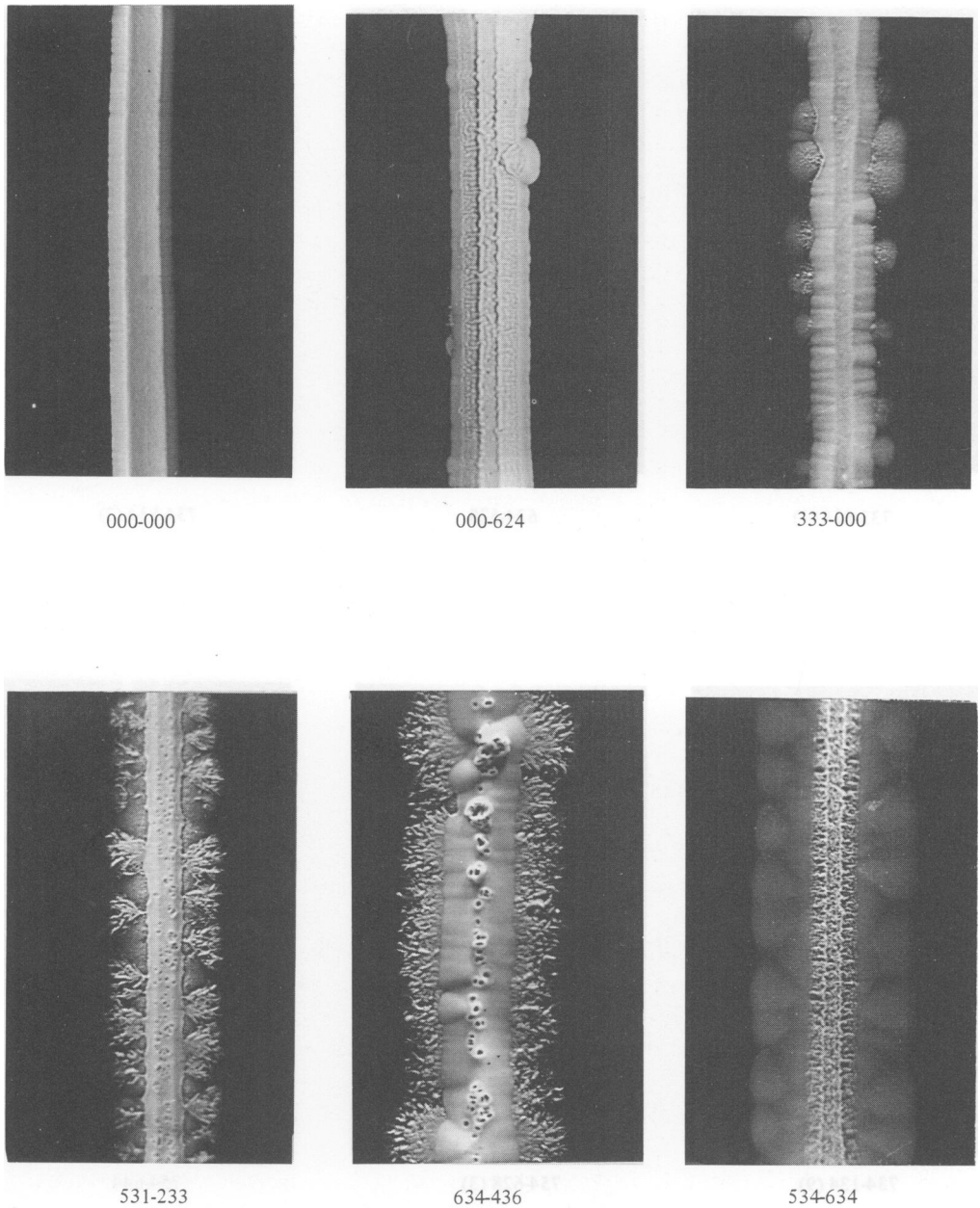

Fig. 2. Morphotypes of *Candida albicans*. Streaks from six different isolates with assigned codes. Note wide range of morphologies.

be avoided, since filamentation is then reduced or absent. Similarly, if plates are incubated within plastic bags or comparable containers, to minimize drying out, the high relative humidity encourages spread of a superficial layer of yeast cells from the edge of the streak, which can obscure features of the fringing. Other features which can influence the colonial development (and hence the coding) include depth and uniformity of thickness of the agar medium and smoothness of

inoculation. Temperature of incubation does not appear to be critical. Results over a wide range of room temperature showed no differences from those at 30 °C.

A control strain, selected because of its distinctive features should always be included with each batch of isolates examined.

Compared with other systems of strain differentiation, morphotyping has both advantages and disadvantages. It is technically simple, involves no special apparatus or expertise, and enables both similarities and differences between strains to be readily appreciated. No precise estimate can be made of the number of morphotypes which can be distinguished by this scheme, but as presently constituted, it has been able to distinguish more than 100 morphotypes in different epidemiological studies.

In common with determinations of resistogram patterns, evaluations of morphotype are subjective and some variations can be anticipated in codes assigned by different observers. In practice this is largely minimized by discounting minor differences in readings and by familiarization with the procedure. Although morphotyping allows a numerical code to be assigned to all isolates in a survey, identical or different strains can almost always be readily distinguished by visual comparison alone. If the requirement is simply to compare isolates of *C. albicans* from different sources (e.g. vagina and rectum, or blood culture and catheter tip), then this simple visual comparison may prove adequate for establishing identity or non-identity of the strains.

Spontaneous alterations in colony morphology of *C. albicans* have recently been described by Slutsky, Buffer & Soll (1985). This 'phenotypic switching' occurs at frequencies of *c.* $1{\cdot}4 \times 10^{-4}$, although high frequency switching has also been reported at frequencies up to 2×10^{-2}.

The observed frequency of this phenomenon makes it unlikely that it will invalidate the morphotyping system described in the present study. Protocols for the systems for strain differentiation described to date generally involve the analysis of single colonies.

DNA fingerprinting methods may prove in time to be the most precise and definitive means for characterizing individual isolates of *C. albicans*, but it is suggested that the overall simplicity and reproducibility of morphotyping make it a valuable tool for epidemiological studies of candida and candidosis.

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