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The frequency distribution and consistency of assimilation biotypes of *Candida albicans*

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SUMMARY

Two hundred and fifty clinical strains of *Candida albicans* and six isolates from a cross-infection outbreak were studied for their ability to assimilate 19 carbohydrates in the API-20C system (API Laboratory Products Ltd., Basingstoke, UK). The assimilation profiles were stable on repeat testing; at intervals in the 72 h duration of the test; and when incubated at different temperatures.

Although not a complete system of biotyping, the API-20C system shows high typability, and fair reproducibility and discrimination, having a limited role in indicating which isolates should be typed by more elaborate methods.

INTRODUCTION

Candida albicans is increasingly implicated as a cause of hospital outbreaks, especially among immunosuppressed patients on oncology wards, surgical and neonatal units. The diagnostic laboratory identification of yeasts as *C. albicans* depends largely on assimilation of carbon sources and several commercial kits are available for this (De Louvois, Mulhall & Hurley, 1979; Buesching, Kurek & Roberts, 1979; Guinet 1985). For establishing the epidemiology of case clustering, Odds & Abbott (1980, 1983) described a biotyping scheme incorporating some carbon-source assimilations in order to distinguish epidemiologically distinct isolates.

Warnock *et al.* (1979) described a resistogram typing system; currently available typing schemes have been reviewed by Warnock (Warnock, 1984).

The API-20C auxanogramme identification scheme (API Laboratory Products Ltd., Basingstoke, UK) employs 19 carbon sources for assimilation and is used extensively in diagnostic laboratories. However, although at least 35 assimilation patterns (or profiles) are listed for *C. albicans*, most isolates conform to a minority of these. The use of assimilation profiles for epidemiology is therefore limited. In addition, assimilation patterns are reported to vary with methodology, incubation temperature and duration (Syverson, 1981).

This study shows the frequency distribution of assimilation profiles using the API-20C auxanogramme kits, and the reproducibility of these profiles on repeat testing to determine their usefulness in epidemiology.

MATERIALS AND METHODS

Isolates of *C. albicans* came from three sources.

(1) A total of 110 isolates were obtained from oral swabs of terminally ill patients on admission to a hospice. Each isolate was epidemiologically distinct.

(2) Six isolates were obtained from a hospital cross-infection outbreak of *C. albicans*. These isolates were considered indistinguishable upon biotyping by the method of Odds & Abbott (1983). The cross-infection outbreak and biotyping are described elsewhere (Burnie *et al.* 1985).

(3) A total of 139 isolates of *C. albicans* encountered in the diagnostic laboratory at the London Hospital, Whitechapel, London, and which were considered epidemiologically distinct were included. No more than one *C. albicans* isolate was taken from any patient.

Identification of Candida albicans

Initial isolation was made on Sabouraud's dextrose agar (Oxoid) incubated at 37 °C aerobically in 10 % CO_2 for 24 h. Isolates were identified as *C. albicans* on germ-tube test and production of chlamydospores on corn-meal agar, and corroborated by assimilation profile. Isolates were processed as received and stored suspended in sterile distilled water at 4 °C for up to 3 months before repeating the assimilation profile.

Assimilation profiles

The isolates were cultured on blood agar (Oxoid blood agar base no. 2, code CM271, with 6 % defibrinated horse blood (Oxoid)) for purity checks; inoculated on to Sabouraud's dextrose agar (Oxoid) and incubated at 35 °C for 18 h. The API-20C auxanogramme kits were prepared and inoculated in accordance with the manufacturers instructions, incubated at 30 °C for up to 72 h, being read at 18, 48 and 72 h.

In addition, for the 6 cross-infection isolates, and for 50 distinct strains, assimilation profiles were repeated after 2 months storage. Kits were prepared in duplicate and incubated at 37 °C and at 30 °C for upto 72 h with daily reading.

Turbidity greater than or equal to that in the growth control (glucose) cupule was taken as indicating assimilation. Only those tests in which the growth control was positive and the basal medium zero-growth control cupule was negative were accepted. Otherwise the procedure was repeated with a heavier inoculum or after plating for purity check as indicated.

RESULTS

A total of 250 epidemiologically distinct strains were tested on isolation and the results are shown in Table 1; 19 of 35 possible profiles were encountered. Only one of the hospital outbreak strains is included in the figures. Nearly 60 % of all strains gave the same profile; 73·7 % and 84 % were given by 2 and 3 profiles respectively. These percentages are not significantly different considering Hospice strains and diagnostic laboratory strains separately.

Table 1. *Frequency distribution of assimilation profiles*

(Figures in parentheses indicate percentages)

Profile code	Diagnostic Laboratory strains (n = 140)	Hospice strains (n = 110)	Overall strains (n = 250)	Cumulative % positive	Data base predictive value
2576170	*84	65	149 (59·8)	59·8	1/2 (50)
2176170	16	19	35 (14)	73·7	1/42 (2·4)
2776170	15	11	26 (10·5)	84	1/57 (1·75)
2156170	4	6	10 (4)	88	1/2074 (0·05)
2556170	0	7	7 (2·8)	90·8	1/74 (1·35)
2572170	3	2	5 (2)	93·8	1/42 (2·4)
2144170	4	0	4 (1·6)	94·4	—
2756170	3	0	3 (1·2)	—	—
2576130	1	0	1 (0·8)	—	—
6756170	1	0	1	—	—
Others	9	0	9 (3·6)	—	—

* Includes outbreak strain: counted once.

Table 2. *Distribution of assimilation profiles by reagent.*

	Number of strains positive (n = 250)								
Substrate	2576170	2176170	2776170	2156170	2556170	2572170	All others	Total	%
Glucose	149	35	26	10	7	5	18	250	100
Glycerol	—	—	—	—	—	—	3	3	1
2-Keto-D-gluconate	149	35	26	10	7	5	18	250	100
L-arabinose	—	—	26	—	—	—	3	29	11
Xylose	149	—	26	—	7	5	13	200	80
Adonitol	149	35	26	10	7	5	12	244	97
Xylitol	149	35	26	—	—	5	8	233	89
Galactose	149	35	26	10	7	5	18	250	250
Inositol	—	—	—	—	—	—	0	—	—
Sorbitol	149	35	26	10	7	5	11	244	97
Methyl-D-glucoside	149	35	26	10	7	—	18	245	98
N-acetyl-glucosamine	149	35	26	10	7	5	18	250	100
Cellobiose	—	—	—	—	—	—	0	—	—
Lactose	—	—	—	—	—	—	0	—	—
Maltose	149	35	26	10	7	5	18	250	100
Saccharose	149	35	26	10	7	5	18	250	100
Trehalose	149	35	26	10	7	5	18	250	100
Melezitose	—	—	—	—	—	—	0	—	—
Raffinose	—	—	—	—	—	—	0	—	—

Table 1 also shows the predictive value of the individual profiles, representing the manufacturer's value for frequency of occurrence of that profile among *C. albicans* species in the API data base.

Table 2 summarizes the percentage of strains assimilating each reagent, and shows the reagents assimilated by the six commonest profiles (i.e. 93·8 % of strains).

All six isolates from the hospital outbreak gave the same profile on initial

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isolation from clinical specimens in nine tests. Similarly, all gave the same profile (2576170) on repeat testing at 37 and at 30 °C (one gave 2556170 with light inoculum but on repeat testing with a heavier inoculum gave profile 2576170).

Thirty-nine of 50 strains stored in sterile water, and retested 2–3 months later, gave unchanged assimilation profiles. Repeat testing on those 11 strains showing changed profiles showed the altered profile to be consistent in 10; and only 1 gave the original profile.

DISCUSSION

Ideally, methods to subdivide species adequately for epidemiological typing should give high typability, wide discrimination, clear reproducible results and be relatively easy to perform.

All isolates produced germ-tubes and chlamydospores. All the *C. albicans* isolates studied yielded an assimilation profile as part of their speciation, thus circumventing the criterion of typability, which nevertheless must exceed 97% (De Louvois, Mulhall & Hurley, 1979; Buesching, Kurek & Roberts, 1979).

The predominant profile was given by 60% of all strains tested, which renders the uniform demonstration of this profile in an epidemiological study of little significance on its own. As with most organisms, *C. albicans* shows discrete groups of strains with common phenotype characters rather than a continuous spectrum of phenotypes representing all possible properties.

Odds *et al.* (1983), biotyping widely dispersed strains from the UK and USA, found under 20 clusters of statistically significantly similar types (analogous to the profiles), with the majority of strains occurring in only 10 of these clusters.

The predominant profile was given by the hospital outbreak strains which were all of the same biotype (Burnie *et al.* 1985). Significantly, these assimilations were performed at their varying times of initial clinical isolation by different people and were identical; with the results of assimilations at 37 and 30 °C, these profiles have been shown to be both consistent and reproducible, provided uniform methodology is performed. Therefore the demonstration of different profiles in a putative outbreak or cluster of isolates does distinguish strains. However, the uneven discrimination militates against the use of API assimilation profiles alone as a typing schema.

The uniform demonstration of a 'less common' profile in a cluster of isolates may well be significant on its own. However, the discriminatory function of different profiles is exaggerated by the profile numerical value. For example, profiles 2576170 and 2176170 differ by only 1 assimilation in 19, i.e. xylose. Thus 73·6% of isolates in the study were placed in one of two profiles by a test which is positive in 80% of all tests. On the other hand, only 11·6% of isolates assimilated arabinose which therefore has greater discriminatory value when positive.

Odds *et al.* (1983) demonstrated a geographical variation among *C. albicans* strains in the ability to assimilate glycine, sorbose and urea. For example, in the USA, 97·5% of strains assimilated glycine compared with 90·0% in UK strains; and 33·2% of US strains assimilated sorbitol compared with 23% of UK strains. The geographical variation may explain the differences in frequency distribution between the API data base and this study (Table 1).

Surprisingly, in the present study none of the profiles differed between 24 and 72 h incubation.

The strict criteria for defining assimilation, designed to limit observer error, excluded some weak or late reactions.

Only three test strains (1·2%) in the present study assimilated glycerol within 72 h. Syverson (1981), however, demonstrated differences in rates of assimilation of glycerol (and of melezitose and sorbose) between strains, with 80% assimilating glycerol within 7 days, rising to 94% at 14 days.

Similarly, with melezitose assimilation, Beusching, Kurek & Roberts (1979) misdiagnosed 3% of *C. albicans* strains according to API-20C criteria whereas Syverson demonstrated assimilation by 7% of isolates within 3 days and 61% by 7 days incubation. It is possible that assimilation patterns may change and hence vary between studies as a result of selection of mutant clones possibly expressing recessive mutations (Kakar, Partridge & Magee, 1983). This phenomenon might explain the delayed onset of assimilation occurring after several days in broth cultures. The methodology and time of reading is therefore critical for speciation, and biotyping.

The consistency of profile of the outbreak strain and other repeated strains suggests the genetic stability of observed assimilation patterns.

In conclusion, judged from the standpoint of reproducibility and discrimination, the ability of API-20C auxanogramme profiles to show strain definition or to identify stable markers within the species is fair, although typability and ease of testing are excellent.

At the least, in epidemiology studies of proven *C. albicans*, auxanogram patterns can indicate which of the isolates should be typed by more elaborate typing methods.

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