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Identification of *Candida albicans* and *C. tropicalis* with an umbelliferyl-labelled galactosaminide

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Summary. The 4-methylumbelliferyl-N-acetyl-β-D-galactosaminide (UAG) test was evaluated in parallel with chlamydospore (CHL) and germ-tube (GT) tests for the identification of *Candida* spp. The UAG test gave 86·6% correct identification of *C. albicans* and *C. tropicalis*. Non-*C. albicans* and non-*C. tropicalis* yeasts gave correct negative results in UAG tests. Only one isolate of *Trichosporon beigelii* gave a "false" positive reaction. The UAG test, which can be completed within 30 min, is a reliable test for screening non-*C. albicans* and non-*C. tropicalis* yeasts.

Introduction

Candidosis caused by several species of the genus *Candida* is the most widespread and prevalent mycotic disease of man.[1] It may be superficial or a systemic infection; superficial infections are the most common. Systemic candidosis is a serious problem, predominantly of temporarily or chronically compromised hosts, and affects the internal organs of vital importance.[2] The most important causative organisms are *C. albicans* and *C. tropicalis* as they are responsible for most of the superficial and systemic mycoses.[3,4]

Most of the biochemical tests used for the identification of yeasts suffer from the disadvantage that the results are obtained relatively slowly. The use of a rapid assay for the identification of *C. albicans* with 4-methylumbelliferyl-N-acetyl-β-D-galactosaminide was first reported by Perry and Miller,[5] and was arbitrarily designated as UAG.

The present study was initiated to evaluate the reliability of the 4-methylumbelliferyl substrate in differentiating *C. albicans*, a principal human pathogen, from *C. tropicalis* and other yeasts. Yeast cultures were tested for the ability to produce blue fluorescence with UAG both as primary and as pure cultures.

The UAG test is a biochemical test that relies on detecting pre-formed or newly formed *C. albicans* β-D-galactosaminidase.[5] This enzyme hydrolyses UAG to 4-methylumbelliferone (4-MU) that produces a blue fluorescence at 365 nm.

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The identity of the clinical isolates tested with UAG was confirmed by tests for chlamydospore (CHL) and germ-tube (GT) formation for *C. albicans* and morphology, fermentation and assimilation tests for *C. tropicalis* and other yeasts.

Materials and methods

UAG test

UAG (0·15 mM) was dissolved in 1 ml of dimethyl sulphoxide and diluted to 100 ml with acetate buffer, pH 5·1. The substrate was sterilised by filtration with a membrane filter (Millipore Corp., Bedford, MA, USA). It was then dispensed in borosilicate tubes (7 × 120 mm) for immediate use or stored at −4°C. Borosilicate tubes were used to avoid metal ions interfering with the reaction, which is likely if ordinary test tubes are used. For the same reason the UAG solution was inoculated with a wooden applicator to give a heavy milky suspension and mixed with a vortex mixer. The suspension was incubated at 37°C and examined for the presence or absence of blue fluorescence with a Wood's lamp (365 nm) in a partially darkened room every 30 min for 2 h. For positive tests, the substrate-yeast mixture produced a blue fluorescence within 30 min; lack of blue fluorescence indicated a negative result.

A positive control strain of *C. albicans*, selected for its ability to produce blue fluorescence with UAG, CHL on rice cream and GTs in both serum and rabbit coagulase plasma, was used. The other controls were an uninoculated tube of substrate and an inoculated tube of solvent without conjugate, to ensure that the

conjugate had not deteriorated and that the solvent had not caused the fluorescence.

Ox serum and rabbit plasma with tryptic soy broth

Many laboratories use non-human serum as the GT induction medium[6] because of safety problems associated with human serum, especially since the recognition of the human immunodeficiency virus (HIV) and the acquired immunodeficiency syndrome (AIDS).

Ox serum (0·3 ml) and Rabbit Coagulase Plasma (RCP; BBL) (0·3 ml), each mixed with 0·2 ml of tryptic soy broth, were investigated for their ability to induce GT. RCP with EDTA was reconstituted with sterile distilled water and used within 14 days. Germ-tube induction medium (0·5 ml) was mixed in a test tube with a yeast grown for 24 or 48 h on Sabouraud dextrose agar. The suspension was incubated at 37°C with a sterile pipette in the test tube which was used to withdraw a drop of yeast suspension to mount on a slide for examination for GT formation. The GT definition used was that of Berardinelli and Opheim.[6]

GT formation was examined at 30-min intervals for 2 h; a minimum of 100 cells was examined by light microscopy at a magnification of × 250.

Identification

Eighty-two clinical yeast isolates were tested for carbohydrate and nitrogen assimilation profiles and glucose fermentation. The yeasts were identified with the aid of a computer program—"Yeast Identification Program".[7] The carbohydrate and nitrogen assimilation profiles used in the program are: D-glucose fermentation; growth or lack of growth on D-galactose, D-xylose, maltose, cellobiose, lactose, raffinose, L-arabinitol, myo-inositol, 2-keto-D-gluconate, DL-lactate, citrate, L-arabinose, D-gluconate, nitrate, nitrite, ethylamine, creatinine, rhamnose and cycloheximide 0·01%; growth at 37°C or 42°C, or both; and the presence of pink pigmentation. Additional tests are available on the program in the event that there is no definitive species identification based on the results of the above tests.

Results

Reaction of C. albicans and C. tropicalis with UAG

Based on three tests—CHL, GT and UAG—the possible result combinations for *C. albicans* and *C. tropicalis* are shown in table I. The theoretically expected results are based on the assumption that the UAG test is positive only with *C. albicans* and always negative with *C. tropicalis*. Therefore, combination no. 1 is theoretically typical of *C. albicans* and combination no. 6 of *C. tropicalis*. The other combinations—nos. 2, 3 and 4—are theoretically possible for *C. albicans* while no. 5 identifies *C. tropicalis*. The results of CHL, GT and UAG tests were used together with the morphology, assimilation and fermentation tests to provide a complete identification profile.

Table I. Theoretically expected result combinations based on chlamydospore (CHL) production, germ-tube (GT) formation and the UAG reaction

Combination no.	CHL	GT	UAG
1	+	+	+
2	−	+	+
3	+	−	+
4	+	+	−
5	−	−	+
6	−	−	−

Combination no. 1 is typical for *C. albicans*; no. 6 is typical for *C. tropicalis*; nos. 2, 3 and 4 are theoretically possible for atypical *C. albicans*; no. 5 is theoretically possible for atypical *C. tropicalis*.

Table II. Experimental results obtained with *C. albicans* and *C. tropicalis* strains

Combination no.	CHL	GT	UAG	Number of strains	Identification
1	+	+	+	48	typical *C. albicans*
6	−	−	−	11	typical *C. tropicalis*
5	−	−	+	18	atypical *C. tropicalis*
2	−	+	+	5	atypical *C. albicans*

Table III. UAG reactions in typical *C. albicans* and *C. tropicalis* strains and atypical *C. albicans* strains

Combination no.*	Number of strains					
	GT+	GT−	UAG colour intensity†			
			3	2	1	0
1	48	0	43	5	0	0
6	0	11	0	0	0	11
5	0	18	14	2	2	0
2	5	0	5	0	0	0
Total	53	29	62	7	2	11

*1, typical *C. albicans*; 6, typical *C. tropicalis*; 2, atypical *C. albicans*; 5, atypical *C. tropicalis*. Only typical *C. albicans* strains formed chlamydospores, while the other yeast isolates were all negative for chlamydospores.
†3, intensity of fluorescence as in positive control; 2, less fluorescence compared to control; 1, much less fluorescence compared to 2 and control; 0, no fluorescence.

The result combinations nos. 3 and 4 were not obtained. As can be seen in table II, 48 isolates gave results typical for *C. albicans* and 11 for *C. tropicalis*. This represents 72% (59) typical strains. Of the remaining strains, none was able to form chlamydospores except for the five isolates that formed a germ tube and were UAG positive. The other 18 isolates were UAG positive only but gave negative results in CHL and GT tests. Two-thirds of the results obtained in this study were those theoretically expected.

Based on the morphology, fermentation and assimilation results, the 18 strains listed in tables II and III were considered to be *C. tropicalis*, although they gave positive UAG results, while five strains con-

Table IV. Results of CHL, GT and UAG reaction for yeasts other than *C. albicans* and *C. tropicalis*

Yeast species	Number of strains						
	CHL negative	GT negative	UAG colour intensity				Total
			3	2	1	0	
C. parapsilosis	30	30	0	0	0	30	30
D. hansenii	19	19	0	0	2	17	19
Cr. neoformans	17	17	0	0	2	15	17
C. guilliermondii	14	14	0	0	0	14	14
C. glabrata	3	3	0	0	0	3	3
S. pombe	2	2	0	0	0	2	2
C. lusitaniae	2	2	0	0	0	2	2
T. beigelii	9	9	1	0	3	5	9
G. candidum	2	2	0	0	0	2	2
T. candida	2	2	0	0	0	2	2
C. kefyr	1	1	0	0	0	1	1
Rh. minuta	1	1	0	0	0	1	1
Cr. lutoelus	1	1	0	0	0	1	1
S. cerevisiae	1	1	0	0	0	1	1
Cr. lipolytica	1	1	0	0	0	1	1
Cr. rugosa	1	1	0	0	0	1	1
Total	106	106	1	0	7	98	106

For UAG colour intensities see table III. C, *Candida*; Cr, *Cryptococcus*; S, *Saccharomyces*; G, *Geotrichum*; T, *Trichosporon*; Rh, *Rhodotorula*; D, *Debaryomyces*.

sidered to be *C. albicans* were unable to form chlamydospores. These results suggest that at least 10% of *C. albicans* isolates may fail to form chlamydospores. However, fermentation and assimilation pattern results, as analysed with the "Yeast Identification Program",[7] confirmed that the five isolates can be considered to be *C. albicans* and the 18 isolates can be considered to be *C. tropicalis* strains that gave "false" positive UAG results. Although the UAG reaction could be read within 30 min, it was monitored for 2 h to ensure that there was no further colour change to that observed after 30 min.

Reaction of other yeasts with UAG

The reactions of other yeasts (non-*C. albicans*, non-*C. tropicalis*) tested with UAG were more reliable. Only one isolate of *Trichosporon beigelii* gave a positive blue fluorescence with UAG similar to the positive control. The other three isolates of *T. beigelii*, and two isolates each of *Debaryomyces hansenii* and *Cryptococcus neoformans* gave only weakly positive reactions (table IV).

Discussion

In recent years, a significant increase in the number of yeast infections has been reported.[4] Most infections are attributed to *C. albicans*. However, other yeast species have been implicated, such as *C. tropicalis*.[1,8] *C. albicans* and *C. tropicalis* are found mostly as the cause of systemic mycoses, especially with the increasing use of antibiotics and immunosuppressive chemotherapy. AIDS has further added to the numbers of immunosuppressed hosts, leading to a rise in the number of cases of candidosis and cryptococcosis.

The differentiation of *C. albicans* from *C. tropicalis* by traditional methods is still problematic because of the formation of germ-tube-like structures by some strains of *C. tropicalis*.[9] The isolates used in this study were from samples of sputum, vaginal swabs, pleural puncture, dental protheses, skin scrapings and stomatitis. A similar selection of isolates was used by Hellstein *et al.*[10] where *C. albicans* from diseased and non-diseased hosts gain differences in biochemical properties and colony phenotypic characteristics. A particular pattern of characteristics may predict the behaviour of candidal isolates in disease.

The reasons for the inability of the five isolates of *C. albicans* to form chlamydospores are not clear; it may be due to the choice of medium, too heavy an inoculum or to some other inhibitory factors. For example, Young *et al.*[11] reported the inhibition of *C. albicans* growth in the pH range 3·3–4·5, especially when the pH was adjusted by adding lactic acid. However, if the pH was adjusted with tartaric acid, inhibition was less. Assimilation of nutrients, especially the utilisation of organic acids, is affected by pH. The five isolates above were tested three separate times on rice cream agar, but no chlamydospores were formed. The possibility of non-chlamydospore-forming *C. albicans* isolates should not be discounted.

When examining a yeast for chlamydospores, especially with *C. tropicalis*, careful observation is essential to differentiate chlamydospore-like structures from the true chlamydospores of *C. albicans*. To induce germ-tube structures, media containing non-human serum are useful. In this study, ox serum was superior to RCP in tryptic soy broth for inducing germ tubes with *C. albicans*. Some isolates of *Cr. neoformans* var. *gattii* of serotype C were able to form germ-tube-like structures. Other studies have also reported formation of germ-tube-like structures in other yeasts, such as *C. tropicalis*, *C. parapsilosis* and *Cr. gastricus*.[5] Therefore, discrepant structures resembling germ tubes must be distinguished.

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