SHORT ARTICLES

The oxidase test in yeasts of medical importance

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Summary. The results of an oxidase test were positive with species of *Cryptococcus* and eight species of *Candida*, but negative with *Candida krusei* and species of *Saccharomyces* and *Torulopsis*, grown on Columbia Agar base and similar media, including Brain Heart Infusion Agar and Mueller Hinton Agar. When grown on Sabouraud Dextrose Agar, all the yeast genera and species were oxidase negative. The test is useful in delineating the genus *Candida* from *Saccharomyces* and *Torulopsis*.

Introduction

The oxidase test, based upon the oxidation of the dimethyl- or tetramethyl-derivatives of *p*-phenylenediamine to an intensely coloured indophenol blue, detects the presence of cytochrome *c* oxidase in bacteria (Morris, 1983). This test was first used in the identification of *Pseudomonas* spp. (Kovács, 1956) and now has wide use in bacterial classification and identification (Smibert and Krieg, 1981).

In yeasts, oxidases act upon a wide range of substrates and *Cryptococcus neoformans* is unique in its oxidasic activity on *o*- and *p*-diphenols (Chaskes and Tyndall, 1975; Polacheck *et al.*, 1982). However, with the exception of this species (Chaskes *et al.*, 1981; Kaufman and Merz, 1982), oxidase reactions appear not to have been used in the identification of yeasts. We observed that *Candida albicans* occasionally gave a positive result in oxidase tests when grown on chocolate agar but gave negative reactions when grown on Sabouraud Dextrose Agar. In the present work we record culture media upon which the yeasts grown were found to be oxidase positive and those on which they were oxidase negative, and we consider the factors responsible for the differences.

Material and methods

Yeast strains

A total of 102 strains was studied; 78 strains were from clinical specimens, mainly vaginal secretions and faeces, submitted to the laboratory at Centro Regional de Saude da Amadora, Amadora, and Instituto Bacteriológico Câmara Pestana, Lisbon. These were: *Candida albicans* 25, *C. parapsilosis* 10, *C. tropicalis* 2, *C. pseudotropicalis* 2, *C. krusei* 8, *C. guilliermondii* 3, *Torulopsis glabrata* 24 and

Saccharomyces cerevisiae 4. The 2 strains of *Cryptococcus neoformans* studied were from the cerebro-spinal fluids of two patients with meningitis, one from the Hospital Pulido Valente, Lisbon, and the other from the Hospital de Curry Cabral, Lisbon. The strain of *Cry. laurentii* was from a patient's sputum. The identification of *C. albicans* was based on filamentation (germ tube formation) in serum. The other isolates were identified by the API C system (API System S.A., La Balme les Grottes, Montalieu-Vercieu, France). The two strains of *Cry. neoformans* grew at 37°C and were virulent for mice by the intravenous route.

The Instituto Gulbenkian de Ciência (IGC), Oeiras, Portugal provided 18 strains and the Institut Pasteur (IP), Paris, 3—*T. glabrata* IGC 2418, *T. colliculosa* IGC 2916, *T. maris* IGC 3398, *T. famata* IGC 3524, and IGC 3063, *C. rugosa* IGC 3523, *C. zeylanoides* IGC 2601, *C. lusitaniae* IGC 2705, *T. pintolopesi* IGC 2569, *Hansenula anomala* IGC 2495, *Pichia membranifaciens* IGC 2458, *S. italicus* IGC 2613, *S. cerevisiae* IGC 3507, *S. exiguus* IGC 2543, *S. telluris* IGC 2663, *S. kluyveri* IGC 2817, *S. rosei* IGC 2521, *Zigosaccharomyces rouxi* IGC 3199, *T. candida* IP 1490–83, IP 1035 and IP 1036.

The following culture media were used: Columbia Agar Base (Oxoid); Blood Agar Base No. 2 (Oxoid); Tryptose Agar (Difco); blood-agar prepared from Columbia Agar Base (Oxoid) with horse blood 5%; chocolate-agar with heated defibrinated horse blood 10% in Columbia Agar Base (Oxoid); Mueller-Hinton Agar (Oxoid); Brain Heart Infusion Agar (Oxoid); Sabouraud Dextrose Agar (Oxoid); Columbia Agar Base (Oxoid) with Bacto Dextrose (Difco) 4% added; a medium similar to Columbia Agar Base but with Mycological Peptone (Oxoid)—Mycological Peptone 10 g, starch (E. Merck, Darmstadt, West Germany) 1 g, NaCl 5 g, Agar No. 1 (Oxoid) 10 g, distilled water 1000 ml, pH 7.3; a medium resembling Sabouraud Dextrose Agar but with starch instead of dextrose—Mycological Peptone (Oxoid) 10 g, starch (E. Merck) 10 g, Agar No. 1 (Oxoid) 15 g, distilled water 1000 ml, pH 5.6.

Oxidase test

Colonies were tested with NNN'N'-tetramethyl-p-phenylenediamine dihydrochloride (BDH Chemicals Ltd, Poole, Dorset) 1% w/v by the technique of Cruickshank *et al.* (1975). Development of a violet colour within 60 s indicated a positive reaction.

Assay of different culture media

The oxidase test was performed with cultures grown at 37°C for 48 h on the different media described.

Results

A positive oxidase reaction was observed in *C. albicans* strains grown on Columbia Agar Base, Blood Agar Base, Tryptose Agar, blood agar, chocolate agar, Mueller-Hinton Agar and Brain Heart Infusion Agar, but a negative oxidase reaction was invariably found when the yeast was cultured on Sabouraud Dextrose Agar. Furthermore, on Columbia Agar Base, yeasts of the genera *Candida* and *Cryptococcus*, with the notable exception of *C. krusei*, always gave a positive oxidase reaction whereas strains of the genera *Torulopsis* and *Saccharomyces*, with

the exception of *T. candida*, gave a negative oxidase reaction. Other genera, from which unique isolates were studied, gave varied results: negative for *Hansenula anomala* and *Pichia membranaefaciens*, positive for *Zigosaccharomyces rouxi* (table).

It was shown that on Columbia Agar Base with dextrose added, *C. albicans* gave the negative oxidase-test result obtained on Sabouraud Dextrose Agar. On Columbia medium prepared with mycological peptone, *C. albicans* gave the same positive oxidase reaction as the fungus grown on Columbia medium of normal composition. Moreover, cultures from Sabouraud agar in which starch had replaced dextrose, gave the same positive result observed when the fungus was grown on Columbia Agar Base.

Discussion

In all the species studied, when the strains were grown on Sabouraud Dextrose Agar, the oxidase test was negative. The result of the oxidase test with yeasts grown on this medium appears to be associated with the presence of 4% dextrose, because the same effect was observed in yeasts grown on Columbia Agar Base containing the same concentration of the sugar. This is

Table. Results of oxidase tests performed with NNN'N'-tetramethyl-p-phenylenediamine dihydrochloride in different yeast species grown on Columbia Agar Base and incubated at 37°C for 48 h.

Species	Number of strains		
	total studied	oxidase-positive	oxidase-negative
<i>C. albicans</i>	25	25	0
<i>C. parapsilosis</i>	10	10	0
<i>C. tropicalis</i>	2	2	0
<i>C. pseudotropicalis</i>	2	2	0
<i>C. rugosa</i>	1	1	0
<i>C. krusei</i>	8	5	3
<i>C. zeylanoides</i>	1	1	0
<i>C. guilliermondi</i>	3	3	0
<i>C. lusitaniae</i>	1	1	0
<i>T. glabrata</i>	25	0	25
<i>T. famata</i>	2	0	2
<i>T. candida</i>	3	3	0
<i>T. maris</i>	2	0	2
<i>T. colliculosa</i>	1	0	1
<i>T. pintolopesi</i>	1	0	1
<i>Cryptococcus neoformans</i>	2	2	0
<i>Cryptococcus laurentii</i>	1	1	0
<i>S. cerevisiae</i>	5	0	5
<i>S. exiguus</i>	1	0	1
<i>S. telluris</i>	1	0	1
<i>S. kluyveri</i>	1	0	1
<i>S. rosei</i>	1	0	1
<i>Zigosaccharomyces rouxi</i>	1	1	0
<i>Hansenula anomala</i>	1	0	1
<i>Pichia membranaefaciens</i>	1	0	1

probably due to the repressive action of dextrose on the synthesis of cytochrome *c* that has been observed in yeasts by Zitomer and Nichols (1978).

The oxidase test has been shown to be useful in the identification of yeasts of medical importance. It is an aid in the delineation of the genus *Candida* from *Saccharomyces* and *Torulopsis*. The reaction has particular interest in relation to the controversy about the validity of the genus *Torulopsis* (Yarrow and Meyer, 1978, McGinnis *et al.*, 1984).

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