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BRIEF COMMUNICATION

CARBOHYDRATE ASSIMILATION PROFILES OF BRAZILIAN *Candida dubliniensis* ISOLATES BASED ON ID 32C SYSTEM

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SUMMARY

The purpose of the present study was to evaluate the identification of 19 Brazilian *C. dubliniensis* based on the biochemical profile exhibited when tested by the commercial identification kit ID 32C (bioMerieux). Thirteen of the isolates were rigorously identified as *C. dubliniensis* and the remaining isolates (six) were considered as having a doubtful profile but the software also suggested that there was 83.6% of chances for them to be *C. dubliniensis*. As well as pointed by the literature the identification obtained by phenotypic tests should be considered presumptive for *C. dubliniensis* due to variability of this new species.

KEYWORDS: *Candida dubliniensis*; ID 32C; Phenotypic identification.

INTRODUCTION

Candida dubliniensis is a newly described species of *Candida*, which was first reported by SULLIVAN *et al.* in 1995[21]. Despite *C. dubliniensis* has been recovered from several body sites in many human populations, it is most often isolated from the oral cavities of patients infected with the Human Immunodeficiency Virus. This new yeast is found all over the world[2,4,10,15,16,18,22] and is similar to *C. albicans* in a number of ways including morphology and metabolism, being considered as an opportunistic yeast pathogen but also recognized as a minor constituent of the normal human oral microbial flora[11,15].

A multicenter surveillance study conducted in Brazil by MILÁN *et al.*[10] showed a 2.8% prevalence rate of HIV-infected/AIDS adult patients harboring *C. dubliniensis* in their oral cavities. In contrast with patients from the north-hemisphere countries where this particular species may be recovered from 27% of asymptomatic HIV-positive patients and 32% of AIDS patients with oral candidiasis[21], different studies from South America suggest that *C. dubliniensis* is more rarely found[2,10,16,18].

C. dubliniensis is phylogenetically related to *C. albicans* and the distinction between *C. dubliniensis* and *C. albicans* remains a challenge for clinical microbiology laboratories. The importance of the correct identification seems to be meaningful for epidemiological proposals and therapeutic interventions. The majority of *C. dubliniensis* isolates show susceptibility to currently used antifungal drugs[12], but it has been demonstrated that they may rapidly develop a stable resistance to fluconazole upon *in vitro* exposure[11].

Various phenotype screening tests have been used to discriminate those organisms, including the colony color on CHROMagar Candida, growth temperature test at 42/45 °C[14], sugar assimilation tests and, β-glucosidase activity[17,23]. In addition, *C. dubliniensis* strains may be recognized by their ability to produce abundant chlamydospores often observed in triplets or in contiguous pairs[21], to coaggregate *in vitro* with *Fusobacterium nucleatum*[8], to produce rough colonies and chlamydospores on Staib agar[20] and also by its intolerance to 6.5% sodium chloride broth[3] and inability to produce an opacity halo at Tween 80 agar[19]. It is important to highlight that no single phenotype test has proven to be highly effective and the use of genotypic tests may be necessary for definitive identification purposes[7,9,22]. Considering that molecular methods are relatively time consuming and expensive there is a need for clinical laboratories to have phenotype tests as reliable as the carbohydrate assimilation profiles.

The present study was undertaken to evaluate the biochemical profile exhibited by nineteen Brazilian *C. dubliniensis* isolates formerly identified by genotyping methods, when tested by the commercial identification kit ID 32C (bioMerieux). This kit provides an evaluation for the assimilation of 30 carbon sources and for the growth of yeasts in the presence of cyclohexemide. The assays were performed according to the manufacturer's instructions. It consists of 32 cupules, each containing a dehydrated carbohydrate substrate. A semi-solid,

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Table 1
Differences of carbohydrate assimilation profiles reported by using ID 32C system when testing 19 Brazilian *C. dubliniensis* strains

Substrates which showed discrepant assimilation	Number of isolates	Brazilian *C. dubliniensis* isolates (Cd)
Palatinose	7	Cd1 ; Cd2 ; Cd8 ; Cd9 ; Cd10 ; Cd14 ; Cd15
Trehalose	1	Cd5
2-keto-gluconate	2	Cd15 ; Cd19
DL-Lactate	1	Cd5
Galactose	1	Cd9
Sorbitol	1	Cd16
N-acetylglucosamine	1	Cd4

chemically defined, minimal medium was inoculated with a suspension of the yeast organism to be tested. After 24-48 hours of incubation, growth in each cupule was detected by visual reading. Identification was obtained using the identification software (bioMerieux).

After 48 h of incubation, the ID 32 C system was able to rigorously identify and classify great part of the isolates as positive (13) for C. *dubliniensis* at three different levels: excellent (seven), very good (five) and as good (one). Almost all the remaining isolates (six) were considered as having a doubtful profile but the software also suggest that there is 83.6% of chances for them to be *C. dubliniensis*. It should be pointed here that in laboratory routine the percentage of 83.6% would be an important indication of the species and should be taken into account.

Some assimilation profiles were not consistent among the strains of *C. dubliniensis* tested once they exhibited variation of results among different isolates tested. The lower agreement rate was found with results generated by palatinose, 2-keto-gluconate, N-acetylglucosamine, lactate, trehalose, galactose and sorbitol (Table 1). Best discrimination and consistence of results were obtained with α-methyl-D-glucoside (MDG) and xylose (XYL) that were not assimilated by 100% of the isolates tested. Lactate (LAT) and trehalose (TRE) were assimilated by one isolate. Our results were slightly different from those reported by PINCUS *et al.*[13] in which 30% of the isolates assimilated trehalose (TRE) and no one of the isolates assimilated lactate (LAT). When SULLIVAN *et al.*[21] described *C. dubliniensis* as a new species, they reported that this organism was not able to assimilate xylose (XYL) and α-methyl-D-glucoside (MDG). However, some positive reactions with this subtract were described previously by testing some strains of *C. dubliniensis* with commercial kits as the Vitek system[5,13].

The results obtained in the present study are in accordance with the results reported by other authors[6,13]. The major identification problem related to the ID32 system was the discrepancy of results generated by some reactions causing misidentification of *C. dubliniensis*. It has been reported that variations in the density of the inoculum may produce false-positive or false-negative results requiring the adjustment of the inoculum to the proper density[5]. Otherwise, one could speculate that different strains of *C. dubliniensis* may exhibit some differences in their metabolic pathways like that involving trehalose and N-acetylglucosamine[24]. The variant phenotypes could also arise from the process of high-frequency switching to adapt to the host environment, like almost observed with *C. albicans*[1].

In conclusion, despite the promising results obtained with the ID 32C system, this method exhibited some limitations in the identification of *C. dubliniensis*. Since variability has been reported in the literature, the results obtained by phenotypic tests should be considered presumptive for *C. dubliniensis* and one or more confirmatory tests should be employed. Therefore, we do not recommend the use of ID 32C system as the only screening test for the identification of *C. dubliniensis*.

RESUMO

Identificação de *Candida dubliniensis* isoladas no Brasil, através do método comercial ID 32C

Dezenove culturas de *C. dubliniensis* isoladas no Brasil, previamente identificadas através de métodos genotípicos, foram avaliadas pelo kit comercial ID 32C (bioMerieux). Treze culturas foram identificadas como *C. dubliniensis*, mas as demais (seis) evidenciaram perfil duvidoso, embora o software do sistema sugerisse 83,6% de chances das mesmas pertencerem à espécie *C. dubliniensis*. A literatura tem registrado grande variabilidade fenotípica com esta espécie e, por isto, as identificações obtidas com este sistema deverão ser consideradas como presuntivas.

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