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Note

Candida dubliniensis and *Candida albicans* differentiation by colony morphotype in Sabouraud-triphenyltetrazolium agar

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ABSTRACT

Background: *Candida dubliniensis* is a germ tube and chlamydoconidia producing *Candida* species that may be misidentified as *Candida albicans*. Molecular-based methods are the most reliable techniques for *C. albicans* and *C. dubliniensis* differentiation. However, accurate, quick and inexpensive phenotypic tests are needed to be used in low-complexity mycology laboratories.
Aims: To evaluate colony morphotypes on Sabouraud-triphenyltetrazolium agar as a tool for *C. dubliniensis* and *C. albicans* differentiation.
Methods: The morphology of 126 *C. albicans* and *C. dubliniensis* strains was evaluated and compared with their identification by molecular methods.
Results: The method showed 100% sensitivity and specificity when color and the presence or absence of large white mycelial halo was evaluated.
Conclusions: Colony morphotype on Sabouraud-triphenyltetrazolium agar should be considered as a new tool to differentiate *C. dubliniensis* and *C. albicans*.

Discriminación entre *Candida dubliniensis* y *Candida albicans* evaluando el morfotipo de las colonias en agar Sabouraud-trifeniltetrazolio

Palabras clave:
Candida dubliniensis
Candida albicans
Sales de trifeniltetrazolio

RESUMEN

Antecedentes: *Candida dubliniensis* es una especie del género *Candida* capaz de producir tubos germinativos y clamidoconidios, y puede ser identificada erróneamente como *Candida albicans*. Las técnicas moleculares de identificación son consideradas las más específicas para diferenciar estas especies. Sin embargo, se siguen necesitando métodos exactos, rápidos y de bajo coste para ser utilizados en laboratorios de micología de baja complejidad.
Objetivos: Evaluar el morfotipo de las colonias de levaduras en agar Sabouraud-trifeniltetrazolio como una herramienta para diferenciar *C. dubliniensis* de *C. albicans*.
Métodos: Se evaluó la morfología de 126 aislamientos de *C. albicans* y *C. dubliniensis* y los resultados fueron comparados con los obtenidos utilizando métodos moleculares.
Resultados: El método utilizado mostró una sensibilidad y una especificidad del 100% cuando se evaluó el color y la presencia o ausencia de un gran halo de micelio blanco.
Conclusiones: La evaluación del morfotipo de las colonias en agar Sabouraud-trifeniltetrazolio puede ser utilizada como una nueva herramienta para diferenciar *C. dubliniensis* de *C. albicans*.

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Candida dubliniensis is a germ tube and chlamydoconidia producing *Candida* species described in 1995[15,17] that may be misidentified as *C. albicans*.[7] Firstly, *C. dubliniensis* was associated with oropharyngeal candidiasis in HIV+ population[15,17] but later it was also isolated from HIV negative patients and from different body sites and fluids, including blood, urine, etc.[3,8,10] Molecular-based methods are the most reliable techniques for *C. albicans* and *C. dubliniensis* differentiation.[2,12,16] However, accurate, quick and inexpensive phenotypic tests are needed to be used in low-complexity mycology laboratories. Different tests were proposed and have been used as screening methods, including differences in carbohydrate assimilation, growth capacity at 42 °C, 45 °C and in hypertonic media, clustered chlamydoconidia production, etc.[1,4–6,9,13,14,16] Any of the described methods are 100% specific for *C. dubliniensis* and *C. albicans* differentiation. In this work, colony morphotype on Sabouraud-triphenyltetrazolium agar (STA) was evaluated as a tool for *C. dubliniensis* and *C. albicans* differentiation.

Colony morphotype data were obtained from 110 *C. albicans* and 12 *C. dubliniensis* clinical strains isolated from different sources (blood, urine, vulvovaginal infections, etc.). *C. albicans* ATCC 90028, *C. albicans* ATCC 36082, *C. albicans* Sc5314 and *C. dubliniensis* NCPF 3949 were included in the study as control strains. All the strains were identified by phenotypic[5] and molecular methods.[3,12] The molecular identification was considered the gold standard when the specificity of the STA method was evaluated.

STA was prepared as follows. Firstly, a basal medium was prepared dissolving 10 g of peptone, 20 g of glucose and 20 g of agar (all from Britania Laboratory, Argentina) in 990 ml of distilled water. The basal medium was sterilized at 121 °C for 15 min. Meanwhile, a stock solution of 2,3,5-triphenyltetrazolium chloride (TTZ) (Sigma–Aldrich, Argentina) was prepared dissolving 1 g in 100 ml of distilled water. This last solution was filter-sterilized. Finally, the basal medium was cooled at 55 °C in a water bath and 10 ml of the TTZ stock solution were added, reaching a 0.1 g/l TTZ final concentration. Then, the media was plated in 90 mm Petri dishes.

For the morphotype evaluation, fresh 24 h-cultures in Sabouraud dextrose agar (peptone 1%, glucose 2%, agar 2%) were used. Four to five colonies were picked to obtain a 0.5 McFarland inoculum in water. Afterwards, 3 μl of each cell suspension were inoculated onto STA plates (16 strains per plate), and incubated for 7 days at 28 °C. The morphotype data collected included color (pink or violet), presence or absence of mycelial halo and colony texture (smooth or rough). The morphotypes were named using the nomenclature published by Quindós et al.[11] The experiments were performed in triplicates in three separate days.

The 113 *C. albicans* strains showed three different morphotypes: pink with no mycelial halo ($n = 65$, 57.5%), pink with mycelial halo ($n = 39$, 34.5%), and violet with no mycelial halo ($n = 9$, 8%). On the other hand, all the *C. dubliniensis* ($n = 13$) strains produced violet colonies with large white mycelial halo (Fig. 1). Colony texture was not informative since *C. albicans* and *C. dubliniensis* showed both phenotypes. The morphotypes were reproducible even after a −86 °C storage and multiple subcultures (data not shown).

The reduction of TTZ has been used as an aid for *Candida* identification since the 1980s.[5] In 1992, Quindós et al. reported the use of colony morphotype on STA as a *Candida* species identification tool.[11] In that report, 93.67% and 3.6% of the strains identified phenotypically as *C. albicans* were pink or violet, respectively, and only 2% of the *C. albicans* isolates showed violet colonies with mycelial halo. It would be possible that those results represented a 2%

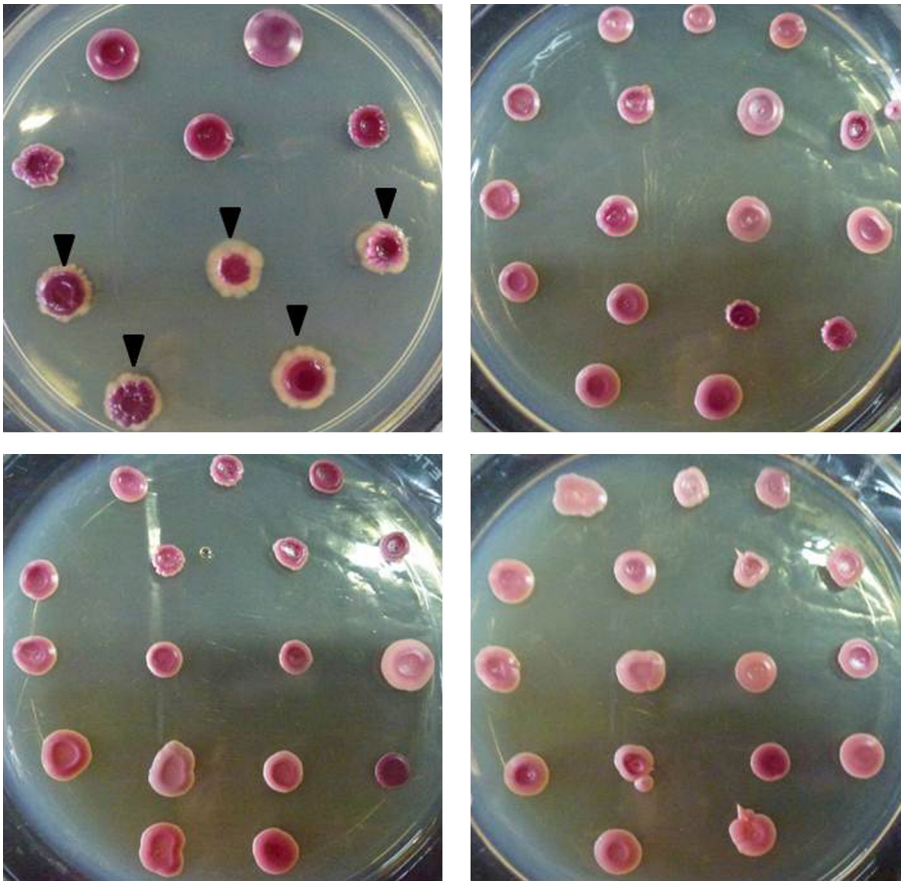

Fig. 1. STA plates inoculated with 3 μl of 0.5 Mc Farland yeast cell suspensions. Arrow heads in the upper left photography show *C. dubliniensis* isolates identified by molecular methods (3,12). The other three photos show different *C. albicans* morphotypes.

incidence of *C. dubliniensis* in the yeast collection used. This incidence was not reported since *C. dubliniensis* was proposed as separate species three years later.

In 1998, the reduction of TTZ was suggested as a useful tool for the differentiation of *C. albicans* from *C. dubliniensis* by Velegraky and Logotheti.[19] However, in the aforementioned work, another basal medium was used; there is no description of the number of isolates tested and how the authors identified the strains as *C. albicans* or *C. dubliniensis*. Accordingly, the specificity and sensitivity evaluation was not performed. Also, Velegraky and Logotheti evaluated the color of the colony as the only characteristic. In the present study, we evaluated three morphotype characteristics on STA as a tool for *C. dubliniensis* – *C. albicans* differentiation using a total of 126 strains (122 isolated from different clinical sources) identified by two different molecular-based techniques. The method described here has 100% specificity and sensitivity when color and mycelial halo is considered together (all the *C. dubliniensis* showed violet colonies with large white mycelial halo). It has to be highlighted that we do not propose this test as a primary isolation media capable to distinguish *C. dubliniensis* and *C. albicans* directly from biological samples since other *Candida* species (e.g. *C. tropicalis*) are also able to reduce TTZ salts.[18] We suggest the use of STA medium as and inexpensive, easy to perform differentiation tool for *C. albicans* and *C. dubliniensis* after a germ tube evaluation or starting from a green colony in Chromagar® *Candida*. Also, the seven days of incubation needed to see the result is an important disadvantage as a useful method in a clinical laboratory. However, STA medium would be useful for large epidemiology studies in reference labs since 16 strains could be studied per each 90 mm Petri dish.

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