Rev. Inst. Med. trop. S. Paulo
48(3):119-121, May-June, 2006

COMPARISON AMONG TOMATO JUICE AGAR WITH OTHER THREE MEDIA FOR DIFFERENTIATION OF *Candida dubliniensis* FROM *Candida albicans*

Sydney Hartz ALVES(1), Érico Silva de LORETO(2), Carlos Eduardo LINARES(2), Carolina P. SILVEIRA(3), Liliane A. SCHEID(3), Daniela I. Brayer PEREIRA(1) & Janio Morais SANTURIO(1)

SUMMARY

The purpose of the present study is to compare the tomato juice agar, a well known medium employed to observe ascospore formation, with niger seed agar, casein agar and sunflower seed agar, applied to a differentiation between *C. dubliniensis* and *C. albicans*. After 48 hours of incubation at 30 °C all 26 (100%) *C. dubliniensis* isolates tested produced chlamydospores on tomato juice agar as well as in the other three media evaluated. However, when we inoculated all media with *C. albicans,* the absence of chlamydospores became resulting in the following percents: tomato juice agar (92.47%), niger seed agar (96.7%), casein agar (91.39%), and sunflower seed agar (96.7%). These results indicate that tomato juice agar is another medium which can also be used in the first phenotypic differentiation between *C. dubliniensis* and *C. albicans*.

KEYWORDS: *Candida dubliniensis*; Tomato juice agar; Phenotypic identification.

INTRODUCTION

Candida dubliniensis is a newly described fungus which was first reported by SULLIVAN *et al.* in 1995[21]. *C. dubliniensis* is phylogenetically closely related to *C. albicans*, thereby sharing many morphological and physiological characteristics as germ tube positive, similar biochemical patterns and the ability to form chlamydospores in rice extract agar and cornmeal agar[21,22].

Routine discrimination between *C. dubliniensis* and the closely related species *C. albicans* has been problematic[21,22]. The most accurate method of identifying *C. dubliniensis* and discriminating it from *C. albicans* requires PCR-based tests[8,12,21,22]; however, these are not readily applicable to the high-volume throughput of isolates in many diagnostic laboratories routine[2,3,14].

The most reliable phenotypic methods for the identification of *C. dubliniensis* include carbohydrate assimilation profile analysis by using commercially available yeast identification systems[5,7] and detection of differential antigen expression through immunofluorescence microscopy[6]. However, they can only be applied after the first isolation.

One of the key features employed in the initial description of *C. dubliniensis* was its ability to produce abundant chlamydospores on corn meal agar and rice-agar-Tween medium[21]. Based on this characteristic, recently new media as niger seed agar[20], caffeic-acid-ferricitrate agar[2], casein agar[14], sunflower seed agar[3] and tobacco agar[10] have been proposed to differentiate both species simultaneously up to the first isolation.

In the last ten years there has been a proliferation of phenotypic tests in the literature for the differentiation of *C. albicans* and *C. dubliniensis* which includes carbohydrate assimilation profile[5,7], appearance on CHROMagar[11], ability to grow at 45 °C[16], inability to grow on Sabouraud dextrose broth with NaCl 6.5%[4], absence of an opacity halo around an inoculated site on Tween 80 medium[19] and some others[6,15,18].

Tomato juice agar or V-8 juice agar is a well known medium widely used for ascospore formation in yeasts as *Saccharomyces cerevisae* and *Hansenula anomala*[1,9,13].

Here we compare the tomato juice agar (V8 agar) with niger seed agar, casein agar and sunflower agar applied to differentiation between *C. dubliniensis* and *C. albicans*.

MATERIAL AND METHODS

Strains: a total of 26 Brazilian *C. dubliniensis* isolates and 93 *C. albicans* isolates were studied. *C. albicans* isolates were from the culture collection of the Laboratório de Pesquisas Micológicas, Department of Microbiology and Parasitology, Universidade Federal

(1) Departamento de Microbiologia e Parasitologia, Universidade Federal de Santa Maria (UFSM). Santa Maria, RS, Brasil. Phone/fax (55) 3220-8906.
(2) Curso de Pós graduação em Ciências Farmacêuticas, UFSM, Santa Maria, RS, Brasil.
(3) Aluno de Graduação em Farmácia e Bioquímica, PIBIC/CNPq.
Correspondence to: Sydney Hartz Alves, Rua Andradas 1985/201, 97010-033 Santa Maria, RS, Brasil. **e**-mail: hartzsa@ccs.ufsm.br

de Santa Maria, Santa Maria city, RS, Brazil. *C. dubliniensis* were from different Brazilian cities. All the *C. dubliniensis* isolates were rigorously identified by phenotypic and genotypic methods[8,12,20,21]. The strains were stored in frozen stocks at -80 °C and were routinely propagated on YPD agar plate (10 g yeast extract, 20 g peptone, 20 g glucose, 15 g agar per litre) at 30 °C[13].

Culture media for chlamydospores production: a) Tomato juice agar[17] (tomato juice 200 mL; $CaCO_3$ 3 g; dextrose 5 g; agar 20 g per liter); b) Niger seed agar[20] (50 g *Guizotia abyssinica* seed pulverized; 1 g glucose; 1 g KH_2PO_4; 1 g creatinine; 15 g agar per liter); c) Sunflower seed agar[3] (extract cooled and filtered from 50 g *Helianthus annuus* pulverized; 1 g glucose; 1 g KH_2PO_4; 1 g creatinine and 15 g agar per liter); d) Casein agar[14] (10 g of skim milk was dissolved in 90 mL of distilled water, and 3 g of agar was dissolved in 97 mL of distilled water. After autoclaving both solutions separately at 121 °C for 15 min, they were allowed to cool to 45 °C to 50 °C and after this were mixed together. All the media after autoclaving were dispensed in 25 mL amounts into 90 mm-diameter Petri dishes. Tomato juice agar, niger seed agar, sunflower agar and casein agar plates were inoculated with culture growth in a 48 h YPD agar followed by incubation at 30 °C, except casein agar, which was incubated at 24 °C. Following the incubation, the plates were directly examined by 10X and 40X objectives lens to observe the chlamydospores. When absent, samples of culture growth were stained with lactophenol cotton blue and were again examined for chlamydospores production by light microscopy.

RESULTS AND DISCUSSION

All the 26 (100%) *C. dubliniensis* isolates tested produced chlamydospores in Tomato juice agar after 48 h of incubation at 30 °C. The majority of *C. albicans* isolates evaluated (92.47%) did not show chlamydospores in the same conditions. However, in the cases that chlamydospores were produced by *C. albicans*, although indistinguishable from those by *C. dubliniensis*, were difficult to observe due to their very low number. When we compared the four media for *C. dubliniensis* identification based on chlamydospore production, we observed that all *C. dubliniensis* isolates tested (n = 26) produced chlamydospores, yielding 100% of sensibility. On the other hand, when we inoculated all media with *C. albicans* isolates (n = 93), the production of chlamydospores was very little: tomato juice agar 7/93, niger seed agar 3/93, sunflower agar 3/93 and casein agar 8/93, which have resulted in specificities ranging from 91% to 97%.

C. dubliniensis is usually isolated in mixed cultures with *C. albicans* and/or other yeasts. The difficulty in detecting mixed cultures in plates with traditional media as Sabouraud dextrose agar has stimulated the development of identification systems and differential media for yeasts[1,9,13,17]. Specific media is being purposed to discriminate *C. dubliniensis* from *C. albicans* such as the niger seed agar (*Guizotia abyssinica*)[20], the sunflower agar (*Helianthus annuus*)[3] and casein agar[14] among others[2,10,11,19]. They are simple and cheap methods for presumptive differentiation of *C. dubliniensis* from *C. albicans,* showing satisfactory results. However, it is well established that *C. dubliniensis* isolates present great phenotypic variability, what hinders the standardization of the identification techniques[2,5,7,11,16,21]; so the search for new and more refined methods are advantageous and should be stimulated. Here we have studied the tomato juice agar which is a traditional medium employed to observe the formation of ascus to differentiate the sexual reproduction as seen in *Saccharomyces cerevisae* from the asexual reproduction in yeasts like *Candida* species[1,9,13,17]. We noticed that tomato juice agar stimulates the chlamydospore production in *C. dubliniensis* similarly to what occurs with other media. Chlamydospores, from greek *chlamys* that means mantle, plus *spora* that means seed or spore, is a thick-walled thallic conidium that generally functions as a resting spore[1]. These fungal structures contain very significant reserves which are activated and consumed during germination until the newly formed colony has developed sufficiently for its own trophic extension[1,9].

Concluding, we emphasize that tomato juice agar is a well known medium used in Mycology laboratories, simple to prepare, cheap and that now, it can have a double usage indicating the sexual differentiation of yeasts and as a presumptive medium for differentiation between *C. albicans* and *C. dubliniensis*.

RESUMO

Comparação do ágar suco de tomate com outros três meios, na diferenciação entre *C. albicans* e *C. dubliniensis*

O presente estudo teve como objetivo comparar o ágar suco de tomate, um tradicional meio utilizado para observação de ascósporos em leveduras, com o ágar semente de niger, ágar caseína e ágar semente de girassol, na diferenciação fenotípica entre *C. albicans* e *C. dubliniensis*. Após 48 h de incubação a 30 °C, os 26 isolados de *C. dublinienis* (100%) evidenciaram a formação de clamidoconídios igualmente em todos os meios comparados. Entretanto, quando semeados com *C. albicans*, a formação de clamidoconídios foi raramente observada, resultando nos seguintes percentuais de ausência destas estruturas: ágar suco de tomate (92,47%), ágar niger (96,7%), ágar caseína (91,39%), ágar semente de girassol (96,7%). Estes resultados permitem-nos sugerir a utilização do ágar suco de tomate como mais um meio que, já no primo-isolamento, é capaz de, presuntivamente, diferenciar *C. albicans* de *C. dubliniensis.*

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Received: 15 August 2005
Accepted: 21 February 2006