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New Simple Media Substrates to Differentiate *Candida dubliniensis* from *Candida albicans*

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Abstract: *Candida dubliniensis* is a recently described as an important pathogenic species, which shares many phenotypic features with *Candida albicans* and therefore microbiological laboratories may be misidentified. The molecular methods can be exhausting and unavailable at many mycological laboratories, with restricted budgets such as those in developing countries. Therefore, the phenotypic techniques have been encouraged in the development of simple differential media with high specificity and sensitivity for the presumptive identification of these species. We examined the colony morphology and chlamydospore production of *C. dubliniensis* (28 isolates) and *C. albicans* (40 isolates). Isolates were cultured on several new proposed media such as Coriander (Coriandrum sativum L), Cumin (Cuminum cyminum), Soybean (Glycine max), lupin (Lupinus albus), flaxseed (Linum usitatissimum), pumpkinseed (Lepomis gibbosus), Basil (Ocimum basilicum L), Peppermint (Mentha piperita) and Marjoram (Origanum majorana L), yerba mate (*Ilex paraguariensis*), thyme (Thymus vulgaris), rosemary (Rosmarinus officinalis), and sage (Salvia officinal) extract agar. In all of these media, over 80% of C. *dubliniensis* isolates showed rough colonies with peripheral hyphae fringes and abundant chlamydospores after 24 to 48 hours of incubation at 25°C. In contrast, under the same conditions, all isolates of *C. albicans* (100%) showed smooth colonies without hyphae fringes or chlamydospores. In conclusion, this media subtracts offer a simple, rapid, and inexpensive screening media for the differentiation of *C. albicans* and *C. dubliniensis*.

Keywords: Candida Albicans, *C. dubliniensis*, Chlamydospore Production, Differential Medium, Seeds, Leaves

1. Introduction

Candida dubliniensis is a newly described fungus, which first reported in 1995 [1]. *Candida dubliniensis* phylogenetically closely related to *Candida 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 or cornmeal agar [1, 2]. Routine discrimination between both species has been problematic and accurate method of identifying and discriminating them only requires PCR-based tests [1, 2, 3, 4]. However, these are not readily applicable to the high-volume throughput of isolates and highly costly in many diagnostic laboratories routine [5, 6, 7].

The most reliable phenotypic methods for the identification of *C. dubliniensis* include the carbohydrate assimilation profile analysis by using commercially available yeast identification systems [7, 8]. The detection of differential antigen expression through immunofluorescence microscopy can only be apply after the first isolation [9]. 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 [1]. Based on this characteristic, recently many new media as Niger seed agar [10], caffic-acid ferric citrate agar [11], casein agar [5], sunflower seed agar [12] and tobacco agar [13] have been proposed to differentiate both species simultaneously up to the first isolation. In the last ten years,

there are a proliferation of phenotypic tests in the literature for the differentiation of *C. albicans* and *C. dubliniensis*. These includes carbohydrate assimilation profile [7, 8], appearance on CHROMagar® [14] ability to grow at 45°C [15], inability to grow on Sabouraud dextrose broth with NaCl 6.5% [16], absence of an opacity halo around an inoculated site on Tween 80 medium and some others [9, 17, 18].

However, none of the proposed and reported biochemical or phenotypic methods can differentiates both species with 100% specificity and sensitivity. Here we compare new different substrates agar media with sunflower seed agar as a well-recommended media to differentiate both species and some other phenotypic tests.

2. Material and Methods

2.1. Media Substrates

Seeds and leaves of the following plants such as Coriander (Coriandrum sativum L), Cumin (Cuminum cyminum), Soybean (Glycine max), lupin (Lupinus albus), flaxseed (Linum usitatissimum), pumpkinseed (Lepomis gibbosus), Basil (Ocimum basilicum L), and Peppermint (Mentha piperita) and Marjoram (Origanum majorana L), yerba mate (*Ilex paraguariensis*), thyme (Thymus vulgaris), rosemary (Rosmarinus officinalis), and sage (Salvia officinal) were used. To 50g (5%) of each grinded type of substrates except the last five substrates in which ten gram was used (1%), one liter of distilled water added. The mixture boiled for 30 minutes, the decoction then cooled and filtered through gauze, and the volume readjusted to 1 liter with distilled water. The pH was adjusted to 6.0, and 20 g agar-agar (Difco) was added before the mixture was autoclaved at 121 °C for 15 min. The media allowed to cool to 45-55 °C and dispensed into sterile Petri dishes [12].

2.2. Isolate Strains

A total of (28) C. *dubliniensis* isolates, (40) *C. albicans* and one isolate each of candida parapsilosis and candida glabrata isolated from oral cavity from AIDS patient and patients with dental disease were enrolled in this studies [19, 20]. All isolates previously identified vigorously by phenotypic test such as germ tube, API 20C AUX yeast identification system, Growth at 42°C, NaCl, chlamydospores production on Corm meal-Tween agar and Sunflower seed agar. The strains routinely propagated on YPD agar plate at 30°C.

2.3. Phenotypic Identification and Characterization

All isolates retested for germ tube formation in horse serum, growth at 6.5% NaCl broth SDA, Growth at 42°C, Corn meal agar plus tween 80, Sunflower seed agar, [1, 12.16] and our agar media substrates for chlamydospores production. Germ tube test result were read at 2 and four hours incubation at 37°C while other phenotypic test was read after 24 hours and up to 96 hours incubation at 28°C.

3. Results

Out of the (28) strains identified as *C. dubliniensis* by API20C AUX yeast identification system (Biomuriex), assimilation of xylose, Growth at 42°C and ability to produce chlamydospores on both corn meal tween 80 and sunflower seed agar. The most, strains showed typical rough colonies with peripheral hyphal fringes and abundant chlamydospores after 24 to 48 hours of incubation at 25°C - 28°Cdegrees on all our substrate media. Figure 1 and 2 show the observations of colonial morphology and chlamydospore formation by *C. dubliniensis* and *C. albicans* on SSA agar, CM-Tween agar and our new media substrates media. However only six strains out of 28 Strains showed yeast cell or yeast and few hyphae on some of our substrates media. In contrast, under the same conditions, all isolates of *C. albicans* (40 strains), *candida glabrata* and *C. parapsilosis s*howed smooth colonies without hyphal fringes or chlamydospores during the incubation periods. Furthermore, even the few strains of *candida albi*cans (12 strains), which produced smooth colonies and few scanty chlamydospores on sunflower seed agar, were only showing yeast or few hyphae on all our substrate media with the exception of (7 strains) produce smooth colonies and few scanty chlamydospores on flaxseed agar media. The test sensitivity and specificity of each phenotypic identification method been show in (Table 1).

Table 1. *The sensitivity and the specificity of the phenotypic methods used for differentiating C. dubliniensis and C. albicans.*

Substrate media	*C. dubliniensis*— sensitivity (%)	*C. albicans*— specificity (%)
Germ Tube	100	100
NaCl	85.7	97.5
API20C AUX	100	100
Growth at 42°C	100	100
Corn meal-Tween 80 agar	100	100
Sunflower seed agar	96.4	70
Lupin	82.1	100
Soybean	82.1	100
Pumpkin	85.7	100
Flaxseed	89.3	82.5
Coriander	78.6	100
Cumin	78.6	100
Marjoram	92.9	100
Basil	82.1	100
Mint	89.3	100
Mata	96.4	100
Thyme	96.4	100
Rosemary	96.4	100
Sage	92.9	100
Total isolate tested	30	40

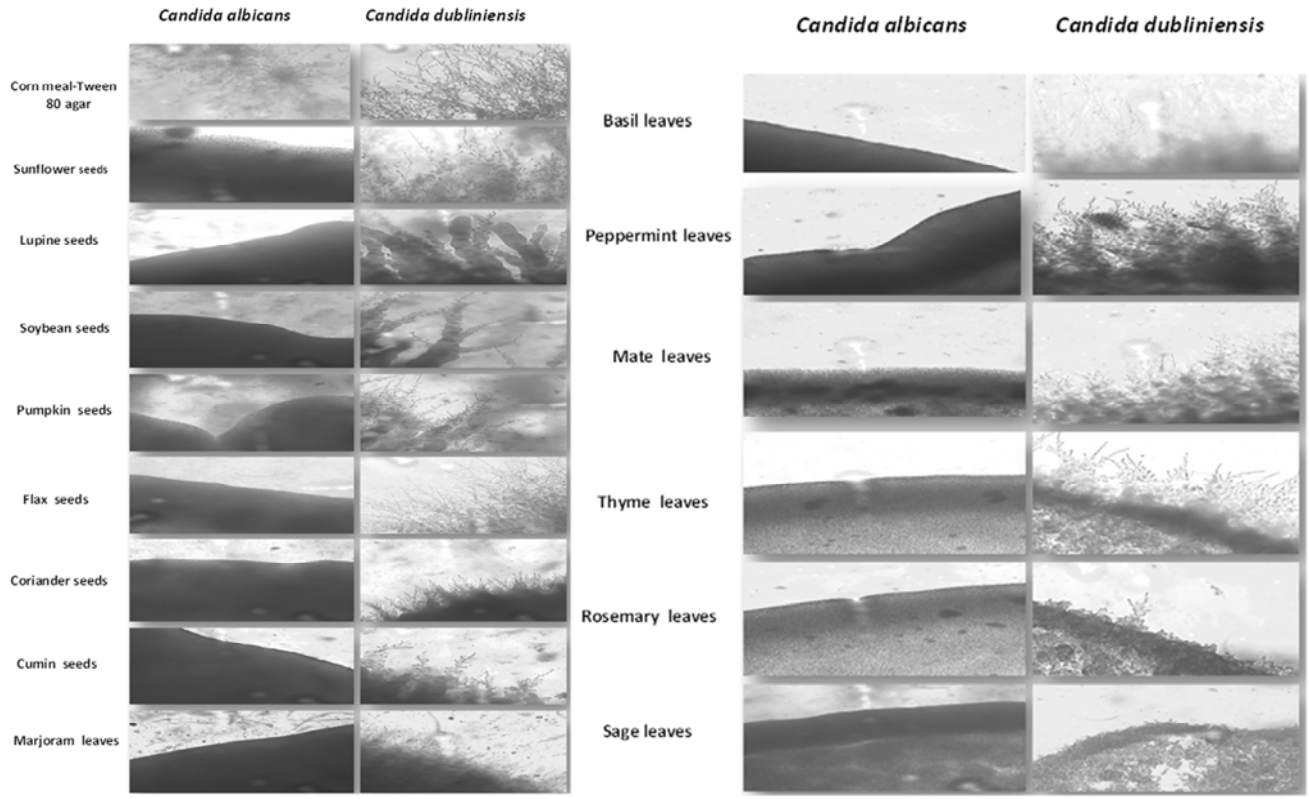

Figure 1. *Microscopic view of C. dubliniensis colony showing hyphal fringe (right side), and yeast cells of C. albicans colony (left side) on our new substrates agar media.*

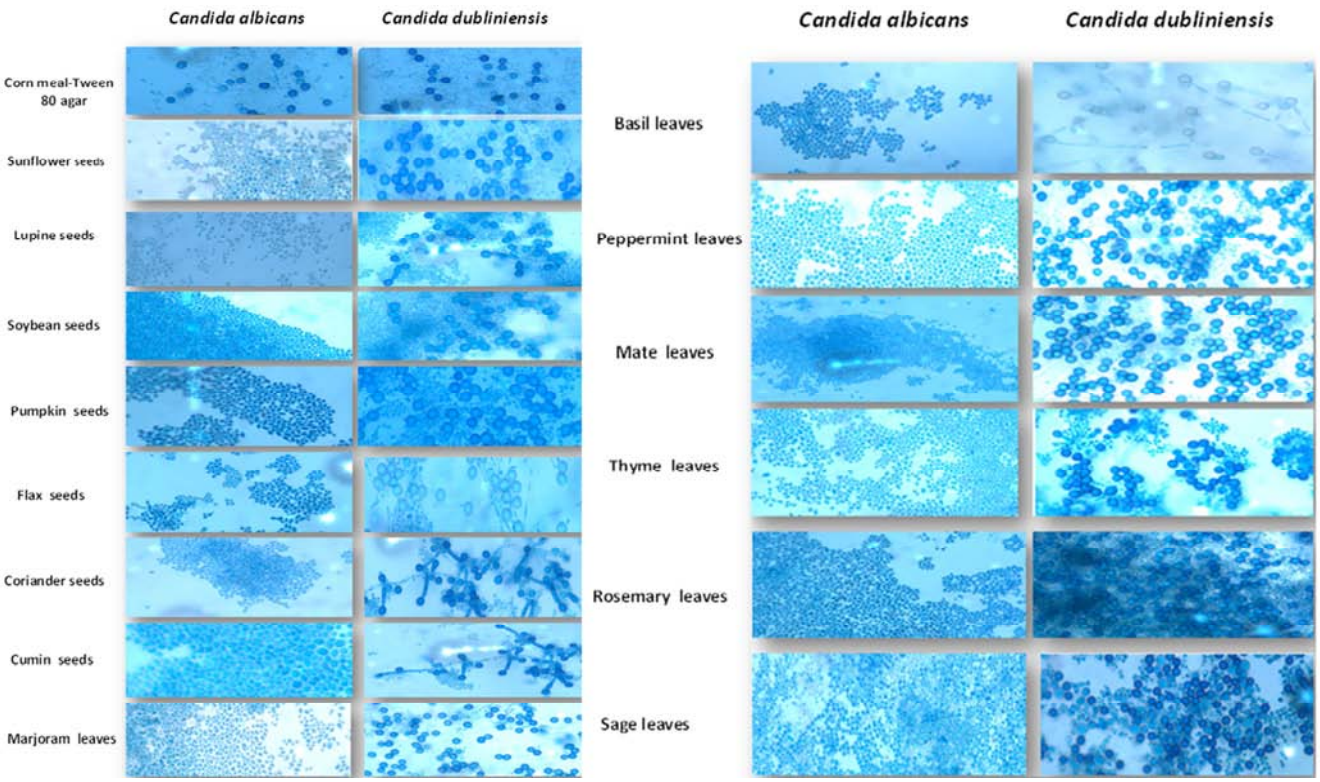

Figure 2. *Slide culture microscopic view on new substrates agar media showing hyphae with chlamydospores of C. dubliniensis (right side) and small yeast of C. albicans (left side)after 2 days of incubation at 25°C-28°C.*

4. Discussion

The phenotypic characteristic of C. albicans to produce germ tubes and chlamydospores has used to differentiate it from other Candida species. However, the recognition of *C. dubliniensis* as a separate species[23] and its similarity to *C. Albicans* in producing chlamydospores and germ tubes. Several studies have been focused attention to develop new and simple inexpensive methods for discriminating the two species in routine clinical microbiology laboratories. The tests that have been reported to be useful for this purpose include production of rough colonies and chlamydospores on sunflower seed agar, casine agar and other media [11, 5, 6], development of dark green colonies on CHROM agar Candida [13], inability to grow at 45°C[8] and in 6.5% sodium chloride [15] by *C. dubliniensis* isolates. Further more recently simple several commercial yeast identification systems based on carbohydrate assimilation profiles have also become available for the identification of C. *dubliniensis* [7, 8]. In an extensive evaluation of commercial yeast, identification systems studies include API 20C AUX, Vitek 2 ID YST, found that useful for the differentiation of *C. dubliniensis* [8]. The result reported that assimilation of a-methyl-D-glucoside, trehalose, and D-xylose might use for the differentiation of *C. dubliniensis*. However, the assimilation results were not consistent with different commercial assimilation systems used and varied with the incubation period [8]. Several investigators have reported that C. *dubliniensis* isolates have the ability to produce chlamydospores more readily and abundantly than *C. albicans* on rice agar-Tween, Tween 80-oxgall-caffeic acid agar or cornmeal-Tween 80agar [2, 21] and this morphologic characteristic used for differentiating the two species. However, identification of *C. dubliniensis* based on abundance of chlamydospores or other phenotypic features were not been shown to be reproducible in some laboratories [14]. Moreover, assessment of relative abundance of chlamydospores is quite subjective in judgment; hence, this feature alone may not serve as dependable criterion for differentiation of *C. dubliniensis* from *C. albicans*. Recently, several studies reported that *C. dubliniensis* on several media [10, 11, 12, 22] under appropriate growth conditions forms rough colonies due to mycelial growth and abundant chlamydospores, which may use as a species-specific marker for identification of this species [6]. However one of the phenotypic test available for the time been can differentiate both species with 100% sensitivity and specify. In this respect research attempt to find new simple inexpensive phenotypic test to differentiate the two closely related species continue. The new substrates media investigated in this study, showed nearly similar results, and allowed the differentiation between *C. dubliniensis* and *C. albicans* isolates. All *C. albicans* (100%) showed colonies without hyphal fringe and yeast was frequently present including the few strains, which produce few scanty chlamydospores on SSA. This is finding given our new media more advantage with 100% specifity except with flaxseed substrate (82, 5%) specifity for the identification of *C. albicans*. All *C. dubliniensis* isolates give sensitivity from (78.6-96.4%) and grew as rough colonies producing hyphal fringes and abundant chlamydospores. This is may be attribute to the fact that chlamydospore production by *C. dubliniensis* depend on strains variation. This is well established that its isolates present great phenotypic variability, what hinders the standardization of the identification techniques, [1, 6, 7, 8, 13, 14]. For that, reason the search for new and more refined method are advantageous and should be stimulated, which can confirmed only by using PCR [23], which are not available in our laboratory. However, in contrast our studies demonstrating that it is possible to use these substrates for a differentiation between these two species, we found that the assay yields a very good sensitivity and specify for the discrimination between *C. dubliniensis* and *C. albicans* similar to other recommended media [6, 10, 11, 12]. Furthermore, these new media substrates were found to useful for the identification of *Cryptococcus neoformans* from other yeast by producing brown pigment colonies [24]. In addition, although there are several media cultivation methods described for the discriminating between *C. dublinie*nsis and *C. albicans* due to abundant chlamydospore formation of the former species. However, some strains of *C. albicans* was found to produces fewer chlamydospores at the end of pseudo hyphae under these growth conditions by many other media even when using SSA [6, 25]. Further studies including a larger number of isolates are necessary to confirm the present results. The results obtained with the new proposed media substrates were the same as those observed with sunflower seed agar, which used here as reference media and even better when rolling out few strains of *C. al* bicans produce few scanty chlamydospores on other media. This media, however, will be more reliable in germ tube-positive Candida producing and it is advisable to confirm the identification with molecular testing.

5. Conclusions

Our findings showed that *C. dubliniensis* could rapidly differentiated from *C. albicans* isolates using new simple and non-expensive media easily obtained and prepared after 24 hours of incubation at 28°C based on chlamydospores production. This method will be also more reliable in germ tube-positive isolates.

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