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Title: *Candida dubliniensis* and *Candida albicans* discrimination by Colony morphotype in Sabouraud-Triphenyltetrazolium Agar.

Article Type: Nota

Keywords: *Candida dubliniensis*; *Candida albicans*; trifeniltetrazolium salt; morphotype

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Abstract: Discriminación entre *Candida dubliniensis* y *Candida albicans* evaluando el morfotipo de las colonias en Agar Sabouraud-trifeniltetrazolium.

Resumen

Antecedentes: *Candida dubliniensis* es una especie del género *Candida* capaz de producir tubos germinativos y clamidoconidios y puede ser identificada erróneamente como *C. 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 costo para ser utilizados en laboratorios de micología de baja complejidad.

Objetivo: Evaluar el morfotipo de las colonias de levaduras en agar Sabouraud-trifeniltetrazolium como una herramienta para diferenciar *C. dubliniensis* de *C. albicans*.

Método: Se evaluó la morfología de 126 cepas de *C. albicans* y *C. dubliniensis* y los resultados fueron comparados con los obtenidos utilizando métodos moleculares.

Resultados y Conclusión: El método evaluado mostró 100% de sensibilidad y especificidad cuando se evaluó el color y la presencia o ausencia de un gran halo de micelio blanco.

Abstract

Background: *Candida dubliniensis* is a germ tube and chlamydoconidia producing *Candida* species that may be misidentified as *C. 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.

Objective: To evaluate colony morphotype on Sabouraud-triphenyltetrazolium agar as a tool for *C. dubliniensis* and *C. albicans* differentiation.

Methods: The morphology of 126 strains *C. albicans* and *C. dubliniensis* strains were evaluated and compared with identification by molecular methods.

Results and conclusion: The method showed 100% sensitivity and specificity when color and the presence or absence of large white mycelial halo was evaluated.

3 de Febrero, 2013

**Dr. Guillermo Quindós,
editor RIAM**

Me dirijo a Usted con el fin de adjuntar las modificaciones requeridas para que el manuscrito "Candida dubliniensis and Candida albicans discrimination by Colony morphotype in Sabouraud-Triphenyltetrazolium Agar." (Ref. RIAM-D-13-00090R1) sea aceptado para publicarse en la revista.
Las modificaciones antes mencionadas incluyen:

- La bibliografía fue adaptada a los requerimientos de la Revista cambiando la forma en que se describían los autores.
- El resumen y abstract fue separado en secciones como se solicita.

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4 Discriminación entre *Candida dubliniensis* y *Candida*
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6 *albicans* evaluando el morfotipo de las colonias en Agar
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12 8 Guillermo Garcia-Effron^{1,2#}.

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Abstract

Background: *Candida dubliniensis* is a germ tube and chlamydoconidia producing *Candida* species that may be misidentified as *C. 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.

Objective: To evaluate colony morphotype on Sabouraud-triphenyltetrazolium agar as a tool for *C. dubliniensis* and *C. albicans* differentiation.

Methods: The morphology of 126 strains *C. albicans* and *C. dubliniensis* strains were evaluated and compared with 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.

Conclusion: Colony morphotype on Sabouraud-triphenyltetrazolium agar would be considered as a new tool to differentiate *C. dubliniensis* and *C. albicans*.

Besuimen

Antecedentes: *Candida dubliniensis* es una especie del género *Candida* capaz de producir tubos germinativos y clamidoconidios y puede ser identificada erróneamente como *C. 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 costo para ser utilizados en laboratorios de micología de baja complejidad.

Objetivo: Evaluar el morfotipo de las colonias de levaduras en agar Sabouraud-triféniltetrazolium como una herramienta para diferenciar *C. dubliniensis* de *C. albicans*.

1 64 Método: Se evaluó la morfología de 126 cepas de *C. albicans*
2 65 y *C. dubliniensis* y los resultados fueron comparados con
3 66 los obtenidos utilizando métodos moleculares.
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5 67 Resultados: El método evaluado mostró 100% de sensibilidad
6 68 y especificidad cuando se evaluó el color y la presencia o
7 69 ausencia de un gran halo de micelio blanco.
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9 70 Conclusión: La evaluación del morfotipo de las colonias en
10 71 agar Sabouraud-triphenyltetrazolium puede ser utilizada
11 72 como una nueva herramienta para diferenciar *C. dubliniensis*
12 73 y *C. albicans*.
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78 **Text**

1 79 *Candida dubliniensis* is a germ tube and chlamydoconidia
2 80 producing *Candida* specie described in 1995 [15,17] that may
3 81 be misidentified as *C. albicans* [7]. Firstly, *C.*
4 82 *dubliniensis* was associated with oropharyngeal candidiasis
5 83 in HIV+ population [15,17] but later it was also isolated
6 84 from HIV- patients and from different body sites and fluids
7 85 including blood, urine, etc. [3,8,10]. Molecular-based
8 86 methods are the most reliable techniques for *C. albicans*
9 87 and *C. dubliniensis* differentiation [2,12,16]. However,
10 88 accurate, quick and inexpensive phenotypic tests are needed
11 89 to be used in low-complexity mycology laboratories.
12 90 Different tests were proposed and have been used as
13 91 screening methods including: differences in carbohydrate
14 92 assimilation, growth capacity at 42°C, 45°C and in
15 93 hypertonic media, clustered chlamydoconidia production,
16 94 etc. [1,4-6,9,13,14,16]. Any of the described methods are
17 95 100% specific for *C. dubliniensis* and *C. albicans*
18 96 differentiation. In this work, colony morphotype on
19 97 Sabouraud-triphenyltetrazolium agar (STA) was evaluated as
20 98 a tool for *C. dubliniensis* and *C. albicans* differentiation.
21 99 Colony morphotype data were obtained from 110 *C. albicans*
22 100 and 12 *C. dubliniensis* clinical strains isolated from
23 101 different sources (blood, urine, vulvovaginal infections,
24 102 etc.). *C. albicans* ATCC 90028, *C. albicans* ATCC 36082, *C.*
25 103 *albicans* Sc5314 and *C. dubliniensis* NCPF 3949 were included
26 104 in the study as control strains. All the strains were
27 105 identified by phenotypic [5] and molecular methods [3,12].
28 106 The molecular identification was considered the gold
29 107 standard when the specificity of the STA method was
30 108 evaluated.

31 109 STA was prepared as follows. Firstly, a basal medium was
32 110 prepared dissolving 10 g of peptone, 20 g of glucose and 20
33 111 g of agar (all from Britania Laboratory, Argentina) in 990
34 112 ml of distilled water. The basal media was sterilized at
35 113 121°C for 15 minutes. Meanwhile, a stock solution of 2,3,5-
36 114 triphenyltetrazolium chloride (TTZ) (Sigma-Aldrich
37 115 Argentina) was prepared dissolving 1 g in 100 ml of
38 116 distilled water. This last solution was filter-sterilized.

117 Finally, the basal medium was cooled at 55°C in a water
118 bath and 10 ml of the TTZ stock solution was added reaching
119 a 0.1 g/l TTZ final concentration. Then, the media was
120 plated in 90 mm Petri dishes.

121 For the morphotype evaluation, fresh 24-h cultures in
122 Sabouraud dextrose agar (peptone 1%, glucose 2%, agar 2%)
123 were used. Four to five colonies were picked to obtain a
124 0.5 McFarland inoculum in water. Afterwards, 3 µl of each
125 cell suspension were inoculated in STA plates (16 strains
126 per plate), incubated for 7 days at 28°C. The morphotype
127 data collected included color (pink or violet), presence or
128 absence of mycelial halo and colony texture (smooth or
129 rough). The morphotypes were named using the nomenclature
130 published by Quindos et al. [11]. The experiments were
131 performed in triplicates in three separate days.

132 The 113 *C. albicans* strains showed three different
133 morphotypes: Pink with no mycelial halo (n=65, 57.5%), Pink
134 with mycelial halo (n=39, 34.5%) and violet with no
135 mycelial halo (n=9, 8%). On the other hand, all the *C.*
136 *dubliniensis* (n=13) produced violet colonies with large
137 white mycelial halo (Figure 1). Colony texture was not
138 informative since *C. albicans* and *C. dubliniensis* showed
139 both phenotypes. The morphotypes were reproducible even
140 after a -86°C storage and multiple subcultures (data not
141 shown).

142 The reduction of TTZ has been used as an aid for *Candida*
143 identification since the 1980's [5]. In 1992, Quindos et
144 al. reported the use of colony morphotype on SAT as a
145 *Candida* species identification tool [11]. In that report,
146 93.67% and 3.6% of the strains identified phenotypically as
147 *C. albicans* were pink or violet, respectively and only 2%
148 of the *C. albicans* isolates showed violet colonies with
149 mycelial halo. It would be possible that those results
150 represented a 2% incidence of *C. dubliniensis* in the yeast
151 collection used. This incidence was not reported since *C.*
152 *dubliniensis* was proposed as separate specie three years
153 later.

154 In 1998, the reduction of TTZ was suggested as an useful
155 tool for the differentiation of *C. albicans* from *C.*

156 *dubliniensis* by Velegraky and Logotheti [19]. However, in
157 the aforementioned work, other basal media was used; there
158 is no description of the number of isolates tested and how
159 they identify the strains as *C. albicans* or *C.*
160 *dubliniensis*. Accordingly, the specificity and sensitivity
161 evaluation was not performed. Also, Velegraky and Logotheti
162 evaluated the color of the colony as the only
163 characteristic. In the present study we evaluated three
164 morphotype characteristics on STA as tool for *C.*
165 *dubliniensis* - *C. albicans* differentiation using a total of
166 126 strains (122 isolated from different clinical sources)
167 identified by two different molecular-based techniques. The
168 method described here has 100% specificity and sensitivity
169 when color and mycelial halo is considered together (all
170 the *C. dubliniensis* showed violet colonies with large white
171 mycelial halo). It has to be highlighted that we do not
172 propose this test as a primary isolation media capable to
173 distinguish *C. dubliniensis* and *C. albicans* directly from
174 biological samples since other *Candida* species (e.g. *C.*
175 *tropicalis*) are also able to reduce TTZ salts [18]. We
176 suggest STA media as and inexpensive, easy to perform
177 differentiation tool for *C. albicans* and *C. dubliniensis*
178 after a germ tube evaluation or starting from a green
179 colony in Chromagar® *Candida*. Also, the seven days of
180 incubation needed to see the result is an important
181 disadvantage as a useful method in a clinical laboratory.
182 However, STA media would be useful for large epidemiology
183 studies in reference labs since 16 strains could be studied
184 per each 90 mm. Petri dish.

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

190

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195 Consejo Nacional de Investigaciones Científicas y Técnicas
196 (CONICET).
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5 30 **Abstract**

6
7 31 Background: *Candida dubliniensis* is a germ tube and
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10 34 the most reliable techniques for *C. albicans* and *C.*
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15 39 Objective: To evaluate colony morphotype on Sabouraud-
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28 52 to differentiate *C. dubliniensis* and *C. albicans*.

29 53 | **Formatted: English (U.S.)**

30 54 **Resumen**

31 55 Antecedentes: *Candida dubliniensis* es una especie del
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33 57 clamidoconidios y puede ser identificada erróneamente como
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41 65 para diferenciar *C. dubliniensis* de *C. albicans*.

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2 **79 Text**
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3 118 Finally, the basal medium was cooled at 55°C in a water
4 bath and 10 ml of the TTZ stock solution was added reaching
5 a 0.1 g/l TTZ final concentration. Then, the media was
6 plated in 90 mm Petri dishes.
7

8 122 For the morphotype evaluation, fresh 24-h cultures in
9 Sabouraud dextrose agar (peptone 1%, glucose 2%, agar 2%)
10 were used. Four to five colonies were picked to obtain a
11 0.5 McFarland inoculum in water. Afterwards, 3 µl of each
12 cell suspension were inoculated in STA plates (16 strains
13 per plate), incubated for 7 days at 28°C. The morphotype
14 data collected included color (pink or violet), presence or
15 absence of mycelial halo and colony texture (smooth or
16 rough). The morphotypes were named using the nomenclature
17 published by Quindos et al. [11]. The experiments were
18 performed in triplicates in three separate days.
19

20 133 The 113 *C. albicans* strains showed three different
21 morphotypes: Pink with no mycelial halo (n=65, 57.5%), Pink
22 with mycelial halo (n=39, 34.5%) and violet with no
23 mycelial halo (n=9, 8%). On the other hand, all the *C.*
24 *dubliniensis* (n=13) produced violet colonies with large
25 white mycelial halo (Figure 1). Colony texture was not
26 informative since *C. albicans* and *C. dubliniensis* showed
27 both phenotypes. The morphotypes were reproducible even
28 after a -86°C storage and multiple subcultures (data not
29 shown).
30

31 143 The reduction of TTZ has been used as an aid for *Candida*
32 identification since the 1980's [5]. In 1992, Quindos et
33 al. reported the use of colony morphotype on SAT as a
34 *Candida* species identification tool [11]. In that report,
35 93.67% and 3.6% of the strains identified phenotypically as
36 *C. albicans* were pink or violet, respectively and only 2%
37 of the *C. albicans* isolates showed violet colonies with
38 mycelial halo. It would be possible that those results
39 represented a 2% incidence of *C. dubliniensis* in the yeast
40 collection used. This incidence was not reported since *C.*
41 *dubliniensis* was proposed as separate specie three years
42 later.
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44 155 In 1998, the reduction of TTZ was suggested as an useful
45 tool for the differentiation of *C. albicans* from *C.*
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3 157 *dubliniensis* by Velegraky and Logotheti [19]. However, in
4 158 the aforementioned work, other basal media was used; there
5 159 is no description of the number of isolates tested and how
6 160 they identify the strains as *C. albicans* or *C.
7 dubliniensis*. Accordingly, the specificity and sensitivity
8 161 evaluation was not performed. Also, Velegraky and Logotheti
9 162 evaluated the color of the colony as the only
10 163 characteristic. In the present study we evaluated three
11 164 morphotype characteristics on STA as tool for *C.
12 dubliniensis* - *C. albicans* differentiation using a total of
13 165 126 strains (122 isolated from different clinical sources)
14 166 identified by two different molecular-based techniques. The
15 167 method described here has 100% specificity and sensitivity
16 168 when color and mycelial halo is considered together (all
17 169 the *C. dubliniensis* showed violet colonies with large white
18 170 mycelial halo). It has to be highlighted that we do not
19 171 propose this test as a primary isolation media capable to
20 172 distinguish *C. dubliniensis* and *C. albicans* directly from
21 173 biological samples since other *Candida* species (e.g. *C.
22 tropicalis*) are also able to reduce TTZ salts [18]. We
23 174 suggest STA media as and inexpensive, easy to perform
24 175 differentiation tool for *C. albicans* and *C. dubliniensis*
25 176 after a germ tube evaluation or starting from a green
26 177 colony in Chromagar® *Candida*. Also, the seven days of
27 178 incubation needed to see the result is an important
28 179 disadvantage as a useful method in a clinical laboratory.
29 180 However, STA media would be useful for large epidemiology
30 181 studies in reference labs since 16 strains could be studied
31 182 per each 90 mm. Petri dish.

40
41 186 **Figure 1 legend:** STA plates inoculated with 3 µl of 0.5 Mc
42 187 Farland yeast cell suspensions. Arrow heads in the upper
43 188 left photography show *C. dubliniensis* isolates identified
44 189 by molecular methods (3,12). The other three photos show
45 190 different *C. albicans* morphotypes.
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Figura (Figure)

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