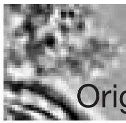

Original

Detection of *Candida dubliniensis* in patients with candidiasis in Caracas, Venezuela

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Summary

Candida species are responsible for 80% of all nosocomial fungal infections. In 1995 a new yeast species was described, *Candida dubliniensis* which shares with *Candida albicans* characteristics. We have studied 109 yeast isolates identified as *C. albicans* to investigate the presence of *C. dubliniensis* by microbiological studies and PCR using DUBR/DUBF primers. Positive results using microbiological tools were between 90 and 98%. Two morphological and physiological of the 80 DNA examined samples (2.5%) showed a PCR product of 288 bp which allow the identification of *C. dubliniensis*. This is the first report in Venezuela of identification of this species using a PCR approach.

Key words

Candida dubliniensis, *Candida albicans*, Candidiasis, Chlamydospore, CHROMagar, PCR, ß-glucosidase

Detección de *Candida dubliniensis* en pacientes con candidosis en el area metropolitana de Caracas, Venezuela

Resumen

Las levaduras de *Candida* causan el 80% de las infecciones nosocomiales por hongos. En 1995, se describió una nueva especie, *Candida dubliniensis*, que comparte con *Candida albicans* características morfológicas y fisiológicas. Estudiamos 109 aislamientos identificados como *C. albicans*, para detectar la presencia de *C. dubliniensis*, por estudios microbiológicos y PCR con cebadores DUBR/DUBF. Las pruebas microbiológicas resultaron positivas entre 90-98%. Dos de los 80 ADN estudiados (2,5%) mostraron un producto de 288 pb, La prueba de PCR utilizada permitió la identificación definitiva de *C. dubliniensis*. Este es el primer reporte en Venezuela de identificación de esta especie por PCR.

Palabras clave

Candida dubliniensis, Candida albicans, Candidiasis, Clamidospora, CHROMagar, PCR, ß-glucosidasa

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Candidiasis is an acute or chronic infection produced by a fungus of the *Candida* genus, generally limited to the skin and mucous membranes, but it could produce a serious systemic disease [14].

The new species *Candida dubliniensis* described by Sullivan et al. [33] shares with *Candida albicans* morphological and physiological characteristics. To differentiate these species, there have been used arrays of controversial criteria [5,24,25,27,30,31,33]. During the last years molecular biology assays have been developed as an aid for the differentiation of these closely related species. For instance, Kurzai et al. [15] have used a rapid PCR identification system that target the structural *C. dubliniensis* gene *PHR1*. Also, the study of the sequence alignment of introns associated to ACTI (actin protein) of both species and the observation that they differ by 16.6%, suggest that this divergence of sequences can be used to identify *C. dubliniensis* [7].

The presence of *C. dubliniensis* has been confirmed in several countries [1-3,8,9,12,20,29,34]. In Venezuela, the first report was made by using phenotypic assays [16]. Another study to differentiate *C. albicans* from *C. dubliniensis* in Maracaibo-Venezuela [19] by means of phenotypic assays, found yeasts suggestive to be *C. dubliniensis* but no confirmation was made using molecular technology such as polymerase chain reaction (PCR). These studies coincide with other published reports on the importance of DNA analysis for the proper identification of *C. dubliniensis* [7,15,33].

Due to discrepancies found on previous studies and the few available published data in Venezuela, our objective was to identify *C. dubliniensis* by the combination of microbiological assays and molecular biology.

We studied 109 isolates recovered from clinical specimens from several health facilities in Caracas-Venezuela, previously identified as *C. albicans* by the fast method of chlamydospore production. As controls for *C. albicans* we used isolates of reference B385 (CDC Atlanta) and for *C. dubliniensis* isolate Cd36, kindly donated by Dr. Derek Sullivan, Dublin, Ireland.

The methods used for the microbiological characteristics were germ tube and chlamydospore production [4,17], growth at 42 and 45 °C [5,33], growth in the medium CHROMagar–*Candida* (CHROMagar-Microbiology, France) [5,21] and ß-glucosidase assay [33].

The genomic assay was carried out by means of a PCR, using specific primers from the ACT1 intron of *C. dubliniensis* (DUBR/DUBF). The obtained primers amplified a DNA segment of approximately 288 pb by PCR assay [7]. DNA extraction was made according to previously described technique [35]. The primers were tested in a total of 80 DNA extracted from previously identified *Candida albicans* strains. As positive control the DNA from Cd36 control strain was used.

For PCR, we used the LPU commercial compound (Fundaim-Venezuela) containing 10 mM Tris HCl, 50 mM KCl, 0.1 % triton x 100, 2 mM Mg_2Cl, 0.2 mM of each of the dNTPs and 1.25U polymerase in 12.5 µl. We added 10 ng of each primer (DUBR/DUBF), 2 µl of the DNA to be tested completing with ultra-pure sterile water up to 25 µl. Amplification was made as previously described [35] in a thermocycler PTC-100 (MJ Research INC, USA). Products were separated in 1% agarose gel and at 75V, stained with ethidium bromide and digitalized by means of the software of the image analyzer FluorS MultiImager (Bio-Rad).

Germ tube production was observed in 94.5% of isolates (Table 1). One of the *C. dubliniensis* identified in this study (*C. albicans* DNA 69) was negative. Literature reports between 5 and 10% of false negatives, which is in agreement with our results [23,26].

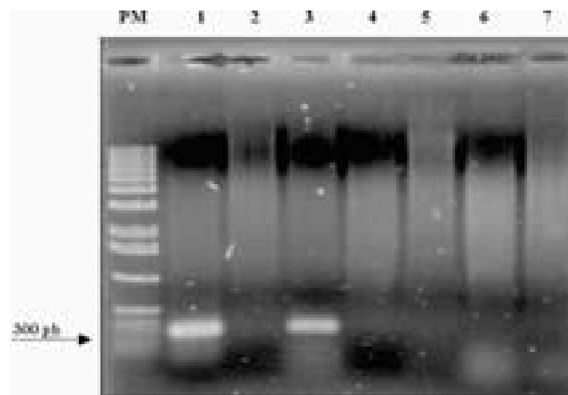

Figure 1. PCR product in agarose 1% gel stained with 0.5 mg/ml Ethidium Bromide using LPU compound (Fundaim-Venezuela) with primers DUBR/DUBF. We observed a band of 288 pb at lanes 1 and 3 corresponding to *C. dubliniensis* Cd36 and *C. dubliniensis* (*C. albicans*-DNA5). Lane 2 corresponds to *C. albicans* B385. PM (1Kb DNA Ladder (Gibco)).

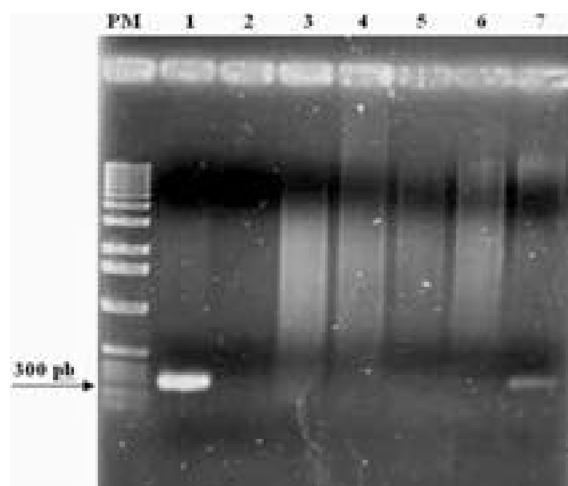

Figure 2. PCR product in agarose 1% gel stained with 0.5 mg/ml Ethidium Bromide using LPU compound (Fundaim-Venezuela) with primers DUBR/DUBF. We observed a band of 288 pb at lanes 1 and 7 corresponding to positive of *C. dubliniensis* (Cd36) and *C. dubliniensis* (*C. albicans*-DNA69). Lanes 2, 3, 4, 5 correspond to *C. albicans*: B385, DNA67, DNA64, DNA65, respectively. Lane 6: blank. PM (1Kb DNA Ladder, Gibco).

Yeasts studied produced chlamydospores in 96.3% of isolates (Table 1). We observed that a low percentage of *C. albicans* (3.7%) did not produce these structures, which is in agreement with previous reports. It has been suggested that this could be due to alterations produced to microorganisms stored for long periods or under chemotherapy [17,22].

The *C. albicans* strains in this study, including those identified as *C. dubliniensis* as well as control isolates, produced solitary and terminal chlamydospores. However, this assay does not discriminate between *C. albicans* and *C. dubliniensis* [25,27,33].

Growth pattern at 42 and 45 °C was similar in 98.2% of yeasts (Table 1). At both temperatures isolate control B385 grew, while Cd36 did not grow. One of the isolates of *C. albicans* (DNA5) identified as *C. dubliniensis* did not grow at 42 or 45 °C, while *C. albicans* (DNA69) also identified as *C. dubliniensis* grew at both temperatures. Growth at 42 and 45 °C was not useful discriminating these two species [6,10,24,27].

Table 1. Positive results of conventional identification tests for *Candida albicans* according to clinical localization.

Clinical localization	Germinal tube		Chlamydospores		Growth at 42 °C		Growth at 45 °C		Total
	n	(%)	n	(%)	n	(%)	n	(%)	
Oral mucosa	54	(100)	54	(100)	53	(98.1)	53	(98.1)	54
Vaginal mucosa	32	(84.2)	37	(97.4)	38	(100)	38	(100)	38
Skin	7	(100)	7	(100)	7	(100)	7	(100)	7
Nails	6	(100)	4	(66.7)	6	(100)	6	(100)	6
Other	4	(100)	3	(75.0)	3	(75.0)	3	(75.0)	4
Total	103	(94.5)	105	(96.3)	107	(98.2)	107	(98.2)	109

Table 2. Color differences in the CHROMagar-*Candida* medium and ß-glucosidase test results for *Candida albicans* according to clinical localization.

Clinical localization	Color differences				ß-glucosidase				Total
	Light green		Purple		Positive		Negative		
	n	(%)	n	(%)	n	(%)	n	(%)	
Oral mucosa	54	(100)	0	(0)	49	(90.74)	5	(9.26)	54
Vaginal mucosa	38	(100)	0	(0)	37	(97.40)	1	(2.60)	38
Skin	7	(100)	0	(0)	7	(100)	0	(0)	7
Nails	2	(33.3)	4	(66.7)	6	(100)	0	(0)	6
Other	3	(75)	1	(25.0)	4	(100)	0	(0)	4
Total	104	(95.4)	5	(4.6)	103	(94.50)	6	(5.50)	109

Table 3. Conventional identification tests and PCR assay for *Candida dubliniensis* and *Candida albicans*.

Isolate	Clinical localization	Germinal tube	Chlamydospores	Growth at 42 °C	Growth at 45 °C	Lactose	Celobiose	Sucrose	Glucose	Maltose	Galactose	CHROMagar	ß-glucosidase	PCR DUBR/DUBF	Final identification
DNA5 *C. albicans*	Oral mucosa	+	+	-	-	-	-	+	+	+	+	Ligh green	-	+	*C. dubliniensis*
DNA69 *C. albicans*	Vaginal mucosa	-	+	+	+	-	-	+	+	+	+	Light green	+	+	*C. dubliniensis*
DNA17 *C. albicans*	Oral mucosa	+	+	+	+	-	-	+	+	+	+	Light green	+	-	*C. albicans*
DNA65 *C. albicans*	Skin	+	+	+	+	-	-	+	+	+	+	Light green	+	-	*C. albicans*
B385 *C. albicans*	ND	+	+	+	+	-	-	+	+	+	+	Light green	+	-	*C. albicans*
3156B *C. albicans*	ND	+	+	+	+	-	-	+	+	+	+	Light green	+	-	*C. albicans*
Cd36 *C. dubliniensis*	Oral mucosa	+	+	-	-	-	-	+	+	+	+	Light green	-	+	*C. dubliniensis*

ND: not determined

Colonies grown in CHROMagar-*Candida* medium were colored as follows: light green 95.4% and purple 4.6% (Table 2). Reference strains B385 and Cd36 produced light green color colonies as well as the two isolates of *C. dubliniensis* identified by PCR assay. With this medium we could not discriminate *C. albicans* from the other yeast in this study. It has been suggested that this medium is of value discriminating between the two species when used in primary growth [5,11,27].

ß-glucosidase production was positive in 94% (Table 2). This assay in controls (Cd36, *C. tropicalis* and *C. guilliermondii*) was negative. The two *C. dubliniensis* identified by PCR, one (*C. albicans*-DNA69) was positive and the other (*C. albicans*-DNA5) was negative for this test. This observation disagrees with reports as to the absence of this enzyme in *C. dubliniensis* [27,31]. Another study reported absence of this enzyme in *C. albicans* [36].

Two of the 80 yeasts studied (2.5%) by PCR assay using DUBR/DUBF primers, showed a product of approximately 288 pb, similar to the band produced by the DNA control of Cd36. The isolates identified as *C. dubliniensis* were *C. albicans*-DNA5 and *C. albicans*-DNA69. DNA of *C. albicans* (B385) did not amplify with the specific primers used (Figures 1 and 2). Yeasts identified *C. albicans*-DNA5 was recovered from mouth and *C. albicans*-DNA69 form vagina, areas where this species has been most frequently isolated [1,9,13,18,28,31-33]. Table 3 includes performance of both isolates identified by PCR assay as *C. dubliniensis*.

We conclude that the molecular biology PCR assay used was the test that permitted the identification of this species. This is the first report of identification of *C. dubliniensis* in Venezuela by PCR assay.

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