

## Rapid Identification of *Candida dubliniensis* by Indirect Immunofluorescence Based on Differential Localization of Antigens on *C. dubliniensis* Blastospores and *Candida albicans* Germ Tubes

JOSEBA BIKANDI,<sup>1</sup> ROSARIO SAN MILLÁN,<sup>1</sup> MARÍA D. MORAGUES,<sup>2</sup> GONTZAL CEBAS,<sup>1</sup>  
MARY CLARKE,<sup>3</sup> DAVID C. COLEMAN,<sup>3</sup> DEREK J. SULLIVAN,<sup>3</sup>  
GUILLERMO QUINDÓS,<sup>1</sup> AND JOSÉ PONTÓN<sup>1\*</sup>

*Departamento de Inmunología, Microbiología y Parasitología, Facultad de Medicina y Odontología,<sup>1</sup> and Departamento de Enfermería I,<sup>2</sup> Universidad del País Vasco, E-48080 Bilbao, Vizcaya, Spain, and Department of Oral Surgery, Oral Medicine and Oral Pathology, School of Dental Science, Trinity College, University of Dublin, Dublin 2, Republic of Ireland<sup>3</sup>*

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**There is a clear need for the development of a rapid and reliable test for the identification of *Candida dubliniensis* and for the discrimination of this species from *Candida albicans*. In the present study we have investigated the potential use of *C. dubliniensis*-specific antigens as a basis for its identification. We produced an anti-*C. dubliniensis* serum which, after adsorption with *C. albicans* blastospores, was found to differentially label *C. dubliniensis* isolates in an indirect immunofluorescence test. In this test, the antiserum reacted with blastospores and germ tubes of *C. dubliniensis* and with blastospores of *Candida krusei* and *Rhodotorula rubra* but did not react with blastospores of several other *Candida* species including *C. albicans*. The antiserum also reacted with *C. albicans* germ tubes. The anti-*C. dubliniensis* adsorbed serum reacted with specific components of 25, 28, 37, 40, 52, and 62 kDa in the *C. dubliniensis* extract and with a variety of antigens from other yeast species. The antigens from non-*C. dubliniensis* yeasts showing reactivity with the anti-*C. dubliniensis* adsorbed serum are mostly expressed within the cell walls of these yeast species, and this reactivity does not interfere with the use of the anti-*C. dubliniensis* adsorbed serum in an indirect immunofluorescence test for the rapid identification of *C. dubliniensis*.**

Oral candidiasis is one of the most prevalent clinical presentations of *Candida* infections, particularly in human immunodeficiency virus (HIV)-infected individuals. Although *Candida albicans* remains the most common cause of oral candidiasis, the incidence of disease caused by other species of *Candida*, including *Candida krusei*, *Candida tropicalis*, and *Candida glabrata*, has been increasing steadily (4, 5). The reason(s) for this epidemiological change is not clear; however, it has been suggested that the reduced susceptibility of these species to commonly used antifungal agents, such as fluconazole, may have led to their selection. Furthermore, different groups of researchers have reported the recovery of atypical *C. albicans* isolates from HIV-infected patients (2, 10, 20, 22). Some of these organisms have been shown to belong to a recently described species, *C. dubliniensis*, which appears to be mainly associated with oral disease in HIV-infected individuals (4, 23, 25). First identified as a new species in Ireland in 1995, this organism has since been identified in laboratories around the world (14, 23, 24). Although the majority of isolates so far examined have been associated with oral candidiasis and HIV infection, isolates have also been recovered from other anatomical sites and from healthy individuals (4, 11, 24).

In order to investigate the significance and incidence of *C. dubliniensis* in clinical disease and to determine the reasons for

its recent emergence, an in-depth epidemiological analysis of this species must be performed. However, before this can occur a rapid and simple means of identification of *C. dubliniensis* must be made available. The development of such a technique has been hampered by the very close phenotypic and genotypic relationships between *C. dubliniensis* and *C. albicans* (24, 25). Indeed, the close similarity between these species has led to the misidentification of isolates of *C. dubliniensis* as *C. albicans* (4). At present, the most accurate means of differentiating between isolates of the two species requires the use of molecular biology-based techniques such as DNA fingerprinting with repetitive sequence-containing DNA probes, PCR, or pulsed-field gel electrophoresis (3, 4, 25). However, these techniques are not readily applicable for use with the large numbers of isolates regularly encountered in clinical mycology laboratories. Several phenotype-based methods for the identification of *C. dubliniensis* isolates have been described. The diagnostic characteristics used in these methods include colonial coloration on differential media, such as CHROMagar *Candida*, atypical carbohydrate assimilation profiles with commercially available kits, such as the API ID 32C system, and a lack of  $\beta$ -glucosidase activity (3, 4, 24). However, there are drawbacks with many of these techniques, since they can be unreliable and/or time-consuming (14, 21, 24). Recently, it was shown that *C. dubliniensis* can be readily differentiated from *C. albicans* on the basis of its inability to grow at 45°C (14). Although easy to perform, this test requires that isolates be incubated for 24 to 48 h before the isolates can be discriminated.

In order to facilitate the development of a rapid and reliable test for the identification of *C. dubliniensis* from clinical spec-

\* Corresponding author. Mailing address: Departamento de Inmunología, Microbiología y Parasitología, Facultad de Medicina y Odontología, Universidad del País Vasco, Apartado 699, E-48080 Bilbao, Vizcaya, Spain. Phone: 4 464 7700, ext. 2746. Fax: 4 464 9266. E-mail: oipposaj@lg.ehu.es.

TABLE 1. Reference yeast strains and their reactivities by indirect immunofluorescence with the rabbit hyperimmune serum raised against *C. dubliniensis* NCPF 3949, the serum adsorbed with *C. albicans*<sup>a</sup> blastospores, and the anti-*C. dubliniensis* NCPF 3949 cell wall surface-specific serum

Species	Strain name <sup>b</sup>	Reactivity		
		Unadsorbed antiserum	Adsorbed antiserum	Surface-specific antiserum
<i>Candida dubliniensis</i>	NCPF 3949, CD57 <sup>c</sup>	+	+	+
<i>Candida albicans</i>	NCPF 3153, NCPF 3156, NCPF 3116, NCPF 3119, NCPF 3155, NCPF 3254, ATCC 60458, ATCC 64548, ATCC 76615, ATCC 90029	+	—	—
<i>Candida stellatoidea</i> type I	ATCC 11006	+	—	—
<i>Candida stellatoidea</i> type II	ATCC 20408 <sup>d</sup>	+	—	—
<i>Candida glabrata</i>	NCPF 3203, ATCC 90030, UPV94387, UPV94308	+	—	—
<i>Candida guilliermondii</i>	NCPF 3099, ATCC 22995, ATCC 58070	+	—	—
<i>Candida krusei</i>	NCPF 3100, UPV94823, UPV94813, UPV95773	+	+/-	+/-
<i>Candida parapsilosis</i>	NCPF 3103, 3104, ATCC 90018	+	—	—
<i>Candida tropicalis</i>	NCPF 3111, UPV94800, UPV94840, UPV96080	+	—	—
<i>Rhodotorula rubra</i>	UPV94921, UPV95093, UPV95487	+	+	+
<i>Trichosporon beigelii</i>	UPV97398	+	—	—
<i>Saccharomyces cerevisiae</i>	NCPF 3191, CECT1478, CECT1678	+	—	—
<i>Cryptococcus neoformans</i>	ATCC 90112, UPV94448, UPV94450	+	—	—

<sup>a</sup> *C. albicans* NCPF 3153.

<sup>b</sup> The reference strains used in this study were obtained from the British National Collection of Pathogenic Fungi, Bristol, United Kingdom (NCPF); the American Type Culture Collection, Rockville, Md. (ATCC); the Colección Española de Cultivos Tipo, Valencia, Spain (CECT); the Universidad del País Vasco strain collection, Bilbao, Spain (UPV); and the Dublin University strain collection (CD).

<sup>c</sup> CD57 is a vaginal isolate of *C. dubliniensis* (11).

<sup>d</sup> ATCC 20408 is listed in the American Type Culture Collection catalog under *C. albicans* strains.

imens, the presence of antigenic differences between *C. dubliniensis* and *C. albicans* was investigated. In this study, a rabbit polyclonal anti-*C. dubliniensis* antiserum was used to identify cell wall antigens specific for *C. dubliniensis*. The antigenic composition of *C. dubliniensis* was found to be very similar to that of *C. albicans*. However, the use of the anti-*C. dubliniensis* antiserum adsorbed with *C. albicans* blastospores allowed a clear-cut differentiation between *C. dubliniensis* and *C. albicans* by indirect immunofluorescence.

#### MATERIALS AND METHODS

**Yeast strains and clinical isolates.** The reference strains used in this study are listed in Table 1, and the clinical isolates of *C. dubliniensis* and *C. albicans* used in this study are listed in Table 2.

**Culture conditions and preparation of antigens.** *C. dubliniensis* and *C. albicans* strains were routinely grown in medium 199 (Sigma Chemical Co., St. Louis, Mo.) as described previously (17). Briefly, 48-h-old blastospores grown at 24°C on glucose-yeast extract-agar plates were transferred to Erlenmeyer flasks containing medium 199 at  $5 \times 10^7$  blastospores/ml, and the flasks were incubated at 24°C for 18 h in a rotary shaker set at 100 rpm. Following incubation, the blastospores were harvested by centrifugation, inoculated into fresh medium, and incubated with shaking as before at 24°C for 24 h to obtain blastospores. The same conditions were used to obtain blastospores of the other yeast species studied. *C. albicans* germ tubes were induced by incubation of similar cultures in medium 199 at 37°C for 4 h. Since *C. dubliniensis* failed to produce germ tubes under these conditions, they were obtained by incubation in horse serum (9). The cell walls of *C. dubliniensis* and the other yeast species studied were extracted in the presence of dithiothreitol (DTT; Sigma) as reported previously (15). To obtain formalin-killed blastospores, the cells of the different *Candida* species were resuspended in a 4% (vol/vol) formaldehyde solution in phosphate-buffered saline (PBS) and were incubated at 4°C for 18 h. Assessment of the viability of the cells was determined by incubating the suspension on Sabouraud agar plates (Difco, Detroit, Mich.).

**Antisera.** Two separate female New Zealand White rabbits with an initial weight of from 2 to 2.5 kg were subcutaneously inoculated with  $5 \times 10^8$  formalin-killed cells of *C. dubliniensis* NCPF 3949 (oral isolate and type strain) (25) and *C. dubliniensis* CD57 (vaginal isolate) (11), respectively, which had been grown at 37°C and resuspended in 0.5 ml of saline and 0.5 ml of Freund's adjuvant. Immunizations were repeated at weekly intervals. Preimmune sera were obtained from each rabbit before the immunization, and hyperimmune sera were obtained 5 weeks after the start of the immunization.

Aliquots of the hyperimmune anti-*C. dubliniensis* serum obtained from each rabbit were each adsorbed with *C. albicans* NCPF 3153 blastospores by previously described methods (17). Briefly, immune sera were mixed and incubated

with an equal volume of a formalin-killed blastospore suspension in saline ( $10^{10}$  blastospores/ml) for 2 h at room temperature. After incubation, the suspension was centrifuged and the supernatant was recovered. This process was repeated up to three times. Additionally, antibodies directed against *C. dubliniensis* cell wall surface components were obtained by a modification of a previously described method (15). Briefly, 0.5 ml of the anti-*C. dubliniensis* NCPF 3949 serum adsorbed three times with *C. albicans* blastospores was incubated with  $10^{11}$  formalin-killed *C. dubliniensis* NCPF 3949 blastospores for 2 h at room temperature in PBS buffer. Unattached serum proteins were removed by three washes in PBS, and the attached antibodies were then eluted by treatment with 2.5 M NaI in PBS for 1 h at room temperature. Blastospores were harvested by centrifugation, and the supernatant was dialyzed against PBS for 24 h at 4°C.

**Immunofluorescence.** Indirect immunofluorescence assay (IFA) was carried out as described previously (1). Briefly, the blastospores from the different yeast species studied were grown on Sabouraud agar plates for 48 h at 24 or 37°C, resuspended in PBS at a cell density of  $10^6$  cells/ml, and placed on Teflon-coated immunofluorescence slides. The slides were incubated with the anti-*C. dubliniensis* serum diluted 1:5 in PBS supplemented with Evans blue (0.05% [wt/vol]) and Tween 20 (0.05% [vol/vol]) and washed, and the reacting antibodies were revealed by incubation with biotin-conjugated anti-rabbit immunoglobulin G (Sigma), followed by incubation with fluorescein isothiocyanate-conjugated ExtrAvidin (Sigma). Slides were mounted with carbonate-glycerol mounting fluid and examined with an epifluorescence microscope.

**Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting.** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of yeast cell wall extracts prepared in the presence of DTT was performed by the method of Laemmli (8) in a minigel system (Bio-Rad Laboratories, Richmond, Calif.). Electrophoresis was carried out in 10% (wt/vol) gels at 200 V. Subsequently, the proteins contained in the gels were electrophoretically transferred to a nitrocellulose membrane (Bio-Rad) with a fast blot system (Biometra, Göttingen, Germany). After the transfer, the nitrocellulose membranes were blocked in 10% (wt/vol) nonfat dry milk in Tris-buffered saline, incubated with the anti-*C. dubliniensis* sera, and finally incubated again with peroxidase-conjugated anti-rabbit immunoglobulin G antibodies (Sigma). Immunoreactive bands were visualized with 4-chloro-1-naphthol by standard procedures.

#### RESULTS

**Reactivities of the anti-*C. dubliniensis* sera by indirect immunofluorescence.** The reactivities of the preimmune sera and the hyperimmune antisera from rabbits immunized with *C. dubliniensis* NCPF 3949 and *C. dubliniensis* CD57 were first studied by IFA with reference strains of different yeast species, including several *Candida* species, *Trichosporon beigelii*, *Saccharomyces cerevisiae*, *Cryptococcus neoformans*, and *Rhodotorula*

TABLE 2. *C. dubliniensis* and *C. albicans* clinical isolates

Species and country of isolation	No. of isolates <sup>a</sup>	Specimen	HIV status of subjects sampled	Reference or source
<i>C. dubliniensis</i>				
Argentina	1	Oral cavity	+	23
Australia	5	Oral cavity	+	10, 25
Belgium	5	Oral cavity	+	14
Canada	6	Oral cavity	+	14
Finland	1	Oral cavity	+	14
Germany	4	Oral cavity	+	14
Greece	1	Oral cavity	+	14
Ireland	23	Oral cavity	+	4, 25
Ireland	4	Oral cavity	-	4
Ireland	4	Vaginal	-	11, 14
Spain	10	Oral cavity	+	14, this study
Switzerland	6	Oral cavity	+	2, 23
United Kingdom	2	Oral cavity	+	14, 23
United Kingdom	7	Oral cavity	-	14
United Kingdom	4	Miscellaneous <sup>b</sup>	-	14, 23
Total	83			
<i>C. albicans</i>				
Australia	1	Oral cavity	+	25
Hong Kong	6	Oral cavity	+	14
Ireland	12	Oral cavity	+	14
	16	Oral cavity	-	14
United Kingdom	9	Oral cavity	-	14
Total	44			

<sup>a</sup> Each isolate was recovered from a separate individual.

<sup>b</sup> These included one isolate each from a blood culture and fecal, sputum, and postmortem lung specimens.

*rubra* (Table 1). The reactivities of the preimmune sera with the *Candida* species studied were negative. In preliminary experiments, both hyperimmune antisera gave identical results and the antiserum against *C. dubliniensis* NCPF 3949 was selected for further studies. The unadsorbed antiserum reacted with all the yeast species studied, showing a homogeneous reactivity. The reactivity of the anti-*C. dubliniensis* serum adsorbed with formalin-killed *C. albicans* NCPF 3153 blastospores was initially studied with cells grown at 37°C on Sabouraud agar. With this culture medium, all species studied grew as blastospores and the antiserum reacted only with *C. dubliniensis*, *C. krusei*, and *R. rubra*. The expression of antigens reacting with the anti-*C. dubliniensis* adsorbed serum in the *C. dubliniensis* and *R. rubra* strains studied (Table 1) was homogeneous, and most cells showed fluorescence (Fig. 1). However, the reactivity of the adsorbed antiserum with the *C. krusei* strains studied (Table 1) was very heterogeneous, with only 10% of the cells showing reactivity (Fig. 1).

When the yeast species studied (Table 1) were grown at 37°C in medium 199, all species except *C. albicans* grew as blastospores. *C. albicans* strains produced a mixture of blastospores and germ tubes. Since the *C. dubliniensis* strains examined did not produce germ tubes in medium 199, these were obtained by incubation in horse serum. The adsorbed antiserum reacted with blastospores of *C. dubliniensis*, *C. krusei*, and *R. rubra* as well as with *C. dubliniensis* and *C. albicans* germ tubes (Fig. 1). Despite the reactivity of *C. albicans* germ tubes with the anti-*C. dubliniensis* adsorbed serum, the blastospores attached to them remained negative. Adsorption of the anti-*C. dubliniensis* antiserum with *C. albicans* germ tubes resulted in a loss of its reactivity with *C. dubliniensis*. The anti-*C. dubliniensis* cell wall surface-specific serum showed a reactivity by IFA identical to that shown by the anti-*C. dubliniensis* adsorbed serum (Table 1).

The ability of the anti-*C. dubliniensis* adsorbed serum to

differentiate clinical isolates of *C. dubliniensis* from clinical isolates of *C. albicans* by IFA was also confirmed with a group of 83 *C. dubliniensis* and 44 *C. albicans* isolates (Table 2). These isolates were recovered mainly from the oral cavities of patients from disparate geographical locations and had been identified by both phenotypic and genotypic methods (23, 25). A number of isolates from nonoral sources were also studied (Table 2). The antiserum allowed the rapid and precise discrimination of the isolates, since all of the *C. dubliniensis* isolates were positive by immunofluorescence assays with the antiserum, while all of the *C. albicans* isolates were negative.

**Identification of *C. dubliniensis* antigens reactive with the anti-*C. dubliniensis* serum.** By immunoblotting, the unadsorbed anti-*C. dubliniensis* serum reacted with antigens in DTT extracts from both *C. dubliniensis* NCPF 3949 and *C. albicans* NCPF 3153 strains spanning a wide range of molecular weights (Fig. 2A). Most antigenic components present in the extracts from both species had similar molecular masses, although differences in banding intensity were observed for some components. Despite the common reactivity observed between *C. dubliniensis* and *C. albicans*, the antiserum reacted with three components of 25, 35, and 50 kDa in the *C. albicans* extract which were not observed in the *C. dubliniensis* extract, while the *C. dubliniensis* extract contained 52- and 62-kDa components which were not observed in the *C. albicans* extract. The unadsorbed antiserum also reacted with a variety of components in cell wall extracts from the rest of the yeast species studied (data not shown).

After the adsorption with *C. albicans* blastospores, the anti-*C. dubliniensis* serum showed a decrease in reactivity with components in the *C. albicans* extract but not with components in the *C. dubliniensis* extract (Fig. 2B). The adsorbed antiserum showed a higher reactivity with extracts from both *C. dubliniensis* NCPF 3949 and *C. albicans* NCPF 3153 cells grown at 37°C than with extracts from the cells grown at 24°C. Further-



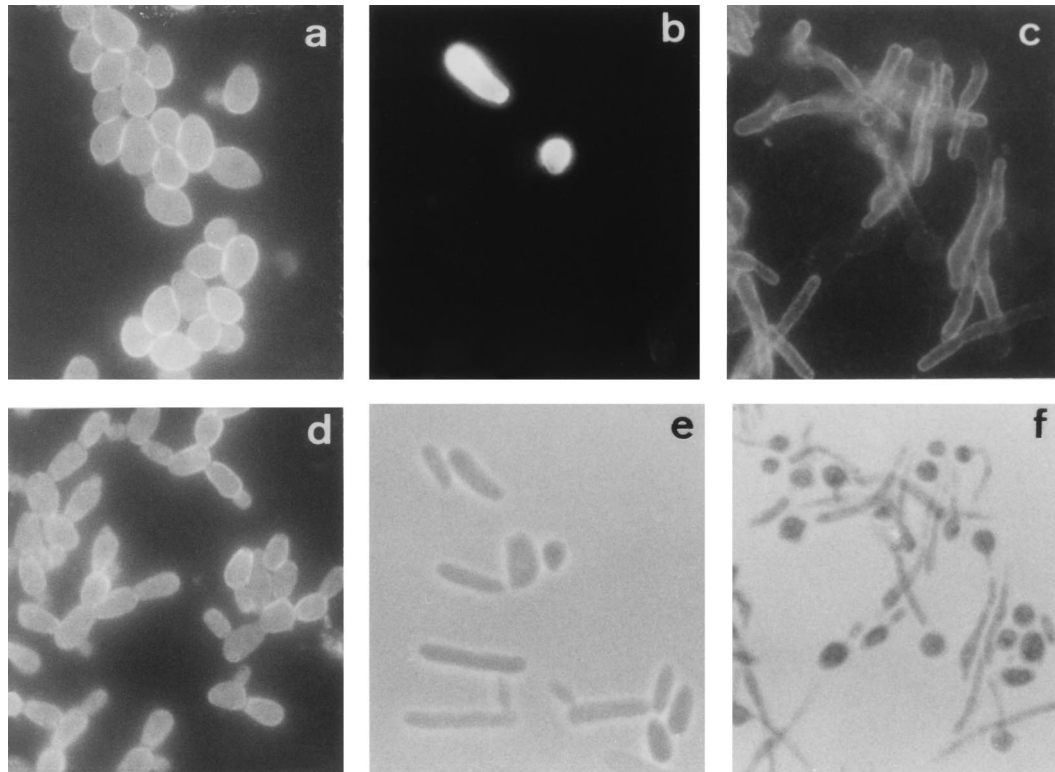


FIG. 1. Immunofluorescence photographs of *C. dubliniensis* NCPF 3949 blastospores (a) and germ tubes (d) stained with the anti-*C. dubliniensis* adsorbed serum. Immunofluorescence (b and c) and phase-contrast (e and f) photographs, respectively, of the same microscopic fields, show *C. krusei* NCPF 3100 blastospores (b and c) and *C. albicans* NCPF 3153 germ tubes (e and f) stained with the anti-*C. dubliniensis* adsorbed serum. Magnification,  $\times 1,000$ .

more, the adsorbed antiserum stained several components of 25, 28, 37, 40, 52, and 62 kDa in the *C. dubliniensis* extracts which were not stained in the *C. albicans* extracts. In contrast, a component of 35 kDa present in the *C. albicans* extracts was not observed in the extracts from *C. dubliniensis* cells grown at

both 24 and 37°C. Similar results were observed when extracts from *C. dubliniensis* CD57 and *C. albicans* ATCC 60458 were examined (data not shown).

The anti-*C. dubliniensis* adsorbed serum also reacted by immunoblotting with antigens from other yeast species (Fig. 3). Major antigens comprised a 66-kDa component in the *C. krusei* NCPF 3100 extract, a 47-kDa component in the *C. tropicalis* NCPF 3111 extract, several components ranging between 29 and 68 kDa in the *Candida parapsilosis* NCPF 3104 extract, two

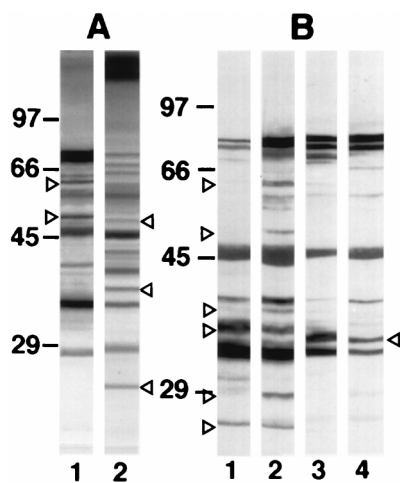


FIG. 2. Western blots of 10% slab gels loaded with extracts from *C. dubliniensis* NCPF 3949 blastospores grown at 37°C (lanes A1 and B2) or 24°C (lane B1) and *C. albicans* NCPF 3153 germ tubes (lanes A2 and B4) or blastospores (lane B3) stained with the anti-*C. dubliniensis* serum (A) or with the anti-*C. dubliniensis* adsorbed serum (B). The molecular masses of standard proteins (in kilodaltons) are listed to the left of the gels. Relevant antigenic bands are indicated by arrowheads.

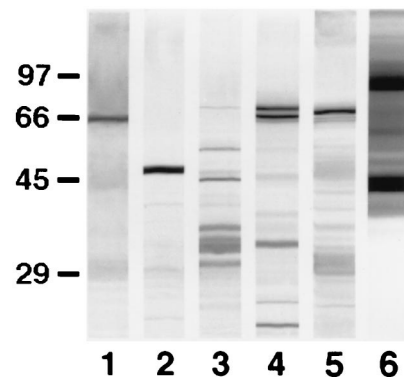


FIG. 3. Western blots of 10% slab gels loaded with extracts from *C. krusei* NCPF 3100 (lane 1), *C. tropicalis* NCPF 3111 (lane 2), *C. parapsilosis* NCPF 3104 (lane 3), *C. guilliermondii* NCPF 3099 (lane 4), *C. glabrata* NCPF 3203 (lane 5), and *R. rubra* UPV94921 (lane 6) stained with the anti-*C. dubliniensis* adsorbed serum. The molecular masses of standard proteins (in kilodaltons) are listed to the left of the gel.

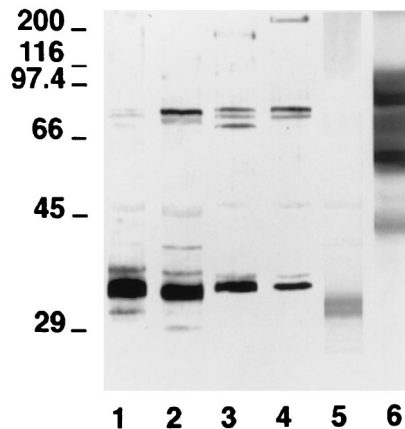


FIG. 4. Western blots of 10% (wt/vol) acrylamide slab gels loaded with extracts from *C. dubliniensis* NCPF 3949 grown at 24°C (lane 1) and 37°C (lane 2), *C. albicans* NCPF 3153 grown at 24°C (lane 3) and 37°C (lane 4), *C. krusei* NCPF 3100 (lane 5), and *R. rubra* UPV94921 (lane 6) stained with the anti-*C. dubliniensis* cell wall surface-specific serum. The molecular masses of standard proteins (in kilodaltons) are listed to the left of the gel.

components of 66 and 68 kDa in the *Candida guilliermondii* NCPF 3099 extract, a component of 67 kDa in the *C. glabrata* NCPF 3203 extract, and several components with molecular masses of >40 kDa in the *R. rubra* UPV94921 extract.

**Identification of *C. dubliniensis* antigens reactive with the anti-*C. dubliniensis* cell wall surface-specific serum.** In order to identify the antigens from the cell wall surface responsible for the fluorescence observed on the *C. dubliniensis* cells, the reactivity of an anti-*C. dubliniensis* cell wall surface-specific serum was studied by immunoblotting with cell wall extracts from *C. dubliniensis*, *C. albicans*, *C. krusei*, and *R. rubra*. The anti-*C. dubliniensis* cell wall surface-specific serum reacted with a component of 33 kDa in the extract from *C. dubliniensis* cells grown at 24°C and with components of 32 and 77 kDa in the extract from *C. dubliniensis* cells grown at 37°C (Fig. 4). This antiserum also stained four components of 33, 70, 74, 77, and 180 kDa in the *C. albicans* blastospore extract and with components of 33, 74, 77, and >200 kDa in the *C. albicans* germ tube extract. The anti-*C. dubliniensis* cell wall surface-specific serum also stained a component of 30 kDa in the *C. krusei* NCPF 3100 extract and several components ranging from 43 to 100 kDa in the *R. rubra* UPV94921 extract. In one experiment, the anti-*C. dubliniensis* cell wall surface-specific serum was diluted in order to identify the most reactive components present in the extracts. Under these conditions, only the component of 33 kDa present in the *C. dubliniensis* NCPF 3949 extract and the components ranging from 60 to 100 kDa present in the *R. rubra* UPV94921 extract were stained (data not shown).

## DISCUSSION

Because species of *Candida* other than *C. albicans* continue to be recovered from patients with infection with increasing frequency, it is clear that routine diagnostic laboratories should be in a position to identify these species rapidly and accurately. Precise identification of these species will allow epidemiological studies to be conducted and may be of benefit in selecting the most effective therapies (12, 13). However, *Candida* species identification can be time-consuming and inaccurate. This is further complicated by the many anomalies in candidal classification (4), particularly with the recent identification of new *Candida* species such as *C. dubliniensis* and *C. fermentatii* (19,

25). As far as possible, species identification techniques should be easy to perform, rapid, reliable, inexpensive, and applicable to a large volume of isolates. In this study, we attempted to develop such a technique for the identification of clinical *C. dubliniensis* isolates. Reliable phenotypic identification of *C. dubliniensis* has proved to be problematic (21, 24). Although *C. dubliniensis* and *C. albicans* are readily distinguishable at the genetic level, the techniques capable of detecting the discriminatory differences are not easily applicable to the large numbers of isolates tested routinely in clinical mycology laboratories. Furthermore, the phenotypic similarities of the two species, including the ability to produce germ tubes and chlamydo-spores, has hampered the development of a rapid phenotypic test that can discriminate between isolates of both species (24).

In this paper, we present evidence, for the first time, of the existence of antigenic differences between *C. dubliniensis* and *C. albicans*, thus providing the basis for the development of a rapid identification test. Anti-*C. dubliniensis* sera were raised in rabbits by inoculation with formalin-killed strains of *C. dubliniensis*. In order to remove antibodies raised against common antigens present on the cell wall surfaces of *C. dubliniensis* and *C. albicans*, the antisera were adsorbed with *C. albicans* blastospores, a technique originally used by Hasenclever and Mitchell (7) to identify the existence of two serotypes in *C. albicans*. These adsorbed sera were then investigated for their ability to identify *C. dubliniensis* in indirect immunofluorescence experiments.

The IFA developed with the anti-*C. dubliniensis* adsorbed serum takes less than 2 h to perform and correctly identified the *C. dubliniensis* reference strains NCPF 3949 and CD57 and a further 83 clinical isolates of *C. dubliniensis* which had been identified previously by both phenotypic and genotypic methods. In all cases, most cells showed fluorescence on the entire cell wall, although differences in reactivity were observed among *C. dubliniensis* isolates. This antiserum showed no indirect immunofluorescence with blastospores of species closely related to *C. dubliniensis*, such as *C. albicans*, *Candida stellatoidea* type I, *C. stellatoidea* type II, and *C. tropicalis*. However, the anti-*C. dubliniensis* adsorbed serum reacted with *R. rubra* and *C. krusei*, the latter showing a heterogeneous reactivity with only 10% of the cells being positive. The reactivity with the anti-*C. dubliniensis* adsorbed serum shown by *C. krusei* and *R. rubra* is not likely to be an important problem when identifying *C. dubliniensis* by indirect immunofluorescence, since *R. rubra* has a distinctive colony color on conventional mycological media and *C. krusei* can be easily differentiated from *C. dubliniensis* by the conventional identification methods used in the clinical microbiology laboratory such as the germ tube or Krusei color tests (6, 9) as well as by growth on CHROMagar *Candida* medium (18).

The anti-*C. dubliniensis* adsorbed serum was also used to identify the antigens responsible for the fluorescence observed on the *C. dubliniensis* cell wall and to study the presence of similar antigens in the cell walls of other yeasts. This antiserum reacted with several cell wall components with molecular masses ranging from 25 to 77 kDa, and when compared with the reactivity observed in the *C. albicans* extract, some of them seemed to be present only in the *C. dubliniensis* extract. Some of the components stained by the anti-*C. dubliniensis* adsorbed serum in the *C. dubliniensis* extract are likely to be located on the *C. dubliniensis* cell wall surface since they were stained with an anti-*C. dubliniensis* cell wall surface-specific serum. Among these antigens, the component of 33 kDa seems to be the most reactive with the anti-*C. dubliniensis* cell wall surface-specific serum, and it may be a good candidate for use in the produc-

tion of monoclonal antibodies to be used in the identification of *C. dubliniensis*.

In addition to the reactivity observed with *C. dubliniensis*, the anti-*C. dubliniensis* adsorbed serum also reacted with other yeast species. On the basis of their reactivities by both indirect immunofluorescence and Western blotting, we can speculate about the distribution of the antigens in the cell wall. Since *C. albicans* blastospores did not react by indirect immunofluorescence with either the anti-*C. dubliniensis* adsorbed serum or the anti-*C. dubliniensis* cell wall surface-specific serum, the antigenic bands observed by Western blotting in the extract from *C. albicans* blastospores stained with these antisera must be located in the inner layers of the cell wall. However, the antigen of >200 kDa observed in the *C. albicans* germ tube extracts is likely to be located on the germ tube cell wall surface since the cell wall of *C. albicans* germ tubes is stained by indirect immunofluorescence with the anti-*C. dubliniensis* adsorbed serum and the adsorption of the anti-*C. dubliniensis* antiserum with *C. albicans* germ tubes abolishes the reactivity of this antiserum with *C. dubliniensis*. The distribution of this antigen in the cell wall of *C. albicans* is in agreement with that shown by the previously described type I antigens, which are specifically expressed on the germ tube cell wall surface (16). The component of 30 kDa and several components ranging from 43 to 100 kDa are likely to be located on the cell wall surface of *C. krusei* and *R. rubra*, respectively, while the components from *C. tropicalis*, *C. parapsilosis*, *C. guilliermondii*, and *C. glabrata* extracts reacting with the anti-*C. dubliniensis* adsorbed serum are located within the cell wall.

In conclusion, our results indicate that *C. dubliniensis* and *C. albicans* share a group of cell wall antigens and that a specific anti-*C. dubliniensis* serum can be prepared after removal of the antibodies against the common antigens present on the cell wall surfaces of both species. The adsorbed anti-*C. dubliniensis* serum reacts with several components expressed specifically on the *C. dubliniensis* cell wall surface. These antigens or other antigens showing cross-reactivity with them are expressed on the cell walls of other *Candida* species and *R. rubra*, but this reactivity does not interfere with the use of the anti-*C. dubliniensis* adsorbed serum in an indirect immunofluorescence test for the rapid identification of *C. dubliniensis*.

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