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Short communication

Prevalence of *Candida dubliniensis* in the BCCM/IHEM Biomedical Fungi/Yeasts Culture Collection (isolates before 1990)

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The BCCM/IHEM Biomedical Fungi/Yeasts collection hosts 1200 *Candida albicans* strains of the Vanbreuseghem mycotheque isolated between 1951 and 1997. From this collection, 469 freeze-dried *C. albicans* strains, producing chlamydospores, germ tubes and forming green colonies on CHROMagar, all isolated before 1990, were screened to identify the *Candida dubliniensis* isolates. Screening was performed in different steps using the growth at 45 °C, the assimilation of xylose, the intracellular ß-glucosidase activity test and *C. dubliniensis*-specific polymerase chain reaction (PCR) with primers from *ACT1* intron sequence. Five isolates (1%) were identified as *C. dubliniensis*: one isolate was not documented, the others were of oropharyngeal origin of which two (1987 and 1990) were from proven human immunodeficiency virus patients.

Keywords *Candida dubliniensis*, culture collection

Introduction

Candida dubliniensis is a recently described yeast species principally associated with carriage and disease in the oral cavities of human immunodeficiency virus (HIV)-infected individuals. This species shows phenotypic characteristics that have long been considered specific for *Candida albicans*, that is the production of germ tubes and chlamydospores. This close similarity has hindered differentiation between the two species in clinical laboratories [1].

Since 1995, advances in phenotypic and genotypic methods for yeast identification have helped define differences between *C. albicans* and *C. dubliniensis*. Whereas *C. albicans* colonies are light bluegreen in colour on CHROMagar (CHROMagar Microbiology, Paris, France), *C. dubliniensis* colonies are a much darker green colour. Yet, this characteristic can be lost following subculture and storage [2].

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Comparative growth analysis at high temperatures, such as 45 °C, has also been suggested as a means of discriminating *C. dubliniensis* from *C. albicans* [3].

Nevertheless, the absence of growth at 45 °C should only be used as a confirmatory test or in conjunction with one or more other identification tests as some *C. albicans* strains also do not grow at this temperature [4]. The second test could be the xylose assimilation test as it is known that *C. dubliniensis*, unlike the great majority of *C. albicans* isolates, is unable to assimilate xylose after a short incubation of 48 h [1].

In an original study by Boerlin *et al.* [5] it was observed that, in contrast to *C. albicans*, *C. dubliniensis* isolates did not appear to produce ß-glucosidase activity. Nevertheless, it was later demonstrated that 12.5% of *C. albicans* isolates were ß-glucosidase negative [6].

Finally, various molecular methods exist to confirm the *C. dubliniensis* identification, for example, restriction fragment length polymorphism (RFLP) hybridization with *C. albicans* probe (27A or Ca3), karyotype analysis, random amplified polymorphic DNA (RAPD), internal transcribed spacer (ITS) sequencing or PCR with primers specific of *C. dubliniensis.* The last was applied to our isolates [7].

Material and methods

The Vanbreuseghem mycotheque is made up of 12,500 fungi kept under freeze-drying. It includes, among others, around 1200 *C. albicans* isolates collected during the last 50 years by Raymond Vanbreuseghem and collaborators at the Institute of Tropical Medicine of Antwerp. Three years ago, this collection was included in the official Belgian Coordinated Collections of Micro-organisms (BCCM)/Institut d'Hygiène et d'Epidémiologie-Mycologie (IHEM) Biomedical Fungi/Yeasts Culture Collection (curator: Nicole Nolard), located at the Scientific Institute of Public Health in Brussels.To establish the historical prevalence of *C. dubliniensis*, a survey of the first 469 chlamydospore-forming isolates recovered between 1952 and 1990 was undertaken.

The yeasts had been stored under freeze-drying since the early 1950s. The isolates had originally been identified phenotypically as *C. albicans* on the basis of the presence of chlamydospores on Rice Cream medium. Among the 469 yeasts, 4 (1%) were originally isolated in the 1950s, 9 (2%) in the 1960s, 150 (32%) in the 1970s and 306 (65%) in the 1980s up to 1990. The majority of the yeasts were isolated in European countries (66%), with those from Belgium being particularly heavily represented (62%). Nevertheless, 147 isolates (31%) came from Rwanda and were mostly from suspected AIDS patients. AIDS had been confirmed in only 70 of those patients. Fifteen of the specimens came from other diverse non-European geographical areas. Among the 469 yeasts, 450 were of human origin: 115 from either the oral cavity or sputum (24%), 42 from faeces, 32 from skin/nail, 29 from vagina, 7 from deep organs and 25 from other clinical settings. The origin of the other 200 isolates (42%) is unknown but they are probably mostly of human origin.

The identification methods chosen for a first screening were growth at 45 °C [3], used in conjunction with the xylose assimilation test performed according to Barnett *et al.* [9]. The second screening relied on the inability of *C. dubliniensis* to produce intracellular ß-glucosidase activity. This test was performed according to Boerlin *et al.* [5].

The final confirmation was a genotypic characterization carried out using PCR. Two sets of primers were used: the universal fungal primers (RNAR and RNAF), which generate a 610 bp fragment for both species, and the species-specific primers from the *ACT1* intron sequence of *C. dubliniensis* (DUBR and DUBF), which generate a 288 bp specific fragment [7].

Results

The results of the screening are presented in Table 1, 464 (99%) of the isolates were *C. albicans*, whereas five isolates (1%) were newly defined as *C. dubliniensis*. Four of these were isolated in Belgium at the Institute of Tropical Medicine in Antwerp. The two oldest strains were isolated respectively in 1974 and 1977, both from sputum, the first from a Belgian patient and the second from a Syrian patient. No underlying disease was recorded for the first, the second had a lesion mimicking facial actinomycosis. The three other strains were isolated in 1987, 1988 and 1990. The isolates from 1987 and 1990 were both from mouthwashings from Belgian AIDS patients, whereas the last was isolated from an

Table 1 Results of the screening of the 469 isolates

	Candida albicans (99%)					*Candida dubliniensis*
	92%	1%	6%			1%
	$n = 433$	$n = 5$	$n = 24$	$n = 1$	$n = 1$	$n = 5$
First screening						
Growth at 45°C	+	+	−	−	−	−
Xylose assimilation	+	−	+	+	−	−
Second screening						
β-Glucosidase test						
PCR		+	+	−	+	−
RNAR-RNAF (universal fungal primers)		+	+	+	+	+
DUBR-DUBF (primers specific for *C. dubliniwnsis*)	−	−	−	−	+	

unknown human source in Amsterdam (The Netherlands).

Discussion

Most isolates of *C. dubliniensis* have been recovered from cases of oral candidiasis in HIV-infected patients [9]. The results are in agreement with this observation as the four well-documented isolates are from this setting with two isolates from AIDS patients. Whereas some studies [10,11] have shown that *C. dubliniensis* isolates dating back to the 1950s existed, no isolate collected before 1970 could be found in the Vanbreuseghem mycotheque, probably as a consequence of the small number of such isolates ($n = 13$).

Regarding the geographical distribution of the isolates, we were unable to find any *C. dubliniensis* among our 147 Rwandan isolates. To date, no African isolate of *C. dubliniensis* has ever been reported. In contrast, one Syrian isolate can be added to the recently isolated Israeli strains [12], confirming that this species is present in the Middle East. Compared with the percentage of *C. dubliniensis* obtained by Odds *et al*. [6] in the archival stock of the Janssen Research Foundation (around 2%), the percentage of *C. dubliniensis* obtained in this study is very low (around 1%). However, the majority of the 2589 isolates studied by Odds *et al*. are of European origin (80.7%) or from North America (3.5%) and if the African isolates are excluded from our collection, taking only the European isolates into account, then we reach a percentage of 1.5%, which is rather similar.

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