Study of Molecular Epidemiology of Candidiasis in Portugal by PCR Fingerprinting of Candida Clinical Isolates

Alexandra Correia, Paula Sampaio, and Judite Almeida and Célia Pais*

Centro de Biologia da Universidade do Minho (CBUM), Departamento de Biologia, Braga, Portugal

Received 22 April 2004/Returned for modification 7 May 2004/Accepted 2 June 2004

PCR fingerprinting was used to type 177 yeast isolates obtained from two medical institutions. Candida albicans was the predominant species found, followed by C. tropicalis, C. glabrata, C. parapsilosis, C. guilliermondii, and C. krusei, which accounted for over 20% of the strains isolated. This survey represents the first study of molecular epidemiology of candidiasis in Portugal.

In the last decade, yeasts belonging to the genus Candida have emerged as major opportunistic pathogens, mainly due to the increase of immunocompromised patients (19, 26, 2, 5). Although Candida albicans is the most frequently isolated species, other species, such as C. tropicalis, C. guilliermondii, C. krusei, C. parapsilosis, and C. glabrata, have increasingly been recognized as pathogens with a wide distribution (6, 4). The significant increase in the frequency of candidiasis has promoted the study and development of a variety of molecular-based techniques aimed at the replacement of traditional methods used for identification and typing of Candida clinical isolates. Among the present molecular techniques for genotyping of yeast strains, PCR fingerprinting is in wide use for its high discriminatory power and reproducibility and because it requires very little starting material and is rapid and simple to perform. PCR fingerprinting with the primer named T3B was first developed for Streptococcus spp. identification (14), but it has been used successfully in the identification of yeast species belonging to the genus Candida (25, 1).

The aim of the present work was to study the diversity and distribution of Candida species among patients suffering from different pathologies in two medical institutions located in Braga in northern Portugal. Approximately two hundred Candida clinical isolates were analyzed by using a PCR-based methodology with primer T3B, representing the first study of the molecular epidemiology of candidiasis in this country.

Yeast clinical isolates were obtained from 123 independent patients during the year 2001 in a hospital and a health center. The yeast strains from the hospital had been previously isolated at the institution of origin and were collected from different body locations. The isolates from the health center were isolated by a rapid identification kit indicated that they all belonged to the genus Candida, although several doubts arose regarding species identity. PCR fingerprinting profiles were obtained with primer T3B for the type strains of C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, C. guilliermondii, C. krusei, and C. lusitaniae, the most common species found among yeast clinical isolates. Results showed that the different species tested could be clearly distinguished by their amplification patterns, because the number and size of the amplification products were characteristic for each species (Fig. 1).

By comparing the PCR profiles of the clinical isolates with those of the reference type strains, all clinical isolates could be identified to the species level, except in the case of four of the strains that did not produce recognizable patterns. Three of them (36 M, 65 M, and 66 M) shared identical profiles, while 153 M presented a different but unique fingerprint. These strains had been preliminarily identified as C. parapsilosis and...
C. glabrata (Fig. 2). Although intraspecies variability was observed, PCR profiles obtained from different strains assigned to the same species were far more similar than those derived from different species. Variability was found for isolates of C. albicans, C. tropicalis, C. guilliermondii, and C. parapsilosis, with C. albicans being the species that exhibited the greatest diversity. On the contrary, no variability was observed in the profiles obtained for both C. glabrata and C. krusei isolates. Our results agree with those of Thanos et al. (25), who used the same methodology to differentiate Candida species. These authors used a condensing step of the amplification products before electrophoresis which was not performed in this study; consequently, variability within a species might have been reduced. However, as our goal was identification at the species level, the methodology without this step turned out to be less time-consuming and produced easily recognizable profiles that maintained high species discrimination. The high power of discrimination of PCR fingerprinting with primer T3B allowed the identification of over 98% of the 177 clinical isolates and the detection of misidentifications made by API 32C.

To investigate the presence of C. dubliniensis, the T3B profiles of two strains, including the type strain, were obtained and compared to the ones found for C. albicans. T3B fingerprinting clearly distinguished these closely related species, because no similarities were observed between the amplification patterns of the two species (Fig. 3). No isolates of C. dubliniensis were found, which is not surprising because this yeast species is commonly reported from oral candidiasis, mainly among human immunodeficiency virus-infected individuals (24), which are not included in this study. Previous reports also refer to the differentiation of these two species by PCR fingerprinting (16), but this was the first time that T3B fingerprints have been applied for this purpose.

To evaluate the taxonomic resolution of T3B amplification profiles, cluster analysis was applied to the data and the dendrogram presented in Fig. 4 was produced, showing a very high correspondence between the clusters and the different Candida species. The calculated cophenetic correlation coefficient (0.97) indicated that the fit for the cluster analysis was very good. This analysis allowed the distribution of the isolates into seven major clusters corresponding to the species studied. The four isolates that did not produce recognizable T3B patterns grouped separately, and the strains that were originally misidentified grouped within the clusters corresponding to their respective T3B profile.

The four strains displaying peculiar banding profiles were
further investigated by sequencing the D1/D2 domain of their 26S rDNA. Sequencing results for strains 36 M, 65 M, and 66 M (GenBank accession no. AY589574) showed a 100% similarity between them and with strain *Candida* sp. strain NRRL Y-17456, which appears to be highly related to *C. parapsilosis* but has been referred to as a new species (9, 13). Furthermore, their T3B profiles did not match with the ones of other *C. parapsilosis* strains (Fig. 2). This species remains a source of

---

**FIG. 4.** Dendrogram showing the degree of similarity of T3B fingerprinting profiles among the clinical *Candida* isolates by using the Dice coefficient and UPGMA cluster method. The unidentified strains are indicated as follows: *, 36 M, 65 M, and 66 M; **, 153 M. An arbitrary line has been drawn at 0.58 delimitating the major groups. r, cophenetic correlation coefficient.
different pathologies were included. We show that over 20% of institutions, is a representative survey, because patients with our study, despite covering a small area and only two medical antant among vaginal isolates, the species other than C. albicans represented mainly from other sources. Little is known from vaginal swabs, 24 were from urine, 23 were isolated from urine and the respiratory tract. While C. albicans was predomin-antly a new species based on the high number of nucleotide origins. C. glabrata species (79.0%), followed by C. tropicalis species (30%), dominated the isolates. The remaining species, C. glabrata, C. parapsilosis, C. guilliermondii, and C. lusitaniae, represented 0.6%, and the isolates whose identification was not conclusive (Candida spp.) represented 2.3% of the total. C. albicans was present in all types of clinical material except blood samples, and C. tropicalis was mainly recovered from blood, C. parapsilosis, and related species. Yeast 9:149–1506.


REFERENCES


