Candida pararugosa isolation from the oral cavity of an Italian denture wearer

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Abstract

Candida pararugosa was first isolated from human feces, but after the initial description no further recovery from humans has been reported. During a study on oral Candida colonization in denture wearers living in Palermo (Italy), we isolated \textit{C. pararugosa} from a 61-year-old woman without signs of oral candidosis. This constitutes, to the authors’ knowledge, the first isolation of \textit{C. pararugosa} from the oral cavity. After six months, colonization by \textit{C. pararugosa} persisted, suggesting that this species could be a component of the normal oral microbiota. The identification procedure we used could be useful in elucidating the epidemiology of \textit{C. pararugosa} and for establishing its clinical significance.

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1. Introduction

Several studies on oral Candida colonization have shown that \textit{Candida albicans} is the most frequently isolated species, but a growing trend toward recovery of non-\textit{albicans} species, including \textit{Candida glabrata}, \textit{Candida tropicalis}, \textit{Candida krusei}, and \textit{Candida dubliniensis}, has been reported in the last decade [3,5,6,15,17]. These species have also emerged as etiological agents in oral candidosis, both as co-infecting organisms with \textit{C. albicans} and as sole pathogens themselves [5,14,18].

Denture wearing is recognized as a predisposing factor for oral colonization by \textit{Candida} species and a relationship between oral hygiene habits, denture cleanliness, yeast carriage and denture stomatitis has been proven [1,2,4,7,11,13,18]. The species isolated in denture wearers did not significantly differ from those encountered in dentate individuals or in edentulous patients not wearing dentures, with \textit{C. albicans} being the most prevalent species [2,12].

\textit{Candida pararugosa}, a species phylogenetically related to \textit{C. rugosa}, has never been recovered from humans since the type strain AJ 4645 (IFO 0966) was first isolated from feces [9]. In the present study we describe the first isolation of \textit{C. pararugosa} from the oral cavity of a systemically healthy denture wearer. Phenotypic and genotypic characteristics of this isolate are described and antifungal susceptibility is assessed.

2. Materials and methods

2.1. Subject

A 61-year-old Italian woman living in the urban area of Palermo (Italy) was enrolled in an investigation aiming to determine the prevalence and carriage of \textit{Candida} species in the oral cavity of denture wearers. The woman was systemically healthy and did not take any drugs. Oral \textit{Candida} colonization was determined by the concentrated oral rinse technique and by swabs [16]. A fecal sample was also obtained and processed for yeast isolation.

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2.2. Yeast isolation

Oral rinses, swabs and feces were cultured on Sabouraud dextrose agar (Becton Dickinson and Company, Franklin Lakes, NJ) supplemented with chloramphenicol (1 mg/ml) (SDA), and on CHROMagar Candida (CAC; CHROMagar, Becton Dickinson and Company) plates at 37 and 25 ºC in ambient air.

2.3. Biochemical characterization

API 20C AUX (BioMérieux, Marcy-l’Etoile, France) was used to study the carbohydrate assimilation profile of the isolates.

2.4. Antifungal susceptibility testing

Antifungal susceptibility of candidal isolates was evaluated by a broth microdilution test carried out by the method outlined in the National Committee for Clinical Laboratory Standards (NCCLS) document M27-A [10]. The medium consisted of RPMI 1640 medium supplemented with 2% glucose, buffered with 0.165 M morpholinepropanesulfonic acid buffer (Sigma–Aldrich, Milan, Italy) and adjusted to pH 7. The following antifungals were tested: amphotericin B (Bristol–Myers Squibb, Princeton, NJ), fluconazole (Pfizer Central Research, Sandwich, England), fluconazole (Fluka, Buchs, Switzerland), itraconazole (Janssen Pharmaceutica, Beerse, Belgium), econazole, ketoconazole, and myconazole (Sigma–Aldrich). C. krusei ATCC 6258 was used as quality control strain.

2.5. Genetic characterization

In order to determine the correct phylogenetic position of our isolate, sequence analysis of the variable D1/D2 domain of the large subunit (26S) ribosomal DNA was performed. This procedure is recognized as a reference method for comparison of phylogenetic relationships among ascomycetous yeasts [8].

The 26S rRNA gene was amplified by PCR and sequenced by GenomeExpress (Montreuil, France). The 26S sequence of our isolate was used for a BLAST search in the EMBL/GenBank database. The 26S sequence of our isolate was further aligned and compared to published 26S sequences with the taxonomy browser of the National Center for Biotechnology Information (NCBI; Bethesda, MD) and retrieved from GenBank. The retrieved sequences belonged to six reference strains of phenotypically close species of Candida, including the C. pararugosa type strain. The EMBL/GenBank accession numbers for 26S rRNA sequences were as follows: C. pararugosa NRRL Y-17089: U62306; C. rugosa NRRL Y-95: U45727; C. maris NRRL Y-6696: U70181; C. silvanorum NRRL Y-7782: U71068; C. magnoliae NRRL Y-2024: U45722; Saccharomyces cerevisiae NRRL Y-12632: U44806.

3. Results

Candidal isolates were obtained from swabs and oral rinses of the oral cavity at the first sampling. Very small colonies of Gram-positive pleomorphic rods were observed after 72 h incubation at 25 ºC on both SDA and CAC. Colonies were two mm in diameter after 96 h incubation and their surface appeared wrinkled. Colony color on CAC was light violet. No growth was observed at 37 ºC.

Biochemical characterization by means of the API 20C AUX system showed that, after 48 h of incubation, the isolate assimilated glucose (GLU), xylitol (XYL), galactose (GAL) and sorbitol (SOR). The biocode for API 20C AUX was 2062000, and resulted in a good identification level for C. rugosa according to the 3.0 release of the identification database.

Genetic analysis, performed by a BLAST search in the EMBL/GenBank database, resulted in a perfect match of the 26S sequence of our isolate with that of the C. pararugosa type strain. The alignment and comparison of the 26S sequence of our isolate to the published 26S rRNA sequences belonging to six reference strains of phenotypically close species of Candida confirmed the 100% correspondence to the C. pararugosa type strain and revealed identity rates <70% with respect to C. rugosa, C. maris, C. silvanorum, C. magnoliae, and S. cerevisiae type strains (Fig. 1).

The antifungal susceptibility test revealed that the isolates were susceptible to all the agents tested.

Following the results of the first sampling, the patient was requested to rinse with 10 ml of 0.2% chlorhexidine digluconate (CHX) mouth rinse twice daily for two weeks. Then she underwent further sampling by oral rinse and swabs. Six months after the first isolation, oral and fecal samples were also taken. C. pararugosa was always present in oral rinses, but no candidal organisms were recovered from swabs and feces. No signs of oral candidosis were observed at any sampling time.

4. Discussion

During a study of oral Candida colonization in denture wearers living in Palermo, Italy, we isolated C. pararugosa from a single subject. This constitutes, to the authors’ knowledge, the first recovery of C. pararugosa from the oral cavity.

The isolate was initially identified as C. rugosa by the API 20C AUX system, but the analysis of genetic divergence in the variable D1/D2 domain of large subunit (26S) ribosomal DNA allowed us to properly identify the isolate as C. pararugosa. This species, phylogenetically close to C. rugosa, is not included in the last (3.0) release of the API 20C AUX identification database.

To gain a better understanding of the role of C. pararugosa in oral colonization and infection, it is essential for microbiology laboratories to be able to identify this
species rapidly and accurately in clinical specimens. Primary CHROMagar screening with 25 °C incubation followed by carbohydrate assimilation tests with commercial yeast identification systems could be the most straightforward identification procedure for C. pararugosa, provided that identification biocodes for this yeast are included in the API 20C AUX identification database. However, two characters described by Nakase et al. [9] for the C. pararugosa type strain were not expressed by our isolates since xylose (XYL) and L-arabinose (ARA) were not assimilated. The recovery of a larger number of C. pararugosa isolates through this identification procedure would improve the effectiveness of the identification database. Though not suitable for routine examination, 26S rRNA sequence analysis has been shown to be an effective method for yeast classification and isolate identification [8]. This method was able to properly identify C. pararugosa, readily differentiating it from the closely related C. rugosa.

There is scarce information about the normal habitat of C. pararugosa and its role as an oral pathogen. Since the unique type strain available (IFO 0966) was isolated from human feces, we also tried to isolate C. pararugosa from the feces of our patient, but the result was negative. Although our C. pararugosa isolate was susceptible to a large panel of antifungal agents, the subject harboring such yeast was healthy and without any signs of oral candidosis. Therefore, she did not undergo systemic antifungal therapy. CHX rinsing for two weeks, however, failed to completely eradicate C. pararugosa.

The absence of oral lesions six months after persistent colonization by C. pararugosa suggests that this species could be a component of the normal oral microbiota. Since commensal yeasts could become opportunistic pathogens in immunocompromised patients, the application of an appropriate identification procedure for C. pararugosa, such as the one we described, would be useful to define the pathogenic role of this species.

References


