Case Report

Recurrence isolation of an uncommon yeast, Candida pararugosa, from a sarcoma patient

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A yeast was repeatedly isolated from the saliva of a sarcoma patient. A relatively uncommon species, Candida maris, was identified based on the API 20C profile. The yeast species most frequently obtained from the patient’s mother and from clinic staff was Candida albicans. A comparison of the yeast obtained from the patient with the type strain of C. maris strongly suggested that the former was not representative of C. maris. Analysis of partial ribosomal DNA sequences of the patient strain and from the type strain of C. maris showed that the two are phylogenetically not closely related. The patient strain was very close to Candida pararugosa, a relatively uncommon asporogenous yeast. DNA reassociation studies among C. pararugosa and patient isolates showed that they were conspecific. We could not determine the source of the yeast infection. This case will alert hospital staff to be aware of the possibility of unexpected environmental microorganisms as causes of infections, colonizations and persistent environmental contamination events in immunocompromised patients.

Keywords API 20C, Candida pararugosa, misjudgement, ribosomal RNA

Introduction

Microbiological management is one of the most important practical issues for immunocompromised patients, such as those with leukaemia or other cancers. Most human pathogenic fungi are also members of the normal microbial flora of the human body surface and opportunistic infection frequently occurs in immunocompromised patients [1]. If fungal infection is established, treatment with antifungal agents must be prompt.

As part of our ongoing infection control research, we recently collected saliva samples from 30 hospitalized children, most of them leukaemia patients, as well as from their families and attending medical staff. The case described here was seen during the course of this research. In this case, a yeast was repeatedly isolated from the saliva of a sarcoma patient who had been periodically treated with anticancer drugs and antifungal agents. At first we were concerned about the emergence of antifungal-resistant species such as Candida glabrata, as well as resistant strains in more common species. However, the API profile (bio-Mérieux, Marcy L’Etoile, France) of the patient isolates showed that all were apparently identical and had a moderately close physiological similarity to Candida maris, an uncommon species in the clinical setting. Based on detailed analyses of the isolates, we ultimately concluded that the yeast was Candida pararugosa, a species originally described by Nakase et al. [2]. In this case, then, recovery of a relatively uncommon yeast led to a misjudgement of species
identity based on use of the API identification system. It should be noted that as the findings described arose from a more general, large research project, not all potentially pertinent case information was available to us at the time the manuscript was in preparation.

**Materials and methods**

**Isolation of the yeasts**

In patients, parents and medical staff, all sampling was done from the saliva using a sterilized swab. An aliquot (approximately 50–100 μl) of saliva obtained from an individual by means of a sterilized swab was plated onto yeast–peptone–dextrose (YPD) solid media (2% glucose, 2% bacto-peptone [Difco, Detroit, MI, USA], 1% yeast extract [Difco]) containing antibiotics. Several representative colonies were chosen and subjected to API identification after purification of each colony.

**Pulsed-field gel electrophoresis**

Pulsed-field gel electrophoresis (PFGE) analysis was performed by Pulsaphor (Pharmacia, Stockholm, Sweden) using the contour-clamped homogeneous field (CHEF) method [3]. The procedure for the sample preparation has been described elsewhere [3]. The running condition was 300 s of pulse time for 24 h at 140 V, followed by 1000 s for 48 h at 90 V on 0.8% agarose in 1 X TBE (89 mm Tris-borate, 2 mm ethylene-diamine tetra-acetic acid [EDTA], pH 8.3) buffer at 12°C.

**Sequence analysis of ribosomal DNA**

The isolation of genomic DNA, nucleotide sequence determination and homology search was carried out as described previously [4].

**Genetic and genomic comparison of the yeast strains**

DNA–DNA reassociation analysis and measurement of GC contents of genomic DNA was carried out as described elsewhere [5].

**Case report and results**

The patient was a 2-year-old Japanese girl with rhabdomyosarcoma. She was periodically treated with anticancer drugs. Fever was detected intermittently, which caused concern. Amphotericin B was administered by inhalation when antibacterial drugs proved ineffective in dealing with the fever. Unfortunately, we were not able to obtain information about the dosages used. Yeast isolates were repeatedly recovered from the saliva of the patient. Yeasts were also recovered from saliva samples from her family and from two nurses who attended her. The number of yeast colonies on plates inoculated with the patient’s saliva reached approximately 1000, suggesting that the yeast populations were established in some part of the oropharyngeal tract (Table 1). Based on micromorphology and colony morphology, all isolates seemed to belong to the same species (Fig. 1). Identification of the isolates was attempted with the API 20 C AUX system. A strain isolated from the patient’s mother as well as isolates from the nurses were confidently identified as *Candida albicans*. However, the probability of the patient strains being *C. maris* was 74–90% (Table 2). *C. maris* is a marine yeast [6] not known to cause human infection. We therefore compared the patient isolates with the type strain of *C. maris*, CBS 5151 (Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands; = IFO 10003, Institute for Fermentation, Osaka, Japan), by several methods. Two patient isolates were deposited in the CBS culture collection as CBS 9121 and CBS 9122. All patient isolates tested grew on YPD medium at 37°C, whereas the CBS 5151 could not. On cornmeal agar plates, the patient isolates grew vigorously by pseudohyphae, whereas CBS 5151 grew exclusively by budding. To compare the chromosomal constitution of the two yeasts, PFGE was carried out. As shown in Fig. 2, five distinct chromosomal bands, ranging from 1.3 to 2.2 Mb were observed in CBS 5151, whereas three bands ranging from 2.2 to 3.5 Mb were found in the patient isolates. All of the patient isolates tested showed the same banding patterns (data not shown), strongly suggesting that the patient isolates and the CBS 5151 are different, notwithstanding the implication of similarity obtained from the API 20 C AUX profiles. However, we were able to establish that the patient isolates belonged to the genus *Candida*, because they formed white to creamy coloured colonies, produce no ascospores on several sporulation media, did not elaborate urease activity, and did not form extracellular polysaccharides (data not shown).

**Table 1** Record of yeast isolations from saliva of the patient.

<table>
<thead>
<tr>
<th>Isolation date</th>
<th>Sample no.</th>
<th>Colony no. formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>22/Feb/1995</td>
<td>2</td>
<td>998</td>
</tr>
<tr>
<td>10/Mar/1995</td>
<td>37</td>
<td>769</td>
</tr>
<tr>
<td>15/Mar/1995</td>
<td>61 (CBS 9122*)</td>
<td>483</td>
</tr>
<tr>
<td>27/Apr/1995</td>
<td>115 (CBS 9121*)</td>
<td>1,206</td>
</tr>
</tbody>
</table>

*CBS (Centraalbureau voor Schimmelcultures) collection no. was assigned after deposition of the isolates to the CBS.

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To investigate which species was most closely related to our patient isolates, nucleotide sequences of the 5.8 s, internal transcribed spacer (ITS) and the 26 s regions of nuclear ribosomal DNA were tested for CBS 9121 and 9122. An homology search revealed that the sequences were closest (99%) to those of *Candida pararugosa*, ex-type isolate CBS 1010 (GenBank accession no. for the 26S rDNA: AF335972; that of partial 18S rDNA, ITS and 5.8S rDNA: AF421856), originally described by Nakase *et al.* [2]. (Accession nos of 26S rDNA for CBS 9121 and CBS 9122 are AB112430 and AB112432, respectively; those of the partial 18S, ITS and 5.8S rDNA are AB112431 and AB112433, respectively.)

Comparison of AF421856, AB112431, and AB112433 is shown in Fig. 3. For additional confirmation, we performed DNA/DNA reassociation tests and GC content measurements using the genomic DNA of CBS 1010 as a reference. Both tests showed that the strains CBS 9121 and CBS 9122 had DNA compatible with an identification as *C. pararugosa* (Table 3).

To determine whether or not the strains recovered from the patient were potentially pathogenic, $1 \times 10^6$ yeast cells of patient strains CBS 9121 and CBS 9122 were intravenously inoculated in mice. As shown in Fig. 4, mice pretreated with cyclophosphamide died within 5 days when inoculated with *C. albicans* strain CA1, while those inoculated with CBS 9121 and CBS 9122 were all alive after 3 weeks, even when pretreated with cyclophosphamide. These results suggest that CBS 9121 and CBS 9122 are much less pathogenic in mice than is *C. albicans*.

Finally, we examined the drug susceptibility of strains CBS 9121 and CBS 9122. As shown in Table 4, both strains were sensitive to amphotericin B, but highly resistant to fluconazole.

Further clinical information about the case patient (e.g. in relation to long-term treatment response and outcome) was not available to us.

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**Table 2** API 20C numerical profiles of the patient isolates.

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Numerical profiles*</th>
<th>Identification (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>6460004</td>
<td><em>Candida maris</em> (74)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>C. rugosa</em> (21)</td>
</tr>
<tr>
<td>37</td>
<td>2460004</td>
<td><em>C. maris</em> (83)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>C. rugosa</em> (17)</td>
</tr>
<tr>
<td>61 (CBS 9122)</td>
<td>2060004</td>
<td><em>C. maris</em> (90)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>C. rugosa</em> (9)</td>
</tr>
<tr>
<td>115 (CBS 9121)</td>
<td>6460004</td>
<td><em>C. maris</em> (74)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>C. rugosa</em> (21)</td>
</tr>
</tbody>
</table>

* The differences among the numerical profiles reflect differences in glycerol and xylose assimilation.
Discussion

Bacterial and fungal nosocomial infection represent a significant medical problem [7–9]. Routine microbiological procedures generally assign aetiological isolates to one of the well-known opportunistic microorganisms. In cases where unusual microorganisms emerge in an immunocompromised patient, it is important to evaluate the route of introduction, the potential pathogenicity of the organism, and the possibility of various modes of transmission, in order to avoid recurrent infection or transmission to other patients. Modes of transmission of such pathogens vary from case to case, and molecular probes detecting repetitive sequences (RPS) are often used to trace the origins of aetiological genotypes (e.g. in *C. albicans*) [10,11]. We previously developed a simple PCR-based method to distinguish *C. albicans* strains, and have successfully used this method to confirm transmission of a strain between patient and bedside visitors [10,12]. No such method is currently available for *C. maris* and *C. pararugosa*.

In the present study, in parallel with isolation of the yeast colonies from the patient, we also obtained several isolates from the patient’s family and from hospital staff members who had had contact with the patient. This was done as part of a prospective study of salivary isolates, and isolates from hands and other body surfaces were therefore not obtained. Strains recovered from family and staff were all identified as *C. albicans* by the API test. Our investigation showed that, in our patient, a relatively uncommon, fluconazole-resistant, experimentally avirulent yeast, *C. pararugosa*, had become established. We could not identify the source of the inoculum or be completely certain that the yeast was truly established in the patient, even though consistent, recurrent and exclusive isolation strongly suggested this. The patient was fond of fresh fruit, a known source of at least some yeast species, and sometimes ate this in addition to hospital meals. The fruit was not tested in regard to its yeast content. It should be noted that *C. pararugosa* appears to be a rare species, and isolates previously deposited in the CBS

![Fig. 3](image-url) Alignment of the nucleotide sequence of a portion of the 18s rDNA, ITSs and 5.8s rDNA of *C. pararugosa*, type strain CBS1010 and patient isolates CBS 9121 and 9122, in a report of recurrent isolation of an uncommon yeast, *Candida pararugosa*, from a sarcoma patient in Japan. Nucleotides not differing from those of the type strain are indicated by ‘.’ and sites of overall consensus are indicated by ‘*’.

![Table 3](image-url) Genomic comparison between the type strain of *C. pararugosa* and patient isolates.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Reassociation value</th>
<th>GC contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBS 1010</td>
<td>(100)</td>
<td>46.5</td>
</tr>
<tr>
<td>CBS 9121</td>
<td>99</td>
<td>46.7</td>
</tr>
<tr>
<td>CBS 9122</td>
<td>100</td>
<td>46.8</td>
</tr>
</tbody>
</table>

Type strain of *Candida pararugosa*.

![Fig. 4](image-url) Virulence assay of the patient isolates, in a report of recurrent isolation of an uncommon yeast, *Candida pararugosa*, from a sarcoma patient in Japan. Mice (*n* = 5) were challenged with 1 × 10⁸ cells of CA9 (*C. albicans*), CBS 9121, or CBS 9122 intravenously. Trials using mice immunodepressed with 200 mg/kg of cyclophosphamide over 5 days before yeast challenge are indicated by (+); those using control mice are indicated by (–). To avoid confusion, the results of four experiments giving identical results (CBS 9121 [–], CBS 9121 [+], CBS 9122 [+], and CBS 9122 [–]) are depicted with a single symbol.

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culture collection include only the ex-type isolate from human faeces and CBS 5849 from *Anolis* lizard intestine. The species is so far unknown from fruit or other food materials.

This case shows that the presence of a medically uncommon yeast in a patient can produce a misidentification when a routine and rapid method such as the API system is used, particularly when only moderately high similarity to the database is indicated. Hospital staff should therefore be warned to maintain an awareness of the possibility of unusual environmental microorganisms in patients, especially as some such isolates may ultimately transpire to be poorly known opportunistic pathogens. As in this case, molecular biological procedures for rigorously correct yeast identification could give useful information.

**References**