Isolation of *Issatchenkia occidentalis* from the esophagus of a leukemic patient

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Summary *Issatchenkia occidentalis* was isolated from an esophageal biopsy of a young leukemic male patient who underwent bone marrow transplantation. At the time the specimen was collected, the patient was also suffering from esophageal herpetic lesions. The identification of the isolate was not possible by the use of the available commercial methods. Thus, its identification was done by PCR and DNA sequencing using panfungal primers.

Key words *Issatchenkia occidentalis*, Leukemic patient, Yeast identification

Aislamiento de *Issatchenkia occidentalis* en el esófago de un paciente con leucemia

Resumen Se ha aislado *Issatchenkia occidentalis* de una biopsia de esófago de un joven con leucemia. El paciente presentaba lesiones esofágicas herpéticas un mes después de recibir un trasplante de médula ósea. La identificación del aislamiento no se pudo realizar por los métodos comerciales existentes, por lo que se recurrió a la secuenciación de un fragmento de DNA amplificado por PCR utilizando cebadores panfúngicos.

Palabras clave *Issatchenkia occidentalis*, Leucemia, Identificación de levaduras

Strains of the genus *Issatchenkia* were first isolated from fruit juices and berries [4]. After studying the ascus morphology, ascospore ultrastructure by scanning electron microscopy, and DNA base sequence, other *Issatchenkia* species were also described [3,6,10]. *Issatchenkia orientalis* was the first reported species in the genus, followed by *Issatchenkia occidentalis*, *Issatchenkia scutulata*, and *Issatchenkia terricola* [6]. *Candida sorbosa* is the anamorph of *I. occidentalis* [6], which is typically isolated from tropical fruits [13]. In this note, we report the isolation of *I. occidentalis* from an esophageal biopsy in a leukemic patient with esophagitis.

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The isolate was recovered on an esophageal biopsy from a young leukemic patient that was suffering from esophagitis one month after undergoing allogenic bone marrow transplantation. The biopsy was taken via gastroscopy and the histopathological study revealed epithelial ulcers, with viral inclusion bodies in the squamous cells and multinuclear cells, findings very consistent with a herpetic esophagitis.

Macroscopically the yeast grew as light creamy dull colored colonies in Sabouraud Dextrose agar (Difco; Becton, Dickinson and Co., France). Growth was better at 30 °C than 37 °C. The isolate was unable to grow at 40 °C. In CHROMagar Candida medium (CHROMagar Microbiology, France), the isolate grew as beige to pale pink colored colonies. White colonies and beige to pale pink rough colonies were produced in Candida ID2 medium (bioMérieux, France) and CHROM-Pal's medium [11], respectively. Microscopically, the cells were ovoid to elongate, single or in pairs, with pseudohyphae. By scanning electron microscopy, the cells showed a rough surface due to the presence of multiple protuberances [6], a morphological feature that contrast with that observed in *Candida albicans* (Figure 1).

The isolate was unable to produce germ tubes in horse serum after 3 h of incubation at 37 °C. Chlamydospores were not developed in any of the tested specialized media (Corn-meal agar, Rice-tween agar, Pal's agar and CHROM-Pal's). Secretory aspartyl proteinases and lipolytic enzymes were not detected in media containing bovine

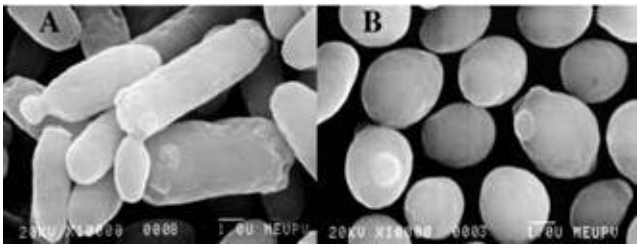

Figure 1. Scanning electron microscopy of *I. occidentalis* (A) and *C. albicans* (B) cells, grown on Malt agar at 30 °C for four days (bar: 1 µm).

serum albumin after nine days of incubation at 37 °C and Tween-80 medium, respectively [7].

Galleries ID 32 C (bioMérieux) and API 20C AUX (bioMérieux) produced doubtful profiles and it was not possible to reach a proper identification using these kits. Antifungal susceptibility was tested by the Sensititre Yeast One test (Trek Diagnostic System Ltd, Imberhorne Lane, East Grinstead, West Sussex, England). The isolate was susceptible to flucytosine (MIC 4 µg/ml), itraconazole (MIC 0.125 µg/ml) and voriconazole (MIC 0.128 µg/ml) and resistant to fluconazole (MIC 128 µg/ml). MICs for amphotericin B and ketoconazole were < 0.008 µg/ml and 0.256 µg/ml, respectively.

We used the primers NL-1 (5'-GCATATCAATAAGCGGAGGAAAAG) and NL-4 (5'GGTCCGTGTTTCAAGACGG) in a PCR assay (36 cycles with annealing at 52 °C and extension at 72 °C for 2 min) to amplify the large subunit (26S) ribosomal DNA gene [5,8]. The amplified DNA (approximately 600 bp) was electrophoresed, purified by the QIAquick Gel extraction kit (QIAGEN GmbH, Hilden, Germany), and sequenced. The sequence data were compared with the sequence database in NCBI Blast GenBank (http://www.ncbi.nlm.nih.gov/BLAST/) rendering a 99.9% homology to the sequence of *I. occidentalis* (GenBank accession no. U76348.1).

Identification of yeasts from clinical specimens is usually performed by using a variety of phenotypic tests, including sugar assimilation, growth on special media, enzymatic profiles and different morphological characteristics. However, with strains of infrequently isolated species, genotypic methods can also be needed. Genotypic methods may be very important when phenotypic characteristics are subjective, unstable or variable [9]. This has been the case in the identification of the *I. occidentalis* isolate presented in this report, since conventional commercial tests were unable to identify it.

The observation of asci in the clinical isolate grown on Potato dextrose agar (Becton Dickinson, Sparks, MD, USA) [12] and cucumber wedges at 25 °C for one month (Figure 2), confirmed the identification of *I. occidentalis*, the teleomorph (sexual stage) of *C. sorbosa*. The isolation of *I. occidentalis* from our patient is exceptional in two ways. Although the clinical significance is not clear, to our knowledge, this is the first report of *I. occidentalis* isolation from a clinical specimen. Searches in PubMed using the key words *I. occidentalis* or *C. sorbosa* yielded no studies related with their isolation from human clinical specimens. Secondly, it was isolated as the teleomorphic form of the fungus, a circumstance which is infrequently observed when dealing with clinical specimens [1,2]. The existence of epithelial ulcers with viral inclusion bodies compatible with herpetic esophagitis suggests that the presence of *I. occidentalis* in this clinical sample was simply colonizing the esophagus of the patient and was not involved as the etiologic agent. In fact, the patient recovered without antifungal treatment.

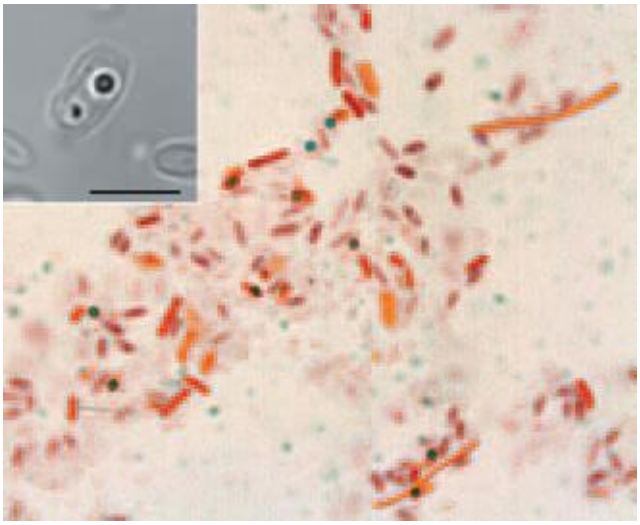

Figure 2. Asci and ascospores of *I. occidentalis* (grown on cucumber wedges at 25 °C for one month) stained with malachite green (1000x). Photograph in the inset was taken with an Olympus FV 500 confocal microscope (bar: 5 µm).

The results presented in this report show that *I. occidentalis* can be isolated from clinical specimens and emphasize the importance of DNA sequencing for the proper identification of rare fungal isolates.

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References

1. García-Martos P, Mira J, Galán F, Hernández-Molina JM. Sexual forms of yeasts in clinical samples. Mycopathologia 1996; 136: 67-70.
2. Hernandez-Molina JM, García-Martos P. Aislamiento de teleomorfos de muestras clínicas. Rev Iberoam Micol 1998; 15: 235-242.
3. Kerger-Van Rij NJW, Veenhuis M. Electron microscopy of ascus formation in the yeast *Debaryomyces hansenii*. J Gen Microbiol 1975; 89: 256-264.
4. Kudriavzev VI. Die Systematik der Hefen. Berlin, Akademie-Verlag, 1960.
5. Kurtzman CP, Robnett CJ. Identification of clinically important ascomycetous yeasts based on nucleotide divergence in the 5' end of the large-subunit (26S) ribosomal DNA gene. J Clin Microbiol 1997; 35: 1216-1223.
6. Kurtzman CP, Smiley MJ, Johnson CJ. Emendation of the genus *Issatchenkia kudriavzev* and comparison of species by deoxyribonucleic acid reassociation, mating reaction, and ascospore ultrastrucure. Int J Syst Bacteriol 1980; 30: 503-513.
7. Malcolm S. Tween 80 opacity test responses of various *Candida* species. J Clin Microbiol 2000; 38: 4626-4628.
8. Peterson SW, Kurtzman CP. Ribosomal RNA sequence divergence among sibling species of yeasts. Syst Appl Microbiol 1991; 14: 124-129.
9. Pontón J. Diagnóstico microbiológico de las micosis. Rev Iberoam Micol 2002; 19: 25-29.
10. Price CW, Fuson GB, Phaff HJ. Genome comparison in yeast systematics: delimitation of species within the genera *Schwanniomyces*, *Saccharomyces*, *Debaryomyces*, and *Pichia*. Microbiol Rev 1978; 42: 161-193.
11. Sahand IH, Moragues MD, Eraso E, Villar-Vidal M, Quindós G, Pontón J. Supplementation of CHROMagar Candida medium with Pal's medium for rapid identification of *Candida dubliniensis*. J Clin Microbiol 2005; 43: 5768-5770.
12. Santos MJS, Trufem SFB, de Oliveira PC. Sexual reproduction in subcultures of *Absidia blakesleeana* after years of preservation under mineral oil. Rev Iberoam Micol 2005; 22:174-176.
13. Trindade RC, Resende MA, Silva CM, Rosa CA. Yeasts associated with fresh and frozen pulps of Brazilian tropical fruit. Syst Appl Microbiol 2002; 25: 294-300.