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Sequence-based identification, genotyping and virulence factors of *Trichosporon asahii* strains isolated from urine samples of hospitalized patients (2011–2016)

G. Hazirolan [a,*], N. Koçak [b], A. Karagöz [c]

[a] *Hacettepe University Faculty of Medicine, Department of Medical Microbiology, Hacettepe Üniversitesi Tıp Fakültesi, Tıbbi Mikrobiyoloji ABD, Sıhhiye, 06100 Ankara, Turkey*
[b] *Selçuk Üniversity Medicine Faculty, Department of Medical Genetics, Selçuk Üniversitesi Tıp Fakültesi Tıbbi Genetik ABD Selçuklu/Konya, Ankara, Turkey*
[c] *Public Health Agency of Turkey, National Molecular Microbiology Reference Centers Laboratory, Türkiye Halk Sağlığı Kurumu, Moleküler Mikrobiyoloji Referans Laboratuvarları, Adnan Saygun Cad. No: 55 Sıhhiye, Ankara, Turkey*

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SUMMARY

Introduction. – *Trichosporon asahii* is the most common species that causes trichosporonosis.
Materials and methods. – In the present study, a collection of 68 *T. asahii* strains recovered from hospitalized patients urine samples between 2011 and 2016 was examined. *T. asahii* strains were identified by sequencing the intergenic spacer 1 region (IGS1) and genotyped. In addition, proteinase, phospholipase, esterase, haemolytic activity, and biofilm formation of a total of *T. asahii* strains were investigated.
Results. – The predominant genotype was 1 (79.3%) and followed by 5 (8%), 3 (6.9%), 6 (3.4%), 4 (1.1%), 9 (1.1%). In none of the 68 strains, proteinase and phospholipase activities could be detected, while all were found to be esterase positive. Biofilm production and hemolytic activity were detected in 23.5 and 97% respectively.
Discussion. – Our results indicated that six genotypes were (1, 5, 3, 6, 4, 9) present among *T. asahii* strains and no property was found to associate with a genotype, in terms of virulence factors.

Introduction

Trichosporon asahii is a yeast-like fungus distributed in the environment and capable of causing localized or systemic infections, especially in immunocompromised patients [1,2]. The emergence of these less common but medically important species has been increased in recent years due to several factors, including the increased occurrence of degenerative and malignant diseases, as well as the higher number of patients exposed to chemotherapy, immunosuppressive therapies, broad-spectrum antibiotics, and invasive medical procedures such as intravenous or urinary catheters, endoscopic forceps, and arteriovenous graft [1–6].

Conventional identification of *T. asahii* mainly depends on cell, colony morphology and biochemical characteristics [2]. With the molecular biologic technique used for the identification of these strains, Sugita et al. demonstrated that the sequence of the intergenic spacer (IGS) 1 region is useful for both identifying and genotyping of *T. asahii* [7]. Based on the IGS 1 sequence, 15 genotypes of *T. asahii* have been identified so far [8].

The pathogenic characteristics of *T. asahii* are remain unclear. To understand pathogenic characteristics of these strains, several virulence factors such as phenotype switching, the ability to produce biofilm, thermotolerance, the expression of cell wall components, and enzyme production and secretion were investigated [9–15]. However, these virulence factors have been reported mostly in Candida species and Cryptococcus neoformans [16–18]. Although *T. asahii* is an important cause of disseminated infections by non-Candida species, several authors have attempted to evaluate the virulence factors of *T. asahii* and the roles of these factors during the course of an *T. asahii* infection [1,2,8,11,12,14,15].

In the present study, we evaluated *T. asahii* genotypes based on IGS 1 region sequence to provide epidemiological data, and determined potential virulence factors (proteinase, phospholipase, esterase, haemolysin and biofilm) to obtain basic information that

* Corresponding author.
E-mail address: drgulsencetin@yahoo.com (G. Hazirolan).

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is needed to understand the pathogenicity of *T. asahii*. In addition, we investigate the relationships between genotypes and virulence factors of *T. asahii*.

Materials and methods

Clinical data

A total of 68 *T. asahii* strains were collected from the urine samples of hospitalized patients with urinary tract infection. Urinary tract infection was defined by the presence of urinary symptoms (dysuria, fever, urgency, frequency, suprapubic or flank pain) associated with significant *T. asahii* ($>$ 10.000 cfu/mL) in urine culture [14]. Sixty-three percent of the strains were from male patients. Seventy-five percent of the patients had urinary catheterization. All patients were immunocompetent and had chronic conditions such as diabetes, hypertension, asthma, and any of the other chronic conditions. All strains in this study were clinically relevant.

Strains identification

Sixty-eight *T. asahii* strains, obtained from urine samples of patients treated at Numune Training and Research Hospital in Ankara, Turkey between 2011 and 2016 were evaluated. The strains were classified to the species level by using both conventional methods, including the colony morphology, microscopic properties on cornmeal Tween 80 agar, urease enzyme activity and Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS). Bruker Microflex LT (Bruker Daltonics, Germany) and VITEK MS (bioMérieux, France) were used for identification of *T. asahii* strains according to the manufacturer's instructions. Strains were preserved in sterile freezing tubes at −80 °C until use.

DNA sequencing and identification

DNA extraction was performed according to the method reported by Sambrook et al. [19]. IGS1 region were amplified using the forward primer 26SF (5_-ATC CTT TGC AGA CGA CTT GA-3_) and reverse primer 5SR (5_-AGC TTG ACT TCG CAG ATCGG -3_) [7]. The PCR products were sequenced by using the ABI 3700 DNA sequencer with an ABI PRISM BigDye Terminator Cycle Sequencing kit (Applied Biosystems, USA) according to the manufacturer's instructions.

Genotyping the T. asahii *and molecular phylogenetic analysis*

Genotyping of *T. asahii* strains were performed by comparison against previously reported strains in GenBank (BLAST; http://www.ncbi.nlm.nih.gov). IGS 1 derived sequences were aligned using Clustal W [20]. For the neighbor-joining analysis, the distances between sequences were calculated using Kimura's two parameter model [21–23]. A bootstrap analysis was conducted with 100 replications [23].

Virulence factors

Yeast suspensions were prepared from the strains included in the study to evaluate proteinase, phospholipase, esterase, hemolytic activities and biofilm formation [14].

Secretion of proteases and phospholipases were detected by the formation of an opaque halo of degradation around the colonies grown in a specific agar plate, according to Rüchel et al. and Price et al., respectively [24,25]. The protease and phospholipase enzymatic activities (Pz) were determined by the ratio between the colony diameter and the colony diameter plus the halo zone [24,25]. The esterase activity of the strains was assessed in Tween-80 agar according to Slifkin et al., this activity was considered to be positive in the presence of a halo viewed with transmitted light around the inoculation site [26]. Hemolytic activity testing was performed on Sabouraud blood medium (65 g/L SGA, 3% w/v glucose and 7% v/v blood). Hemolytic index (Hi) was calculated by dividing the total diameter of the colony plus the translucent halo by the diameter of the colony [27]. Biofilm formation was assessed in Congo red agar plates prepared by adding 0.8 g of Congo red and 36 g of saccharose (Sigma, Germany) to 1 L of Brain hearth infussion agar (Oxoid, England). Rough black colonies were classified as biofilm procuders, while smooth red colonies were classified as non-biofilm producers [28]. *C. albicans* ATCC 90028 and *C. albicans* ATCC 10231 were used as a positive controls and sterile deionized water was used as a negative control for all enzymatic assays.

Results

Sixty-eight *T. asahii* strains were isolated from urine samples of hospitalized patients. These patients were hospitalized in the intensive care units (63%), urology (14%), nephrology (12%), oncology (5%), hematology clinics (4%), and neurology (2%). The median age of patients was 65 years old (range 23–94). The majority of patients are men (63.2%). Fifty-one (75%) patients with positive urine culture results for *T. asahii* had urinary catheterization. All strains were identified as *T. asahii* by macroscopic (white to cream-coloured cerebriform and radial yeast colonies) and microscopic (blastoconidia, true mycelia, arthroconidia) colony morphology, urease activity (positive) and MALDI-TOF MS systems. VITEK MS (confidence level of $>$ 99.9%) and, Bruker Microflex LT (confidence score of $>$ 2.000) correctly identified all *T. asahii* strains by using formic acid extraction.

DNA sequencing and identification

Of the 68 strains that were identified as *T. asahii* with macroscopic, microscopic colony morphology, urease activity and MALDI-TOF MS systems, 68 (100%) were found to be *T. asahii*.

Genotyping the T. asahii *strains*

Molecular phylogenetic trees constructed using the DNA sequence of the IGS 1 region were shown in Fig. 1. Among the 68 *T. asahii* strains, six IGS 1 sequence genotypes were identified. The predominant genotype was 1 (53 strains, 79.3%), followed by genotypes 5 (6 strains, 8%), genotypes 3 (5 strains, 6.9%), genotypes 6 (2 strains, 3.4%), genotypes 4 (1 strain, 1.1%) and, genotypes 9 (1 strain, 1.1%). Genotype 1 *T. asahii* strains were found only in patients with urinary catheters.

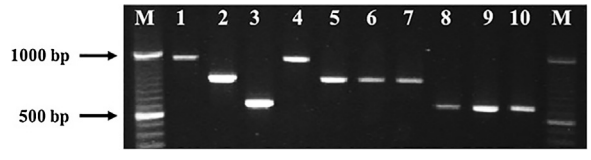

Figure 1. Molecular phylogenetic trees constructed using the DNA sequence of the IGS 1 regions of clinical and reference *T. asahii* strains. The code numbers correspond to the GenBank accession numbers. The numbers indicate the confidence level from 100 replicate bootstrap samplings.

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