Original Article

The first isolation of *Trichosporon coremiiforme* from soil in Iran

Jamali S[1]*, Gharaei M[2]

[1] Assistant Professor of Mycology, Department of Plant Protection, School of Agriculture, Razi University, Kermanshah, Iran
[2] MS student, Department of Plant Protection, School of Agriculture, Razi University, Kermanshah, Iran

***Corresponding author: Samad Jamali,** Department of Plant Protection, School of Agriculture, Razi University, Kermanshah, Iran. Email: jamali454@yahoo.com

(Received: 11 July 2015; Revised: 27 September 2015; Accepted: 13 October 2015)

Abstract

Background and Purpose: *Trichosporon* is a genus of anamorphic basidiomycetous yeast which is widely distributed in nature and is found in tropical and temperate areas. The aim of this work was to study the isolation, identification and molecular analysis of *Trichosporon* species in soil.

Materials and Methods: In order to isolate and identify *Trichosporon* species in soil, 30 samples were collected from 30 different locations across Iran. The isolates were identified by means of the standard methods of yeast identification. To confirm morphological identification, genomic DNA was extracted and the hypervariable D1/D2 domain of the large-subunit (LSU) ribosomal DNA (rDNA) gene was amplified by polymerase chain reaction (PCR), using primer pair NL-1/NL-4, and then the sequences were analyzed.

Results: According to the morphological and physiological assessments, isolates were identified as *T. coremiiforme*. The isolates formed chlamydospore after one week on yeast-malt (YM) agar medium. Using Blast program, we found that the D1/D2 sequences of the *T. coremiiforme* isolates from Iran (accession no: KP055040 and KP055041) showed 99% homology with the *T. coremiiforme* deposited in GenBank. All the *T. coremiiforme* isolates placed in the Ovoides cluster were well-supported by bootstrap values.

Conclusion: The present study is the first attempt to survey *Trichosporon* in soil of Iran. To the best of our knowledge, this is the first investigation of *T. coremiiforme* in Iran.

Keywords: Basidiomycota, DNA Ribosomal/analysis, *Trichosporon* metabolism

➢ How to cite this paper:
Jamali S, Gharaei M. The first isolation of *Trichosporon coremiiforme* from soil in Iran. Curr Med Mycol. 2015; 1(3): 3-10. DOI: 10.18869/acadpub.cmm.1.3.3

Introduction

Trichosporon is a genus of anamorphic basidiomycetous yeast which is widely distributed in nature and is found in tropical and temperate areas [1-3]. Members of the genus *Trichosporon* may also present in soil, water, air and organic substrata [1]. In human beings, these fungal species are known as the causative agents of cutaneous infections and are involved in systemic, localized and disseminated mycosis, particularly in immune-compromised patients [4, 5].

Trichosporon species have been identified in the mucosal surfaces of the human respiratory and gastrointestinal tracts, as well as stool, sputum and hair [6, 7].

Currently, 13 *Trichosporon* species have been reported to be human pathogens including *Trichosporon cutaneum*, *T. asahii*, *T. asteroides*, *T. mucoides*, *T. inkin*, *T. jirovecii*, *T. dermatis*, *T. domesticum*, *T. montevideense*, *T. japonicum*, *T. coremiiforme*, *T. faecale*, and *T. loubieri* [8-10].

The genus *Trichosporon* is characterized by the ability to form hyphae and pseudohyphae, yeast cells, blastoconidia and arthroconidia that disarticulate from true hyphae. The presence of multilamellar cell walls [11] and dolipore septa with or without parenthesomes are important characteristics of *Trichosporon* spp. (except for *T. brassicae* and *T. pullulans*) [12]. Gueho et al. [13] examined the ribosomal RNA (rRNA) D2 region and reported that *T. pullulans* was phylogenetically distinct from the other members of the genus *Trichosporon*.

T. pullulans is now classified as new member of genus *Guehomyces* [14]. Procedures commonly used for species identification are based on the appearance of cellular

morphology and distinctive reactions in a standardized set of assimilation tests. These tests are laborious and sometimes the accuracy for identification seems to be limited due to strain variability [13, 14].

The taxonomy of the genus *Trichosporon* has been revised based on sequence analysis of partial large-subunit (LSU) rRNA sequences and the 19 accepted species [13]. DNA-based methods have been extensively used for the accurate identification of *Trichosporon* spp. [15]. For the specific identification of the genus *Trichosporon,* Sugita et al. designed the primer pair TRF and TRR, which amplifies part of the small-subunit (SSU) region. These oligonucleotides do not amplify conserved regions in the ribosomal genes of other medically important yeasts [16].

In 1999, Sugita et al. sequenced and analyzed the interspacer region (ITS1 and ITS2) genes of the ribosomal DNA (rDNA) from *Trichosporon* spp., and proposed 17 species and five varieties for this genus [17]. In the present study, we surveyed *T. coremiiforme,* isolated from soil in Guilan province of Iran, by sequencing the D1/D2 domain of the LSU rDNA gene.

Material and Methods

Isolation

During 2014, the frequency of *Trichosporon* species was studied across different areas of Iran. A total of 30 soil samples were collected from 30 different locations. In each location, samples were collected from 0–20 cm soil depth and passed through 2 mm, 40 mm and 60 mm mesh sieves. Using soil plating method [18], 10 g of soil samples were placed in 90 ml of 0.1% water agar containing 100 ppm naproxen (NPX) (nonyl phenyl polyethylene glycol ether containing a concentration of 10.5 moles of ethylene oxide). The mixture was stirred and serially diluted to 10^{-2}-10^{-5}, and then 1 ml of each solution was flooded on YM agar (3 g yeast extract, 3 g malt extract, 5 g peptone and 10 g glucose per liter of distilled water) by an L-shaped rod. These media were amended with HCl (0.7 ml/L) and chloramphenicol (25 µg/ml). Plates were incubated at 25–27 ºC for 3–5 days for colony development.

Biochemical and morphological identification

All the suspicious isolates were sub-cultured onto YM agar, amended with chloramphenicol for 10 days at 25 ºC. The isolated yeast was first identified by means of standard microbiological methods [19], based on morphological features and physiological tests such as culture on carbon and nitrogen sources (assimilation patterns) [20], growth at various temperatures, ability to hydrolyze urea and growth in cycloheximide 0.1% [21].

Molecular identification

DNA extraction

DNA extraction was performed using the above mentioned methods [22, 23]. Briefly, isolates were grown for approximately 24 hours at 25 °C in YM medium. For each isolate, one loop of cells was suspended in 100 µl distilled water, and heated for five minutes at 99 °C. The amount of obtained DNA was estimated by a spectrophotometer (CARY100 scan Varion, Australia).

DNA amplification and sequencing

D1/D2 region was amplified with NL-1 (5-GCATATCAATAAGCGGAGGAAAAG) and NL-4 (5-GGTCCGTGTTTCAAGACGG) primers [24]. Amplifications were performed in a T-Personal thermocycler (Biometra, Germany). The polymerase chain reaction (PCR) mixture contained: 10–20 ng of template DNA, 1 µM of each primer, 100 µM of dNTPs, 0.4 U *Taq* DNA polymerase (CinnaGen, Iran), 1.5 mM of $MgCl_2$, 2.5 µl of 10× PCR buffer in a reaction volume of 25 µl. All the PCRs consisted of one cycle at 95 °C for five minutes; 35 cycles at 95 °C for one minute, 55.5 °C for two minutes, 72 °C for two minutes; and a final cycle at 72 °C for ten minutes. Successful amplification was confirmed by gel electrophoresis (one hour at 80 Volts) on 1.0% agarose gels in 1× TBE buffer. Gels were stained using ethidium bromide, and DNA fragments were visualised under ultra-violet light.

Sequencing of the amplified product

To remove excess primers and nucleotides, the amplification products of all the specimens were purified through GenJET PCR purification kit (Fermentas, UK). PCR products were sequenced (Tech Dragon, Hong Kong) in forward and reverse orientations using the primers applied for amplification and Dye Terminator Cycle Sequencing kit (Big Dye sequencing kit, Applied Biosystems, USA) on an ABI377-96 automated sequencer (Applied Biosystems, USA) according to the manufacturer's instructions.

All the obtained sequences were sent to the GenBank genome database at the NCBI website (NCBI, http://www3.ncbi.nlm.nih.gov/Entrez) (Bethesda, MD, USA), using BLAST, to compare gene sequences.

Phylogenetic analysis

Closest matches to each sequence were determined using the BLASTN sequence similarity search tool in GenBank [25, 26]. Multiple alignments were performed with ClustalW [26] using the default settings, and then they were manually edited with BioEdit v.7.0.9 [27]. Phylogenetic analyses were performed with MEGA4 using maximum parsimony (MP) [28] and neighbor-joining (NJ) with the Kimura 2-parameter (K2P) models. Insertions and deletions were taken into account. The complete deletion method was employed in gap handling for all the alignment sites. All the sites containing alignment gaps were removed from the analysis before calculations, and then treated as missing data.

The confidence of branching was assessed through computing 1,000 bootstrap re-samplings [27, 29]. *Cryptococcus humicola* (GenBank accession number AF189836) were used as outgroups. The final tree and matrix of sequences were submitted to TreeBASE (University at Buffalo, USA; http://www. treebase.org).

Results

After three days at 25 °C, the colonies were cream-colored, dry, mat, nearly flat, odorless and three mm in diameter, with a finely fimbriate margin. Hyphae were present and mainly disarticulated into arthroconidia (5-7 µm) (Figure 1A). After three weeks, chlamydospore formed on YM agar medium (Figure 1B). Chlamydospores were thin-walled, globose to ellipsoidal and 3.5-5 µm in diameter.

Growth characteristics of isolates on standard carbon and nitrogen are shown in Tables 1 and 2, respectively. According to the morphological and physiological assessments, isolates were identified as *T. coremiiforme*. PCR-based analysis of D1/D2 region was performed for reconfirmation of *T. coremiiforme.*

Sequence comparisons

The D1/D2 sequences of *T. coremiiforme* isolates were amplified using NL-1 and NL-4 primers. Isolates produced a *ca* 630 base pair(bp) fragment of DNA, which was similar to those of GenBank in terms of length. The

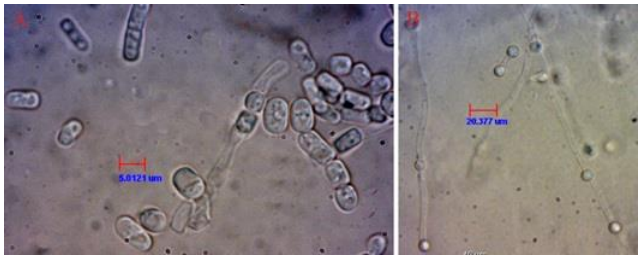

Figure 1. *Trichosporon coremiiforme*: *Arthroconidia* (A), *Chlamydospore* (B)

Table 1. Assimilation of carbon compounds by *Trichosporon coremiiforme* isolates

Glucose	+	N-Acetyl-D-glucosamine	+
Galactose	+	Methanol	-
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	-
Trehalose	+	Galactitol	v
Lactose	+	D-Mannitol	+
Melibiose	-	D-Glucitol	+
Raffinose	-	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	-	D-Gluconate	+
Soluble starch	+	DL-Lactate	+
D-Xylose	+	Succinate	+
L-Arabinose	+	Citrate	v
D-Arabinose	+	Inositol	+
D-Ribose	+	Hexadecane	n
L-Rhamnose	+	Nitrate	-
D-Glucosamine	-	Vitamin-free	-

Table 2. Assimilation of nitrogenous compounds by *Trichosporon coremiiforme* isolates

Cadaverine	+	L-Lysine	+
Nitrite	+	Creatinine	-
Ethylamine	+	Glucosamine	+

D1/D2 domain of the LSU sequences of *T.coremiiforme* isolates from Iran showed 99% homology with *T. coremiiforme* (CBS 8261, GenBank JN939454), [30] deposited in GenBank using the Blast program (http://blast.ncbi.nlm.nih.gov/blast.cgi). Accession numbers of isolates that were submitted to GenBank are KP055040 and KP055041.

Phylogenetic Inferred Trees

Both distance-based and cladistic methods were applied for phylogenetic reconstruction of 31 isolates. The *Cryptococcus humicola* (AF189836) was used as the outgroup. The D1/D2 phylogenetic trees, inferred by both distance-based (Figure 2) and cladistic methods (Figure 3), showed the same topology, although there were differences in percent bootstrapping. In the cladistic method, the tree length=226; consistency index (CI)=0.55; retention index (RI)=0.83; rescaled consistency index (RCI)=0.46 for all the sites; iCI=0.49 for parsimony informative sites and iRI=0.83. With this, 38 trees were retained.

Based on D1/D2 sequences, our isolates were clustered in a distinct monophyletic group related to *T. coremiiforme*, which was quite in line with the results of other studies. Moreover, our isolates showed high genetic relatedness within the group (99% NJ and 100% MP) and shared a common branch with *T. faecale*. All *T. coremiiforme* isolates placed in the Ovoides cluster (Figures 2, 3) were well-supported by bootstrap values (100% NJ and 100% MP). The present study was the first attempt to survey *Trichosporon* in soil of Iran.

Discussion

Trichosporon species are widely distributed in rotting wood, sludge, soil, mushrooms, plants, leaf-cutting ants, birds and mammals. In humans, these species are occasionally isolated from skin, pharynges, urine and stool [31]. These fungi are also considered as emerging fungal pathogens responsible for causing

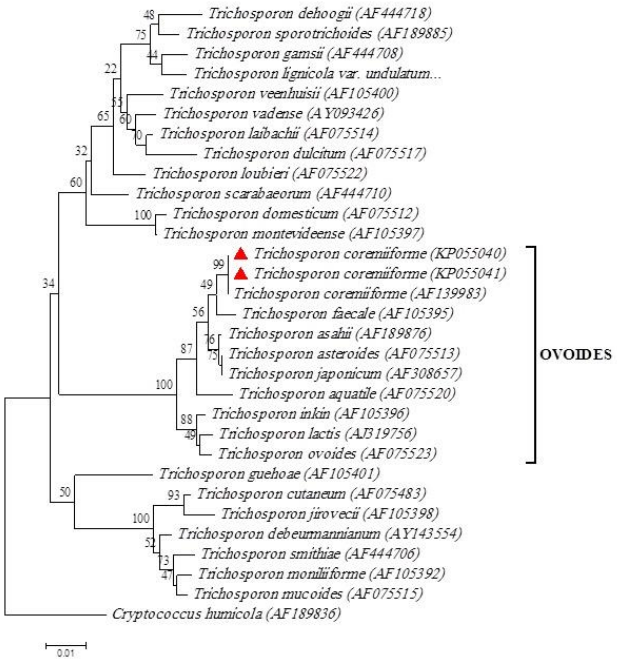

Figure 2. Consensus tree resulting from 500 bootstrap replications of the parsimony analysis of the D1/D2 domain of large subunit ribosomal DNA gene sequences (consistency index (CI)=0.55; retention index (RI)=0.83; rescaled consistency index (RCI) = 0.46). Numbers on the branch are the bootstrap values (%). The red triangle refers to *Trichosporon coremiiforme* in Iran

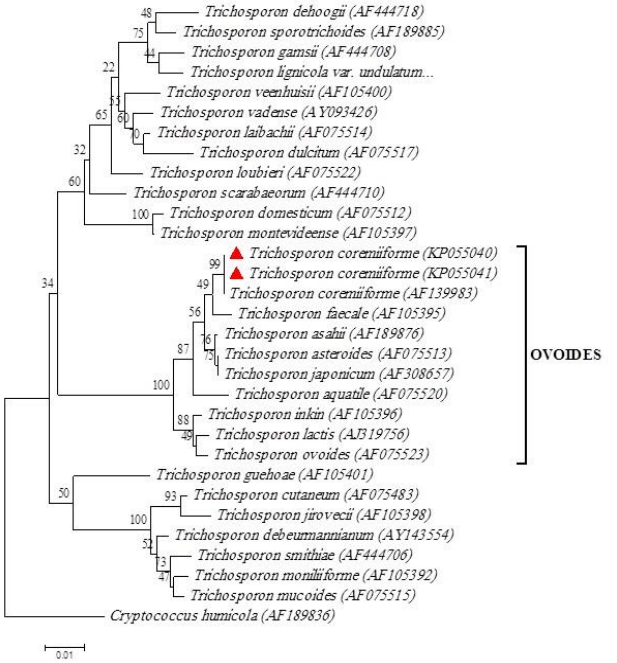

Figure 3. Neighbor joining phylogram generated in Mega from the alignment of 31 D1/D2 domain of large subunit of the ribosomal RNA sequences of *Trichosporon* species using Kimura 2-parameter model with complete deletion gap handling and 1000-replication bootstrapping. The red triangle refers to *Trichosporon coremiiforme* in Iran

the tree length=226; localized or systemic mycoses. However, there are very few published instances where *T. coremiiforme* is described as the cause of human diseases.
Interestingly, despite the fact that Sugita et al. refer to this species as nonpathogenic [32],recently one type of *T. coremiiforme* strain (CBS 2482) has been isolated and reported from a patient with a fungal infection of the head [33]. In 2005, Rodriguez-Tudela et al. recovered *T. coremiiforme* from subcutaneous abscesses and urine [10], and Chagas-Neto et al., isolated it from bloodstream infections [34]. Laachari et al., showed that *T. coremiiforme*is able to produce a lipolytic enzyme [35].

In this study, the isolates obtained from soil were identified as *T. coremiiforme* by means of the standard microbiological method [19], in which cell morphology, colony appearance, pseudo-true hyphae formation, substrate assimilation, physiological characteristics and biochemical activities were accounted for. Although *Trichosporon* species include a heterogeneous group of arthroconidia-forming yeasts, this characteristic by itself has very low distinctive value in the identification of different species, since other yeasts are also able to form these structures [34].

Identification based on carbon and nitrogen assimilation tests may not be completely reliable [36] and need to be repeated several times until the results are confirmed. As phenotype-based methods are not sensitive enough for identification of *Trichosporon* species [37], a 600 bp PCR product of the D1/D2 LSU of rDNA region was sequenced. Using PCR with the NL-1 and NL-4 primers, a fragment of about 600 bp was obtained for *T. coremiiforme* isolates. Based on D1/D2 gene sequences, these isolates showed 99% homology with *T. coremiiforme* isolates deposited in GenBank. Subsequently, both phenotypical and molecular data confirmed the identification of the *Trichosporon* isolates as *T. coremiiforme*.

The isolates formed chlamydospore after one week on YM agar medium; however, no reference to chlamydospore formation was made in the more recent standard description of *T. coremiiforme* [38], because the standard description is based on observations of cells from relatively young cultures. For the phylogenetic analysis, we applied both distance-based and maximum parsimony methods. Although, the comparison of the two types of trees shows their concordance, maximum parsimony tree demonstrates better separation and more intuitive grouping of the taxa (Figures 2, 3).

The final expected transition/transversion ratio in our results was 2.5. These results could be suitable for phylogenetic analysis and resolving species boundaries in constructed phylogram (Figures 2, 3). Phylogenetic analysis of the isolates based on 26S domain D1/D2 rDNA put them into Ovoides cluster. In 1994 and 1995, Sugita et al. proposed to classify the genus *Trichosporon* into 17 species and 5 varieties. In 2002, Sugita et al. proposed 25 species for the genus *Trichosporon* [32].

In 2004, Middlehoven et al. proposed that the *Trichosporonales* can be separated into four clusters with the names of Gracile, Porosum, Cutaneum and Ovoides [36]. Our results were in agreement with the identity of these groups, but Porosum cluster included in the Gracile cluster (Figures 3). Sugita et al. also analyzed the sequences of IGS1, in 25 isolates of *Trichosporon* [32]. They reported that the rDNA internal transcribed spacer (ITS) region is not suitable for *Trichosporon* species identification [32].

The first scientific reference to the presence of *Trichosporon* in Iran was made by Fallahi et al. who recorded *T. faecale* [39]. More recently, PCR-based methods have been extensively used for the accurate identification of *Trichosporon* species. Rodriguez-Tudela et al., accurately identified 49 *Trichosporon* clinical isolates using intergenic spacer (IGS) region sequencing and tested their susceptibility to antifungal agents [40]. Nakajima et al., used a PCR technique and identified *T. asahii* as the causative agent of infection [41]. *Trichosporon* spp. can also be detected from biological fluids using PCR assays [42].

Sugita et al., constructed a phylogenetic tree using the SSU region sequences of rDNAs

from different pathogenic yeasts obtained from DNA libraries [16]. Subsequently, Sugita et al., sequenced and analyzed the ITS1 and ITS2 genes of the rDNA from *Trichosporon* spp. and proposed 17 species and five varieties for this genus [17]. Based on the ITS1 and ITS2 sequences, Leaw et al. developed an oligonucleotide array to identify 77 species of clinically relevant yeasts belonging to 16 genera [43]. Real-time quantitative PCR [44] and sequence of mitochondrial cytochrome b [45] were also developed for identification of *Trichosporon* species.

Considering strain variability, identification of *Trichosporon* species on the basis of their morphology and distinctive reactions in a standardized set of assimilation tests can be difficult or misleading at times [46, 47]. Therefore, application of molecular phylogenetics for accurate identification of these species is inevitable.

Conclusion

The present study is the first attempt to survey *Trichosporon* in soil of Iran. To the best of our knowledge, this is the first investigation of *T. coremiiforme* in Iran

Authors' Contributions

M. Gh. obtained the specimen and performed all the tests, S. J. wrote, designed, reviewed and edited the article.

Conflicts of Interest

The authors state no conflict of interest.

Financial Disclosure

The authors declare no financial interests related to the materials of the study.

References

1. Colombo AL, Padovan AC, Chaves GM. Current knowledge of Trichosporon spp. and Trichosporonosis. Clin Microbiol Rev. 2011; 24(4):682-700.
2. Fell JW, Boekhout T, Fonseca A, Scorzetti G, Statzell-Tallman A. Biodiversity and systematics of basidiomycetous yeasts as determined by large-subunit rDNA D1/D2 domain sequence analysis. Int J Syst Evol Micr. 2000; 50(Pt 3):1351-71.
3. Sood S, Pathak D, Sharma R, Rishi S. Urinary tract infection by Trichosporon asahii. Indian J Med Microbiol. 2006; 24(4):294-6.
4. Gabriel F, Noël T, Accoceberry I. Fatal invasive trichosporonosis due to Trichosporon loubieri in a patient with T-lymphoblastic lymphoma. Med Mycol. 2011; 49(3):306-10.
5. Moreno-Coutiño G, Aquino MA, Vega-Memije M, Arenas R. Necrotic ulcer caused by Trichosporon asahii in an immunocompetent adolescent. Mycoses. 2012; 55(1):93-4.
6. Nakagawa T, Nakashima K, Takaiwa T, Negayama K. Trichosporon cutaneum (Trichosporon asahii) infection mimicking hand eczema in a patient with leukemia. J Am Acad of Dermatol. 2000; 42(5 Pt 2):929-31.
7. Xia Z, Yang R, Wang W, Cong L. Genotyping and antifungal drug susceptibility of Trichosporon asahii isolated from Chinese patients. Mycopathologia. 2012; 173(2-3):127-33.
8. Chagas-Neto TC, Chaves GM, Colombo AL. Update on the genus Trichosporon. Mycopathologia. 2008; 166(3):121-32.
9. Pasqualotto AC, Sukiennik TC, Severo LC, Amorim CS, Colombo AL. An outbreak of Pichia anomala fungemia in a Brazilian pediatric intensive care unit. Infect Control Hosp Epidemiol. 2005; 26(6):553-8.
10. Rodriguez-Tudela JL, Diaz-Guerra TM, Mellado E, Cano V, Tapia C, Perkins A, et al. Susceptibility patterns and molecular identification of Trichosporon species. Antimicrob Agents Chemother. 2005; 49(10):4026-34.
11. Kreger-van Rij NJW, Veenhuis M. A comparative study of the cell wall structure of basidiomycetous and related yeasts. J Gen Microbiol. 1971; 68(1):87-95.
12. Guého E, Smith MT, de Hoog GS, Billon-Grand G, Christen R, Batenburg-van Vegte WH. Contributions to a revision of the genus Trichosporon. Antionie Van Leeuwenhoek. 1992; 61(4):289-316.
13. Gueho E, de Hoog GS, Smith MT. Neotypification of the genus Trichosporon. Antionie Van Leeuwenhoek. 1992; 61(4):285-8.
14. Fell JW, Gueho-Kellermann E. Guehomyces Fell & Scortzetti. In: Kurtzman C, Fell JW, Boekhout T, editors. The yeasts: a taxonomic study. 3th ed. New York: Elsevier; 2011. P. 1773-5.
15. Pfaller MA. Epidemiology of fungal infections: the promise of molecular typing. Clin Infect Dis. 1995; 20(4):1535-9.
16. Sugita T, Nishikawa A, Shinoda T. Rapid detection of species of the opportunistic yeast Trichosporon by PCR. J Clin Microbiol. 1998; 36(5):1458-60.
17. Sugita T, Nishikawa A, Ikeda R, Shinoda T. Identification of medically relevant Trichosporon species based on sequences of internal transcribed spacer regions and construction of a database for

Trichosporon identification. J Clin Microbiol. 1999; 37(6):1985-93.
18. Waksman SA, Fred EB. A tentative outline of the plate method for determining the number of microorganisms in the soil. Soil Sci. 1922; 14(1):27-8.
19. Kurtzman CP, Droby S. Metschnikowia fructicola, a new ascosporic yeast with potential for biocontrol of postharvest fruit rots. Sys Appl Microbiol. 2001; 24(3):395-9.
20. Kurtzman C, Fell JW. The Yeasts: a taxonomic study. North-Holland, Amsterdam: Elsevier; 1999. P. 1055.
21. de Hoog GS, Guarro J, Gené J, Figueras MJ. Atlas of clinical fungi. 2th ed. Utrecht, Netherlands: Centraalbureau voor Schimmelcultures (CBS); 2000.
22. Esteve-Zarzoso B, Belloch C, Uruburu F, Querol A. Identification of yeasts by RFLP analysis of the 5.8S rRNA gene and the two ribosomal internal transcribed spacers. Int J Syst Bacteriol. 1999; 49(Pt 3):329-37.
23. Suh SO, Chen Y, Zaman MS, Hirata H, Yamamura S, Shahryari V, et al. MicroRNA-145 is regulated by DNA methylation and p53 gene mutation in prostate cancer. Carcinogenesis. 2011; 32(5):772-8.
24. O'Donnell K, Cigelnik E. Two Divergent Intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus fusarium are nonorthologous. Mol Phylogenet Evol. 1997; 7(1):103-16.
25. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 1997; 25(17):3389-402.
26. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 1997; 25(24):4876-82.
27. 27. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Axford: Nucleic acids symposium series; 1999. P. 95-8.
28. Kumar S, Tamura K, Nei M. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform. 2004; 5(2):150-63.
29. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution. 1985: 39(4):783-91.
30. Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge Jl, Levesgue CA, et al. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc Natl Acad Sci U S A. 2012; 109(16):6241-6.
31. Obana Y, Sano M, Jike T, Homma T, Nemoto N. Differential diagnosis of trichosporonosis using conventional histopathological stains and electron microscopy. Histopathology. 2010; 56(3):372-83.
32. Sugita T, Nakajima M, Ikeda R, Matsushima T, Shinoda T. Sequence analysis of the ribosomal DNA intergenic spacer 1 regions of Trichosporon species. J Clin Microbial. 2002; 40(5):1826-30.
33. Moore M. Head infection caused by a new Hemispora: H. coremiiforme. Ann Mo Bot Gard. 1935; 22(2):317-32.
34. Chagas-Neto TC, Chaves GM, Melo AS, Colombo AL. Bloodstream infections due to Trichosporon spp.: species distribution, Trichosporon asahii genotypes determined on the basis of ribosomal DNA intergenic spacer 1 sequencing, and antifungal susceptibility testing. J Clin Microbiol. 2009; 47(4):1074-81.
35. Laachari F. Purification and characterization of a Novel Thermostable Lipase from Aspergillus flavus. Int J Res. 2015; 2(2):342-52.
36. Middelhoven WJ, Scorzetti G, Fell JW. Systematics of the anamorphic basidiomycetous yeast genus Trichosporon Behrend with the description of five novel species: Trichosporon vadense, T. smithiae, T. dehoogii, T. scarabaeorum and T. gamsii. Int J Syst Evol Microbiol. 2004; 54(Pt 3):975-86.
37. Guo LN, Xiao M, Kong F, Chen SC, Wang H, Sorrll TC, et al. Three-locus identification, genotyping, and antifungal susceptibilities of medically important Trichosporon species from China. J Clin Microbiol. 2011; 49(11):3805-11.
38. Kurtzman C, Fell JW, Boekhout T, Sugita T. Trichosporon Berhend. Lodder J, Kreger-van Rij NJW, editors. In: The yeasts: a taxonomic study. New York: Elsevier; 2011. P. 2015-61.
39. Fallahi AA, Moazeni M, Noorbakhsh F, Koorbakhsh P, Zaini F, Mirchendi H, et al. A case report of tinea pedis caused by Trichosporon faecale in Iran. Med Mycol Case Rep. 2012; 1(1):49-51.
40. Fujita SI, Senda Y, Nakaguchi S, Hashimoto T. Multiplex PCR using internal transcribed spacer 1 and 2 regions for rapid detection and identification of yeast strains. J Clin Microbiol. 2001; 39(10):3617-22.
41. Nakajima M, Sugita T, Mikami Y. Granuloma associated with Trichosporon asahii infection in the lung: Unusual pathological findings and PCR detection of Trichosporon DNA. Med Mycol. 2007; 45(7):641-4.
42. Nagai H, Yamakami Y, Hashimoto A, Tokimatsu I, Nasu M. PCR detection of DNA specific for Trichosporon species in serum of patients with disseminated trichosporonosis. J Clin Microbiol. 1999; 37(3):694-9.
43. Leaw SN, Chang HC, Barton R, Bouchara JP, Chang TC. Identification of medically important Candida and non-Candida yeast species by an oligonucleotide array. J Clin Microbiol. 2007; 45(7):2220-9.
44. Makino H, Fujimoto J, Watanabe K. Development and evaluation of a real-time quantitative PCR assay for detection and enumeration of yeasts of public

health interest in dairy products. Int J Food Microbiol. 2010; 140(1):76-83.
45. Biswas SK, Wang L, Yokoyama K, Nishimura K. Molecular phylogenetics of the genus Trichosporon inferred from mitochondrial cytochrome B gene sequences. J Clin Microbiol. 2005; 43(10):5171-8.
46. Sugita T, Nishikawa A, Shinoda T. Reclassification of Trichosporon cutaneum by DNA relatedness by the spectrophotometric method and the chemiluminometric method. J Gen Appl Microbiol. 1994; 40(5):397-408.
47. Walsh T, Groll A, Hiemenz J, Fleming R, Roilides E, Anaissie E. Infections due to emerging and uncommon medically important fungal pathogens. Clin Microbiol Infect. 2004; 10(Suppl 1):48-66.