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Research Article

GENOMIC PROFILE OF A NEW NOVEL FUNGAL ISOLATE, *COCHLIOBOLUS* Sp. FROM HUMAN EYE INFECTIONS

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ABSTRACT

Identification of fungi by morphological traits alone is often difficult because mycelial pigmentation, shape and size of conidia are unstable and highly dependent on composition of media and environmental conditions. Molecular techniques such as Internal Transcribed Spacer (ITS) ribosomal DNA (rDNA) have been reliable and are highly suitable tool for identifying fungal species and for assessing genetic variation within populations. The correct identification of fungal isolates from infected eye will help to develop drug against those microbes and also to avoid the possible source of such infections. From the ocular sample collected from infected patients, *Cochliobolus sp* was identified by basic culture techniques and its genomic profile was evaluated ITS r DNA technology.

Keyword: Internal Transcribed spacer (ITS), *Cochliobolus* sp, Genomic tools, Molecular identification.

INTRODUCTION

Human pathogenic fungi are a diverse group morphologically, ecologically and clinically (Rippon, 1988). Many species of fungi commonly classified as fungal ophthalmic pathogens have been reported to cause eye infections (Eduardo and Alfonso, 2008). The warm and moist environment of the eye is subject to mycotic infections from fungal species such as *Fusarium* , *Aspergillus*, *Acremonium* and the *Candida* species of Yeasts. Due to the preferences for moisture – rich environment and warm weather, the prevalence of fungal infections of the eye is higher in trophical areas (Alcazar - Fouli *et al*., 2008). Accurate identification of these agents is critical for rapid diagnosis. This will also help in advancing our understanding of each species and may lead to more identification methods and effective antifungal therapy and improve clinical outcomes. Molecular techniques such as Internal Transcribed Spacer (ITS) ribosomal DNA (rDNA) have been reliable and are highly suitable tool for identifying variable species and for assessing genetic variations within populations (Galgiani and Ampal, 1990).

The internal transcribed spacer (ITS) region of the nuclear ribosomal repeat unit is the most popular locus for species identification and sub generic phylo genetic inference in sequence – based mycological research. The ITS spacers 1 and 2 which are found between the small (18S) and the large (28S) ribosomal subunit genes can show variability at an intraspecific level (Geln *et al.,* 2001, Nilsson *et al.,* 2008). Hence in the present study the fungus *Cochliobolus sp* isolated from eye infected patients was characterized using molecular techniques.

MATERIALS AND METHODS

Clinical sample from ocular patients

Samples collected from clinically suspected infectious patients (with pain, redness, and a history of trauma due to stick injury for a week)

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visited an Eye Care hospital, at Tirunelveli, India. Intra ocular sampling was performed under aseptic conditions by an ophthalmologist. After collection the samples were immediately taken to a microbiological laboratory under appropriate condition (Therese *et al.*, 1998) and processed for fungal culture. The sample obtained were inoculated on to Sabouraud Dextrose Agar(SDA) as streak plates and incubated at 28˚C. After 72h of incubation growth of fungi were observed. The samples were stored at - 20˚C until further study.

Genomic DNA isolation, PCR amplification and sequencing

Total genomic DNA was extracted from mycelia of the unique fungus grown on Sabouraud Dextrose Agar medium by using TAB method (Cai *et al*.,2006). DNA amplification was performed by PCR. The PCR was set up using the following components. 5µl Buffer (10x), 5µl $MgCl_2$ (25mM), 1µl dNTPs (10mM), 1.5µl Taq Polymerase (5uM), 1.5µl Forward primer (10µM), 1.5.µl Reverse Primer (10µM), 3µl DNA Template and 34.7µl distilled water. The PCR condition was run in such a way, where initial denaturation was at 95˚C for 5 min. Denaturation, annealing and elongation were done at 95˚C for 1 min, 52˚C for 30 sec. and 72˚C for 1 min. respectively in 45 cycles. Final extension was done at 72˚C for 10min and hold at 4˚C forever. For amplification of ITS – rDNA region ITS4 and ITS5 primers were used according to the method described by White (*et al.* 1990). The PCR product, spanning approximately 500-600bp was checked on 1% agarose electrophoresis gel. It was then purified using quick spin column and buffers (Washing buffer and elution buffer) according to the manufacturer's protocol (QIA quick gel extraction kit, Cat No. 28708). DNA sequencing was performed using the above mentioned primers in an Applied Biosystem 3130xL analyzer.

Sequence and phylogenetic analysis

The sequence was annotated using sequin software and submitted to NCBI Gen Bank database. BLAST homology search was performed to find the closest homology and sequence analysis was done by comparison of ITS rDNA sequence of ten other fungal species obtained from Gen Bank database. The evolutionary tree was reconstructed using the Neighbor – Joining (NJ) method.

RESULTS AND DISCUSSION

In this study molecular tools based on ITS rDNA have been used to identify fungus from eye infected patients. The ITS sequence based identification showed that the unreported fungal species is *Cochliobolus sp*. The sequence of the fungi has been deposited in the Gene Bank database with Accession Number KJ913671. The EMBL date of release of the data was Sep. 1.2014. A summary of the project information is given below.

Table 1. The sequence of the fungi (EMBL date).

.S.No.	Property	Term
1.	EMBL ID	KJ913671
2.	EMBL Date of Release	September 01.2014
3.	Sequencing Technology	Sanger dideoxy sequencing
4.	LOCUS	455 bp
5.	DNA	Linear PLN 27-AUG-2014
6.	Definition	*Cochliobolus sp.* Sumitha – 01 Internal transcribed spacer – 1,

		Partial sequence, 5.8S ribosomal RNA gene and internal transcribed spacer. 2 complete sequence and 28 S ribosomal RNA gene, Partial sequence.
7.	Source Organism	*Cochliobolus sp.* Sumitha – 01. Eukaryota, Fungi – Dikerys, Ascomycota, Pezizomycolina; Dothideomycetes; Plesoporomycetidae; Pleosporales; Pleosporineae; Pleosporaceae; *Cochliobolus sp.*
8.	Reference	1 (bases 1 to 455)
9.	Location / Qualifiers Source	1.455
10.	Source	Eye pathogen from eye infected patients
11.	Tax on	1539479
	RNA	<1..>455
12.		Contains internal transcribed spacer 1, 5.8S ribosomal RNA, internal transcribed spacer 2, and 285 ribosomal RNA.
13.	Project Relevance	Study of new sp isolated from ocular patients
14.	ORIGIN	1 cccgccacca ggaccacacc ataaaccttt tttatgcagt tgcaatcagc gtcagtataa 61 caaatgtaaa tcatttacaa ctttcaacaa cggatctctt ggttctggca tcgatgaaga 121 acgcagcgaa atgcgatacg tagtgtgaat tgcagaattc agtgaatcat cgaatctttg 181 aacgcacatt gcgccctttg gtattccaaa gggcatgcct gttcgagcgt catttgtacc 241 ctcaagcttt gcttggtgtt gggcgtcttt tgtctttggc ctcgcccaaa gactcgcctt 301 aaaacgattg gcagccggcc tactggtttc gcagcgcagc acatttttgc gcttgcaatc 361 agcaaaaagg acggcaatcc atcaagacta cattttacg tttgacctcg gatcaggtag 421 ggatacccgc tgaacttaag catatcaata agcgg

The present study demonstrated that identification of unknown fungal samples is possible using Genomic tools characteristics of the present fungi KJ913671 and their reference and related species were described.

A querry against the National Center of Biotechnology Information homology search was performed to find the closest homologs and sequence analysis was done by comparison of ITS rDNA sequences of ten other pathogenic *cochliobolus sp* obtained from Gene Bank database. Figure 1. explained the graphic summary of the BLAST query sequence. The fungal ITS rDNA sequences were aligned. Figure 2. gave a detail arrangement of the fungus *cochliobolus* ITS rDNA sequence. The Fungal sample Clustal analysis was given in Figure 3. The distinguishing characteristics of the present fungi KJ913671 and their reference and related species were described.

The bioinformatics tools BLAST, similarity search tool, CLUSTAL W-PHYLODRAW were used to evaluate the similarity among the query sequence and the reference sequences in the genbank. The newly identified eye pathogen *Cochliobolus* has been sequenced for its ITS rDNA. This query was used for running BLAST. Many red coloured hits were produced against query sequences. Among the hits, more than ten hits have shown 100% similarity with query ITS rDNA sequence. The top ten hits were identified as ITS rDNA sequences of fungi were fungi endophyte culture STRI,*Cochliobolus sp* sumitha-01,*Curvularia lunata* A2S2-1, *Curvularia sp V1JC-2012 UTHSC;09-2085 Curvularia sp V1JC-2012 uthsc;09-3124 Cochliobolus lunatus Curvularia aeria FMR 11512, Curvularia aeria FMR11502, Curvularia aeria-2859and Curvularia lunata.* These fungi were known to be close relatives of *Cochliobolus sp* in terms of molecular taxonomy.

Ten sequences in the graphic hits were used to draw the phylogenetic tree using the bioinformatics tool PHYLODRAW. The result page has shown the tree view with fungi in respective branches. The distances have shown the percentage of relativeness among them. The PHYLODRAW utilized the syntax neighbour joining. There were different types of trees – Single, Slanted, Radial and Force. The type of tree selected here was FORCE.

From this tree it was inferred that *Cochliobolus sp* might have originated from *Curvularia lunata* and has close relationships with *Curvularia sp.*

The fungal genus *Cochliobolus* include 55 species including plant pathogenic species. The genus *Cochilobolus* is distinguished by the presence of dark ascomata with a unilocular, globose pseudothecium and a short cylindrical neck (Manamgoda *et al.*, 2011). It is a destructive plant pathogen that causes severe crop loses. The fungi can survive as mycelium in soil and crop debris. Any ocular trauma due to stick injury or soil may lead to *cochliobolus* infection on eyes. Several highly divergent fungal species have been documented within the ITS region of ribosomal RNA (rRNA) of fungi. Tsui et al., (2011) in their studies have revolutionized research in fungal detection and identification using latest technologies like FLSH, LAMP, multiplex tandem PCR, Isothermal system etc. this endophytic fungi – *cochliobolus sp* have been identified from the fruits of Chinese boxhorn (Lycium chinense MILL) using PCR and Gene sequencing Techniques by Paul *et al.*, (2014).

Figure 4. showed phylogenetic tree analysis and their evolutionary relationships of ten pathogenic fungal species. The tree was drawn to scale with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The radial method of phylogenetic three showed the node which differ with various species.

The identified fungal species is close to *Cochliobolus* group and it is a new report of this type of fungi from human eye infection. The survival of this plant based fungal strain in human eye shows that agricultural workers must take precaution to protect their eyes from possible eye infections.

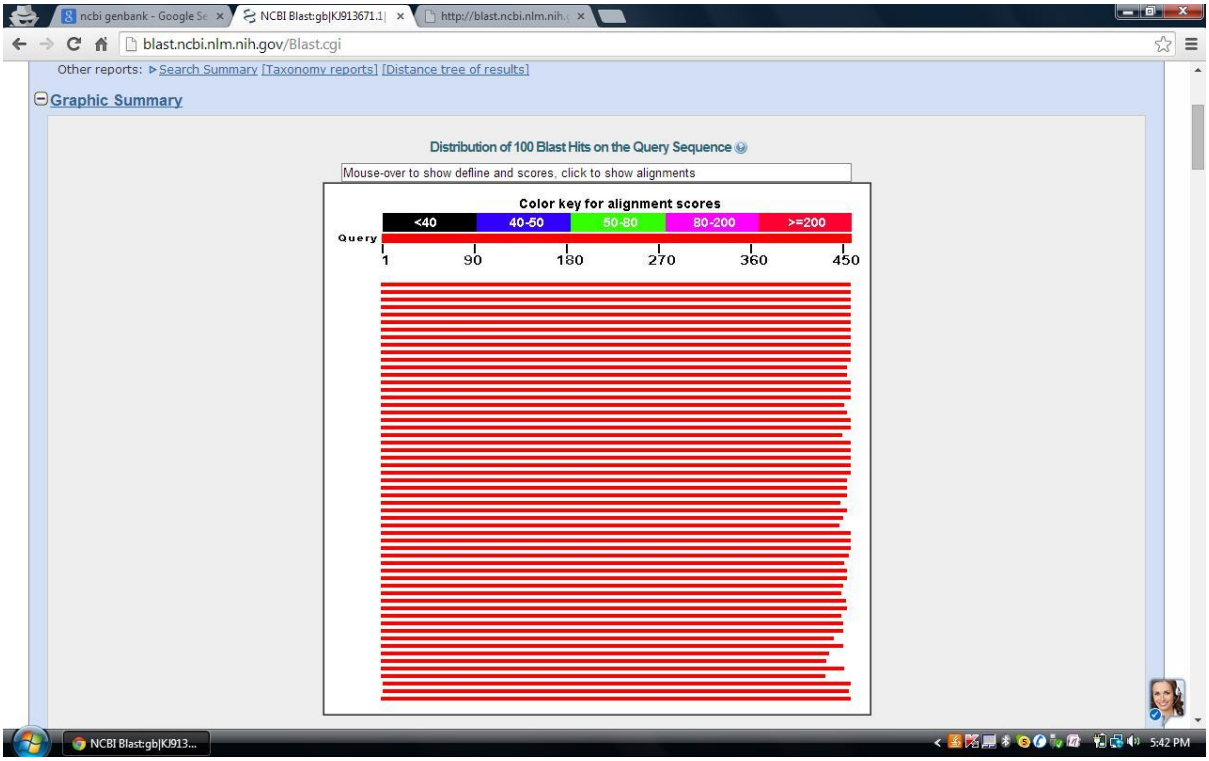

Figure 1. Graphic summary of Blast Query Sequence.

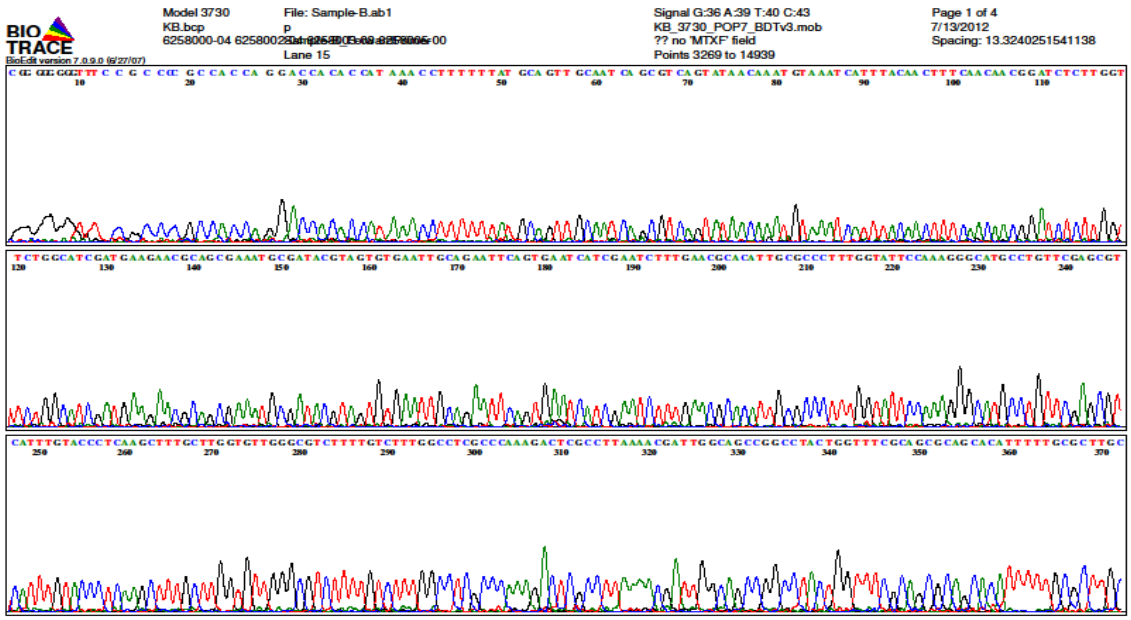

Figure 2. Arrangement of fungal DNA sequence.

Cochliobolus sp. Sumitha-01 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Sequence ID: gb|KJ913671.1|Length: 455Number of Matches: 1
Related Information
Range 1: 1 to 455GenBankGraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
841 bits(455)	0.0	455/455(100%)	0/455(0%)	Plus/Plus

```
Query  1
CCCGCCACCAGGACCACACCATAAACCTTTTTTATGCAGTTGCAATCAGCGTCAGTATAA  60

||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  1
CCCGCCACCAGGACCACACCATAAACCTTTTTTATGCAGTTGCAATCAGCGTCAGTATAA  60

Query  61
CAAATGTAAATCATTTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGA  120

||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  61
CAAATGTAAATCATTTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGA  120

Query  121
ACGCAGCGAAATGCGATACGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG  180

||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  121
ACGCAGCGAAATGCGATACGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG  180

Query  181
AACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCGTCATTTGTACC  240

||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  181
AACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCGTCATTTGTACC  240

Query  241
CTCAAGCTTTGCTTGGTGTTGGGCGTCTTTTGTCTTTGGCCTCGCCCAAAGACTCGCCTT  300

||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Sbjct  241
CTCAAGCTTTGCTTGGTGTTGGGCGTCTTTTGTCTTTGGCCTCGCCCAAAGACTCGCCTT  300

Query  301
AAAACGATTGGCAGCCGGCCTACTGGTTTCGCAGCGCAGCACATTTTTGCGCTTGCAATC  360

||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  301
AAAACGATTGGCAGCCGGCCTACTGGTTTCGCAGCGCAGCACATTTTTGCGCTTGCAATC  360

Query  361
AGCAAAAAGGACGGCAATCCATCAAGACTACATTTTTACGTTTGACCTCGGATCAGGTAG  420

||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  361
AGCAAAAAGGACGGCAATCCATCAAGACTACATTTTTACGTTTGACCTCGGATCAGGTAG  420

Query  421  GGATACCCGCTGAACTTAAGCATATCAATAAGCGG  455
             |||||||||||||||||||||||||||||||||
Sbjct  421  GGATACCCGCTGAACTTAAGCATATCAATAAGCGG  455
```

Figure 3. Fungal sample cluster analysis.

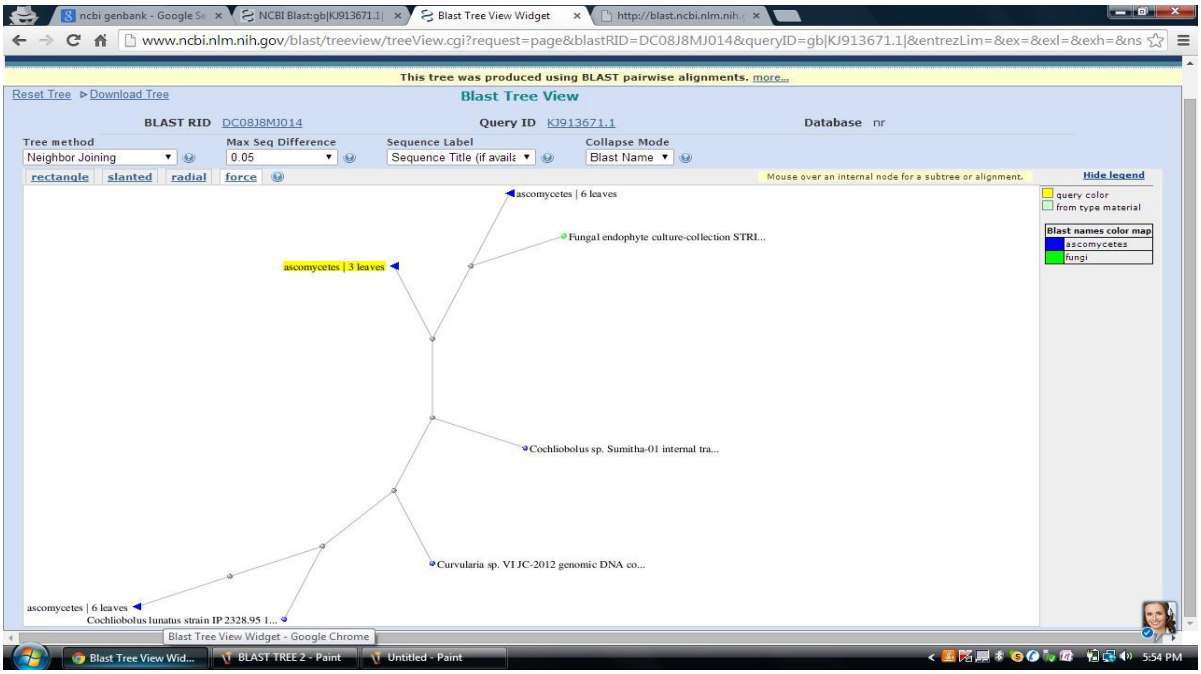

Figure 4. Phylogenitic tree of Fungal Isolate.

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REFERENCES

Alcazar Fouli. L., Emilia Mellado, A., Alastruey-Izquierdo, Manuel Cuenca-Estrella and Juan L. Rodriguez-Tudela, 2008. *Aspergillus* Section *Fumigati*: Antifungal sustainability patterns and sequence-based identification. *Antimicrob. Agents Chemother*., 52(4): 1244 -1251.

Cai, L., Jeewon, R., Hyde, K.D., 2006. Phylogenetic investigation of sordariaceae based on multiple gene sequences and morphology. *Mycol. Res.,* 110: 137-150.

Eduardo, C. and Alfonso, M.D., 2008. Gentotypic identification of *Fusarium* species from ocular sources: Comparison to morphologic classification and antifungal sensitivity testing. *Transac. Ame. Ophthalmol. Soc.*, 106: 227-239.

Galgiani, J.N., and Ampel, N.M. 1990. Coccidioidomycosis in human immunodeficiency virus-infected patients. *J. Infect. Dis.*, 162: 1165-l 169.

Geln, M., Tommerup, I.C., Bougher, N.L. and O'Brien, P.A., 2001. Interspecific and intraspecific variation of ectomycorrhizal fungi associated with Eucalyptus ecosystems as revealed by ribosomal DNA PCR-RFLP. *Mycological Res.*, 105: 843-858.

Manamgoda, D., Cai, L., Buhkali, A., Chukeatirote, E. and Hyde, K., 2011. *Cochliobolus*, an overview and current status of species. *Fungal Diversity,* 51: 3-42.

Nilsson, R.H., Kristiansson, E., Ryberg, M., Hallenberg, N. and Larsson, K.H., 2008. Intraspecific ITS variability in the kingdom fungi as expressed in the international sequence databases and its implications for molecular species identification. *Evol. Bioinformatics,* 4: 193-201.

Paul Narayan Chandra, Hyang Burm Lee, Ji Hye Lee, Kyu Seop Shin , Tae Hee Ryu, Hye Ri Kwon, Yeong Kuk Kim, Young Nam Youn and Seung Hun Yu, 2014. Endophytic Fungi from *Lycium chinense* mill and characterization of two new Korean records of *Colletotrichum. Int. J. Mol. Sci.* 15: 15272-15286.

Rippon, J. W. 1988. Medical mycology: the pathogenic fungi and the pathogenic actinomycetes, 3d ed. W. B. Saunders, Philadelphia.

Therese, K.L., Anand, A.R., Madhavan, H.N., 1998. Polymerase chain reaction in the diagnosis of bacterial endophthalmitis. *Br. J. Ophthalmol*. 82: 1078-1082. 10.1136/bjo.82. 9.1078.

Tsui Clement, K.M., James Woodhall, Wen Chen, C. André Lévesque, Anna Lau, Cor, D. Schoen, Christiane Baschien, Mohammad J. Najafzadeh, and G. Sybren de Hoog, 2011. Molecular techniques for pathogen identification and fungus detection in the Environment. *IMA Fungus*, 2: 177-189.

White, T.J., Bruns, T.D., Lee, S., and Taylor, J.W. 1990. Amplification and direct sequencing of fungal rRNA genes for phylogenetics. In: M.A. Innis, D.H/ Gelfand, J. Sninsky, T.J. White (Eds.): Protocols-A Guide to Methods and PCR Application. San Diego, Academic Press, p. 315-322.