■ Brief Communication ■

A Comparison of the Ability of Fungal Internal Transcribed Spacers and D1/D2 Domain Regions to Accurately Identify *Candida glabrata* Clinical Isolates Using Sequence Analysis

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Candida glabrata is the second most prevalent causative agent for candidiasis following *C. albicans*. The opportunistic yeast, *C. glabrata*, is able to cause the critical bloodstream infections in hospitalized patients. Conventional identification methods for yeasts are often time consuming and labor intensive. Therefore, recent studies on sequence-based identification have been conducted. Recently, sequencing the D1/D2 domain of the large subunit ribosomal RNA gene and the internal transcribed spacers (ITS) 1 and ITS2 regions of the ribosomal DNA has proven useful for DNA-based identification of most species of fungi. In the present study, therefore, fungal ITS and D1/D2 domain regions were targeted and analyzed by DNA sequencing for the accurate identification of *C. glabrata* clinical isolates. A total of 102 *C. glabrata* clinical isolates from various clinical samples including bloodstream, catheterized urine, bile and other body fluids were used in the study. The results of the DNA sequence analysis showed that the mean standard deviation of species identity percent score between ITS and D1/D2 domain regions was 97.8% ± 2.9 and 99.7% ± 0.46, respectively. These results revealed that the D1/D2 domain region might be a better target for identifying *C. glabrata* clinical isolates based on DNA sequences than the ITS1 and ITS2 regions. However, in order to evaluate the usefulness of D1/D2 domain region for species identification of all *Candida* species, other *Candida* species such as *C. albicans*, *C. tropicalis*, *C. dubliniensis*, and *C. krusei* should be verified in further studies additionally.

Key Words: *Candida glabrata*, Sequence analysis, D1/D2 domain, Internal transcribed spacers domain, Clinical isolates

Candida species are the most common cause of fungal healthcare-associated infections and, in particular, nosocomial bloodstream infections (BSIs). These organisms are part of the commensal microorganisms in the human body and, rarely cause superficial or systemic fungal infections. Although,

Candida albicans is the most frequently isolated pathogenic yeast in human, the incidence of non-*C. albicans* infections is also increasing (Katiyar et al., 2016; Amanloo et al., 2018; Kim et al., 2018).

Aside from *C. albicans*, *C. glabrata* is presently consi-

dered to be the most frequently isolated *Candida* spp. and constitutes a normal floral yeast in the human gastrointestinal tract, urogenital tract, and oral cavity. *C. glabrata* causes critical BSIs in hospitalized patients (Byun et al., 2018) and yet, despite its increased incidence, little is known of its molecular structure, epidemiology, and basic biological properties. Its prevalence has increased in the last decade and these species now rank as the second most frequently isolated *Candida* spp. of all reported candidiasis cases. The increase in the incidence of *C. glabrata* infections is noteworthy because of its decreased susceptibility to fluconazole and the high crude mortality rate associated with BSIs (Andrew et al., 2003).

Traditionally, the identification of *Candida* spp. has been performed using biochemical analysis, substrate assimilation methods, morphological examinations, or various combinations thereof. To increase ease of identification of *Candida* spp., commercial tests that use these methods have been created. However, despite the convenience inherent to these methods, the identification of yeasts by conventional applications remains time consuming and labor intensive.

Sequencing the D1/D2 domain of the large subunit RNA gene and the internal transcribed spacer (ITS) 1 and ITS2 regions of the ribosomal DNA (rDNA) has proven useful for the identification of most fungi based on the DNA sequencing. Recent studies have also examined the effectiveness of various molecular identification methods for yeasts using rRNA genes. The ITS1 and ITS2 regions that span the D1/D2 domain have been shown to be the most useful for species-level identification of yeasts as a result of variability within these regions. In particular, DNA sequence analysis of these regions has shown great promising in the practice of clinical mycology. The ability to differentiate between clinical yeast isolates obtained from humans has been demonstrated in several large-scale studies following analysis of these regions (Garner et al., 2010).

Therefore, the current study objective was to target and analyze the fungal ITS and D1/D2 domains using DNA sequencing in order to accurately identify *C. glabrata* clinical isolates.

A total of 102 *C. glabrata* clinical isolates were sourced from the Korean Culture Collection of Medical Fungi (KCMF), Daejeon, the Republic of Korea. Clinical isolates were isolated from various clinical samples, including blood, catheterized urine, bile, and other body fluids (Table 1).

Genomic DNA (gDNA) from *C. glabrata* clinical isolates was extracted using an I-genomic BYF DNA Extraction Mini Kit® (iNtRON Biotechnology, Seongnam, Republic of Korea) according to the manufacturer's instructions. The concentration and purity of the gDNA was checked by absorbance (Optical density ratio of 260/280) using a NanoDrop™ 2,000 Spectrophotometer® (Thermo Scientific, Waltham, MA, USA) (Da Silva-Rocha et al., 2014). The extracted gDNA was stored at -20 ℃ until use.

The fungal ITS regions, the conserved regions between

Table 1. Sources of *Candida glabrata* clinical isolates

Clinical specimens	Number of samples, n (%)
Peripheral blood	64 (63)
Catheterized urine	14 (14)
Others[a]	14 (14)
Bile	9 (9)
Unknown[b]	1 (1)
Total	102 (100)

[a] Others: ascitic fluid, joint fluid, pleural fluid, tissue etc.
[b] No information

Table 2. Oligonucleotide primers for the sequence analysis of the fungal ITS and D1/D2 domain regions

Target regions	Primers	Nucleotide sequences (5' to 3')	Amplicon size (bp)	Tm (℃)	References
ITS	ITS-F	GTCCTAACAAGGTTAACCTGCGG	970~980	62.4	Pryce et al. (2003)
	ITS-R	TCCTCCGCTTATTGATATGC		55.3	
D1/D2	NL-F	GCATATCAATAAGCGGAGGAAAAG	600~650	59.3	Hesham et al. (2014)
	NL-R	GGTCCGTGTTTCAAGACGG		58.8	

the 18S and 28S rRNA, and the D1/D2 domain of the 28S rDNA region were amplified and sequenced using each primer pairs (Fig. 1, Table 2). Target amplification was carried out in 20 μL of sequenced reaction mixture containing 10 μL of Prime TaqPrimix® (GeNet Bio, Daejeon, Republic of Korea), 5 μL of distilled ultra-pure water, 1 μL of each primer (10 pmol/μL), and 3 μL of gDNA template.

The polymerase chain reaction (PCR) protocol comprised initial denaturation at 94℃ for a minute, 30 cycles of subsequent denaturation at 94℃ for 30 seconds, annealing at 57℃ for 30 seconds, and extension at 72℃ for 45 seconds, followed by final extension at 72℃ for seven minutes. Two microliters of the PCR products were analyzed by DNA electrophoresis in a 1.5% agarose gel in tris borate-EDTA (TBE) buffer. Agarose gel electrophoresis of the DNA was conducted using $0.5 \times$ TBE buffer to confirm the presence of the desired product. Thereafter, the amplified product was visualized by staining with SYBR Safe™ (Invitrogen, Grand Island, NY, USA) under blue-light transillumination. Approximate amplicon sizes were determined using a standard molecular weight marker (100 bp DNA Ladder®; iNtRON Biotechnology). The resulting amplicon was purified and
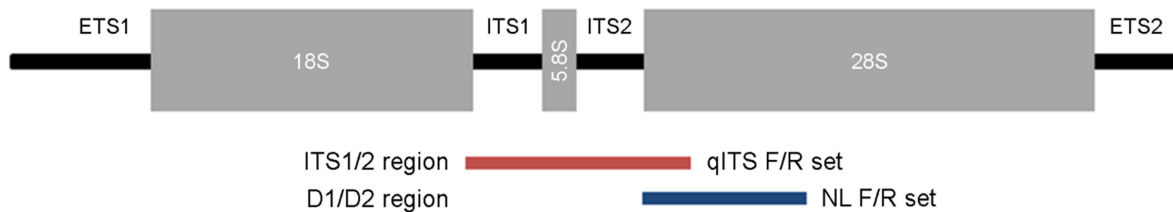

Fig. 1. The target regions of the fungal ITS and D1/D2 domain for the sequence analysis of *Candida glabrata* clinical isolates.
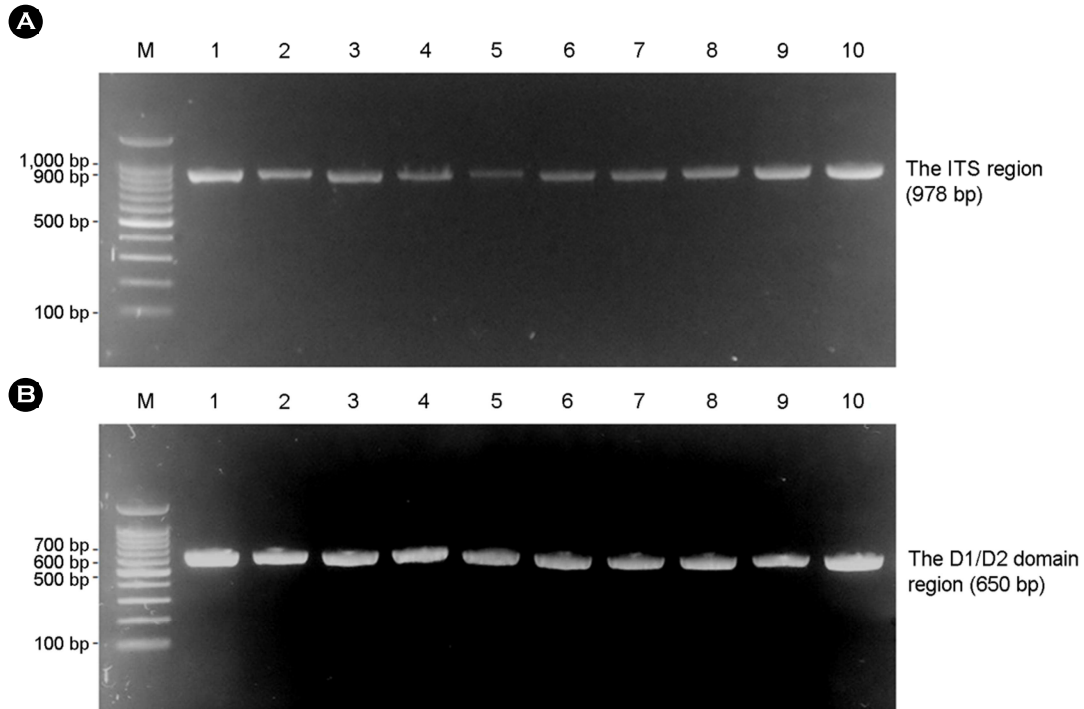

Fig. 2. The results of agarose gel electrophoresis to confirm the target region amplification results before sequence analysis. (A) The amplified fungal ITS domain region analyzed using PCR. M; DNA size marker, lanes 1~10; PCR products (978 bp). (B) The amplified fungal D1/D2 domain region on 28S rDNA analyzed using PCR. M; DNA size marker, lanes 1~10; PCR products (650 bp).

Fig. 3. Results of NCBI Blast analysis with ITS and D1/D2 DNA sequence data. As the result that sequence were aligned with NCBI Blast database, the identity of ITS gene and D1/D2 gene was 94% and 100% in the same sample.

sequenced using reverse sequence primer supplied by Macrogen (Daejeon, Republic of Korea). All sequences with low-quality bases in the chromatogram were resequenced to obtain high-quality results. The obtained sequences were aligned with reference sequences in the GenBank database using the Basic Local Alignment Search Tool (BLAST) at the National Center for Biotechnology Information (NCBI) (Fig. 3). The percentage of homology was determined to precisely identify the *C. glabrata* clinical isolates.

The results of PCR and sequencing analysis of the fungal ITS and the D1/D2 domain regions for species identification of *Candida* spp. were compared. The 1.5% DNA agarose gel electrophoresis data showed that the sizes of the amplified fungal ITS and D1/D2 domain regions were 978 bp and 650 bp, respectively, and that the amplicons had one clear band (Fig. 2). In order to verify the amplified PCR products, they were sequenced and analyzed using the GenBank BLAST. During the comparison, all culture-confirmed clinical isolates used in this study were identified as *C. glabrata*, with high agreement value.

In addition, a comparison was made of the ability of the ITS and D1/D2 domain regions to accurately identify a yeast species using sequence analysis. To this end, both the ITS and the D1/D2 regions of 102 *C. glabrata* clinical isolates were amplified and sequenced, and the results were analyzed with BLAST. The mean standard deviation (expressed as a

Table 3. Comparison of the accuracy of sequence analysis (BLAST) between in the fungal ITS and D1/D2 domain region for the identification of *C. glabrata* 102 clinical isolates

Targeting region	ITS domain	D1/D2 domain
Species identity percent score Mean ± SD (%) for species	97.8% ± 2.9	99.7% ± 0.46

SD: standard deviation

percentage) for the ITS and D1/D2 domain regions for species identification was 97.8% ± 2.9 and 99.7% ± 0.46, respectively (Table 3). These results suggest that although the specificity of the results for both regions was similar (over 97%) and even the amplicon size targeting ITS was much longer than that of D1/D2, the D1/D2 domain region was demonstrated to be a better target for the identification of *C. glabrata* than the ITS region using DNA sequence analysis.

In conclusion, although the D1/D2 domain region was demonstrated to be a better target than the ITS region in identifying *C. glabrata* clinical isolates, other *Candida* spp. including *C. albicans*, *C. tropicalis*, *C. dubliniensis*, and *C. krusei* should also be evaluated in future studies in order to evaluate its usefulness in the identification of all *Candida* spp.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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