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RESEARCH ARTICLE

Polymorphism of ITS4 Gene of *Candida guilliermondii*

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Abstract

The aim of this study is investigate the polymorphisms of *Candida guilliermondii* based on ITS4 gene sequencing. To achieve this goal, samples were collected from patients with dermal infection symptoms, were applied for microbiological analysis using SDA media DNA was isolated from *Candida guilliermondii* and the ITS4 gene were amplified by using specific primer, then sequencing of nucleic acid of genes was performed by machine is AB13730XL, Applied Biosystem, Macro gen company, the DNA sequencing results of flank sense of ITS4 gene from 5 strains of *Candida guilliermondii* was found to be compatible 99% and score 1517 and expect 0.0 of the ITS4 gene of *Candida guilliermondii* strain BO418 from the Gene Bank, The expected substitution were found after the analysis of the sequences there were 5 Transversion and one transition in the 5 *Candida guilliermondii* with Sequence ID emb|AJ3tf10144.1| location at range of nucleotide from 5128 to 5408, compared with data obtained from Gene Bank, these finding lead to conclusion, our assay allows rapid detection of Polymorphism in *Candida guilliermondii*.

Key words: *Candida guilliermondii, ITS4 gene, Polymorphism, and Sequencing*.

Introduction

The ITS4 gene sequence is about 1,550 bp long and is composed of both variable and conserved regions [1]. The gene is large enough, with sufficient interspecific polymorphisms of ITS4gene, to provide distinguishing and statistically valid measurements. Universal primers are usually chosen as complementary to the conserved regions at the beginning of the gene and at either the 540-bp region or at the end of the whole sequence (about the 1,550-bp region), and the sequence of the variable region in between is used for the comparative taxonomy [2].

The genus *Candida* contains about more than 150 different species; however, only a few are known to cause human infections, the medically significant *Candida* species include the following: *C. albicans,* the most common species identified (50-60 %), *C. glabrata* (previously known as *Torulopsis glabrata*) (15-20%), *C. parapsilosis* (10-20%),*C. tropicalis* (6-12%), *C. krusei* (1-3%), *C. kefyr* (<5%), *C. guilliermondi* (<5%) and *C. lusitaniae* (<5%) [3]. *C. guilliermondi* Colonies on SDA are White to cream-coloured smooth, glabrous yeast-like colonies [4] and with pale pink purpule [5] or light purpule to lilac colour on CHROMagar [6] with spherical to sub spherical budding yeast-like cells or blastoconidia, 2.0- 4.0 x 3.0-6.5 µm. Branched pseudohyphae with dense verticals of blastoconidia .germ tube negative yeast and sugar assimilation pattern. *Candida guilliermondii* has been isolated from numerous human infections, mostly of cutaneous origin. It is also found from normal skin and in sea water, faeces of animals, fig wasps, buttermilk, leather, fish, and beer [7].

This species is more frequent in candidemia [8]. The aim of the present study was to detection Polymorphism of ITS4 gene of *Candida guilliermondii.*

Materials and Methods

100 patients with dermal infection representing different age groups from both sexes were under want samples were obtained from Al-jimhory hospital in Samaraa, Iraq. Samples culured on SDA and identified microscopically and microscopically for using it in molecular study.

DNA extraction and Polymerase Chain Reaction

A single colony of cultivated *Candida guilliermondii*, which had been incubated overnight, suspended into 1ml of distilled water, centrifuged at 14000xg for 2 min., then the supernatant discarded, after that 120µL of lysostaphin (10 mg/L; Sigma) was added. DNA extracted using mini DNA extraction kit (Promega) according to manufacture instructions. Specific primers were designed for amplification by using a forward primer (*16s RNA* F: 5′- AGA GTT TGA TCC TGG CTC AG -3′) and a reverse primer (*16s RNA* R: 5′ GGT TAC CTT GTT ACG ACT T -3′) (Primers set supplied by IDT (Integrated DNA Technologies company, Canada).

PCR reaction was conducted in 25µl of a reaction mixture containing 2µl of DNA, 12.5 µl *GoTaq0T*® *Green* Master (Promega, CA), (0.5 µl) 25mM MgCl2, 2µl of (10 Pmol\ µl) of each primer, 2µl of distilled water. Amplification program was 1 cycle at 94°C for 1 min; 35 cycles of 94°C for 1min, 63°C for 1min, 72°C for 1min; 72°C for 10min, using the Mastercycler (Eppendorf). The amplified product was subjected to 1.5% agarose gel electrophoresis, and visualized under UV (Imagemaster VDS, Pharmacia Biotech, USA) after Red safe staining. Positive PCR product samples were sent for sequence analysis; nd 25 µl (10 pmol) from the forward primer. The samples were treated with AB13730XL APPLIED BIOSYSTEMS machine in national instrumentation centre for environmental management NICM/USA company online at (http://nicem.snu.ac.kr/main/?en_skin=index.html).The result of the sequence analysis was analysed by blast in the National Centre Biotechnology Information (NCBI) online at (http:// www.ncbi.nlm.nih.gov) and Bio Edit program .

Results and Discussion

Patients with dermal infection, aging between 19-70 years and mean age 45.They only five have infection with *Candida guilliermondii*. Those isolates were collected had been identified at the species level using morphological characteristics and biochemical tests like urease production 100% of the isolates gave positive results for test. All the processes of DNA amplification were performed with the use of ITS4 gene for the confirmation of *Candida guilliermondii* stains following the procedure published by [9, 10].

ITS4 gene was successfully amplified using specific PCR primer amplification of ITS4 gene of 5 strains of *Candida guilliermondii* collected in the present study to confirm the presence of ITS4 gene in the strains. The DNA extracts was subjected to PCR analysis to confirm the possible presence of ITS4 gene. As expected DNA from all *Candida guilliermondii* produced clean bands upon amplification with ITS4 gene set of specific primer. Figure (1) appeared that molecular weight of ITS4 gene was 1500 bp in the PCR product of *Candida guilliermondii* strains was exclusively used to proceed for the sequencing analysis assay to detect the polymorphism in gene content.

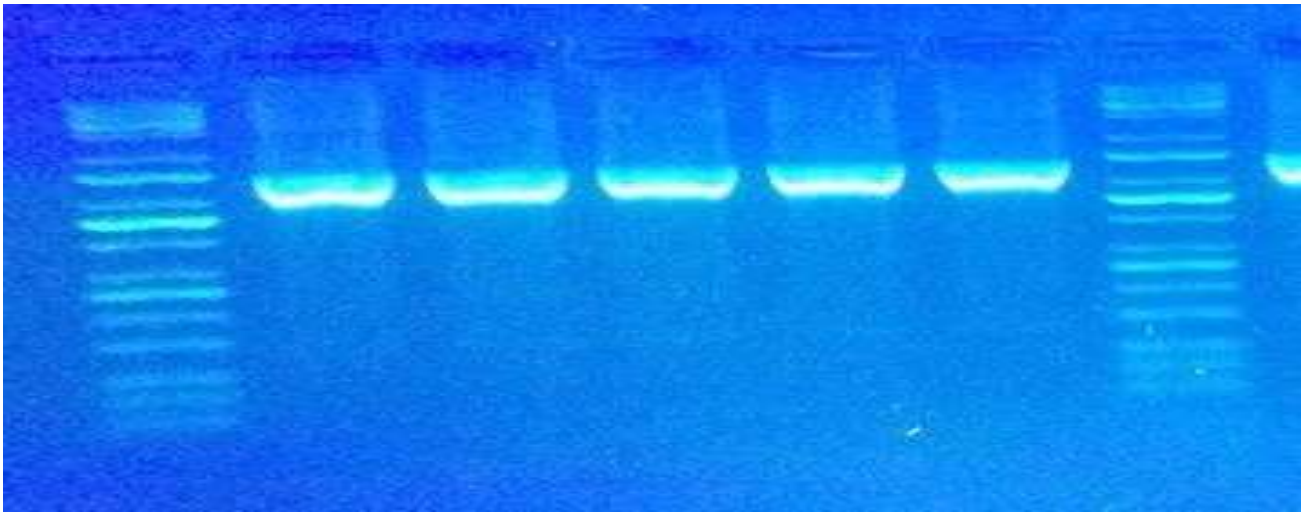

Figure 1: the product was electrophoresis on 2% agarose at 5 volt/cm².1x TBE buffer for 1:30 hours. N: DNA ladder (100), lane (1-10) PCR product of band size 1500bp, visualized under U.V light

Sequencing of this gene was performed to detect mutation related to development of polymorphism. Sequences alignment using BLAST and Bio Edit showed that the 99% similarity with NCBI, score 1517 and expect 0.0 of the ITS4 gene of *Candida guilliermondii* strain BO418 from the Gene Bank (Figure 2).

Helicobacter pylori partial ITS4gene, strain BO418

Sequence ID: emb|AJbv310144.1|

Score	Expect	Identities	Gaps	Strand
1517 bits(821)	0.0	833/839(99%)	0/839(0%)	Plus/Plus

```
Query                                                                 1
CTGGCGGCGTGCCTAATACATGCAAGTCGAACGATGAAGCTTCTAGCTTGCTAGAATGCT
60
        |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct                                                                 1
CTGGCGGCGTGCCTAATACATGCAAGTCGAACGATGAAGCTTCTAGCTTGCTAGAATGCT
60

Query                                                                61
GATTAGTGGCGCACGGGTGAGTAACGCATAGGTCATGTGCCTCTTAGTTTGGGATAGCCA
120
        |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct                                                                61
GATTAGTGGCGCACGGGTGAGTAACGCATAGGTCATGTGCCTCTTAGTTTGGGATAGCCA
120

Query                                                               121
TTGGAAACGTTGATTAATACCAGATACTCCCTACGGGGGAAAGATTTATCGCTAAGAGAT
180
        |||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct                                                               121
TTGGAAACGATGATTAATACCAGATACTCCCTACGGGGGAAAGATTTATCGCTAAGAGAT
180

Query                                                               181
CAGCCTATGTCCTATCAGCTTGTTGGTAAGGTAATGGCTTCCCAAGGCTATGACGGGTAT
240
        |||||||||||||||||||||||||||||||||||| ||||||||||||||||||
Sbjct                                                               181
CAGCCTATGTCCTATCAGCTTGTTGGTAAGGTAATGGCTTACCAAGGCTATGACGGGTAT
240

Query                                                               241
CCGGCCTGAGAGGGTGAACGGACACACTGGAACTGAGACACGGTCCAGACTCCTACGGGA
300
        ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct                                                               241
CCGGCCTGAGAGGGTGAACGGACACACTGGAACTGAGACACGGTCCAGACTCCTACGGGA
300

Query                                                               301
GGCAGCAATAGGGAATATTGCTCAATGGGGGAAACCCTGAAGCAGCAACGCCGCGTGGAG
360
        ||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Sbjct                                                                301
GGCAGCAGTAGGGAATATTGCTCAATGGGGGAAACCCTGAAGCAGCAACGCCGCGTGGAG
360

Query                                                                361
GATGAAGGTTTTAGGATTGTAAACTCCTTTTGTTAGAGAAGATAATGACGGTATCTAACG
420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct                                                                361
GATGAAGGTTTTAGGATTGTAAACTCCTTTTGTTAGAGAAGATAATGACGGTATCTAACG
420

Query                                                                421
AATAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAC
480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct                                                                421
AATAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAC
480
Query                                                                481
TCGGAATCACTGGGCGTAAAGAGCGCGTAGGCGGGATAGTCAGTCAGGTGGGAAATCCTA
540
            |||||||||||||||||||||||||||||||||||||||||||||||||||| |||||||||
Sbjct                                                                481
TCGGAATCACTGGGCGTAAAGAGCGCGTAGGCGGGATAGTCAGTCAGGTGTGAAATCCTA
540
Query                                                                541
TGGCTTAACCATAGAACTGCATTTGAAACTACTATTCTAGAGTGTGGGAGAGGTAGGTGG
600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct                                                                541
TGGCTTAACCATAGAACTGCATTTGAAACTACTATTCTAGAGTGTGGGAGAGGTAGGTGG
600
Query                                                                601
AATTCTTGGTGTAGGGGTAAAATCCGTAGAGATCAAGAGGAATACTCATTGCGAAGGCGA
660
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct                                                                601
AATTCTTGGTGTAGGGGTAAAATCCGTAGAGATCAAGAGGAATACTCATTGCGAAGGCGA
660

Query                                                                661
CCTGCTGGAACATTACTGACGCTGATTCCGCGAAAGCGTGGGGAGCAAACAGGATGAGAT
720
            |||||||||||||||||||||||| |||||||||||||||||||||||||| ||||
Sbjct                                                                661
CCTGCTGGAACATTACTGACGCTGATTGCGCGAAAGCGTGGGGAGCAAACAGGATTAGAT
720
Query                                                                721
ACCCTGGTAGTCCACGCCCTAAACGATGGATGCTAGTTGTTGGAGGGCTTAGTCTCTCCA
780
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct                                                                721
ACCCTGGTAGTCCACGCCCTAAACGATGGATGCTAGTTGTTGGAGGGCTTAGTCTCTCCA
780
Query                                                                781
GTAATGCCACTAACGCATTAAGCATCCCGCCTGGGGAGTACGGTCGCAAGATTAAAACT
839
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||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 781
GTAATGCCACTAACGCATTAAGCATCCCGCCTGGGGAGTACGGTCGCAAGATTAAAACT
839

Figure 2: Sequencing of sense flanking the partial ITS4 gene in *Candida guilliermondii* compared with standard ITS4 obtained from Gene Bank. Query represents of sample; Sbject represent of database of National Center Biotechnology Information (NCBI)

The expected polymorphisms were found after the analysis of the sequences as listed in Table (1). There are 5 Transversion substitution in the 5 *Candida guilliermondii* with Sequence ID emb|AJ310144.1| location at Range of nucleotide from 5128 to 5408 that caused change Adenine to Thiamin, Adenine to Cytocine, Thiamin to Guanine, Guanine to cytosine and Thiamin to Guanine, and transition, resulted in a change of Guanine to Adenine compared with data obtained from Gene Bank as show in Figure (2).

Table 1: Types of mutations detected in partial ITS4 gene in *Helicobacter pylori*

Type of mutation	Nucleotide	Range of nucleotide	Sequence ID	
Transversion	**A>T**	**5128 to 5408**	emb|AJ310144.1|	
Tansversion	A>C	**5128 to 5408**	emb|AJ310144.1|	
Transition	G>A		emb|AJ310144.1|	
Transversion	T>G		emb|AJ310144.1|	
Transversion	G>C		emb|AJ310144.1|	
Transversion	**T>G**			

The ITS4gene is used as the standard for classification and identification of microbes, because it is present in most microbes and shows proper changes. Type strains of ITS4gene sequences for most bacteria and archaea are available on public databases such as NCBI. However, the quality of the sequences found on these databases is often not validated.

Therefore, secondary databases that collect only ITS4sequences are widely used [8]. Conventional methods to assess levels of Polymorphism of *Candida guilliermondii* are based on culture in combination with agar dilution [11, 12]. Since sequencing analysis seems to be restricted to the occurrence of specific mutations in a small region of the ITS4molecule [13, 14, 15], molecular methods an attractive and alternative. In the present study a PCR-based on sequencing analysis was used to detect the presence of the substitution in the ITS4genes. This assay distinguishes the high-level of Polymorphism in isolates from the data from sequencing analysis of ITS4genes in Candida *guilliermondii* strains. Since all the isolates show high-level of Polymorphism that's may be linked to therapy failure [16, 17, 18], this sequencing analysis approach is useful for the detection of clinically relevant levels of polymorphism in *Candida guilliermondii*. It is striking that all characterized H. pylori isolates contain mutations in the exact same ITS4region, especially because these isolates were obtained from dyspeptic patients living in same geographic regions [19].

This observation suggests that *Candida guilliermondii* require mutations within the ITS4primary binding site for antibiotic resistance. Probably this resistance arises by mutations, although the acquisition of mutant ITS4alleles through horizontal gene transfer cannot be excluded [20, 21], these finding lead to conclusion; our assay allows rapid detection of Polymorphism in *H. pylori*.

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