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Research Article

Molecular Identification of *Talaromyces islandicus* Isolated from Clinical Sample (First Record in Iraq)

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ABSTRACT

The analysis of ITS4 gene sequences has been the technique generally used to study and confirm the identification and taxonomy of fungi However, fungal species cannot always be distinguished from each other using cultural methods. Thus, clinical samples were collected from cases with aspergillosis infections, were applied for microbiological and molecular identification. DNA was extracted from *Talaromyces islandicus* 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 *Talaromyces islandicus* was confirm the identification into species level: *Talaromyces islandicus.* analysis of the sequences appeared that there two substitution (Transversion, Transition) in the *Talaromyces islandicus* strain with Sequence ID LC540.1 location at Range of nucleotide from 9 to 77, 100% compatibility with NCBI while no substitution appeared in the *Talaromyces islandicus* Gene Bank accession number: KY30.1.

Keywords: *Talaromyces islandicus*, ITS4, and Sequencing, Gen Bank.

INTRODUCTION

Talaromyces islandicus. is a versatile, opportunistic pathogen able to cause a wide range of diseases in humans. It is considered to be a major pathogen that colonize and infects respiratory system and cause disease like aspergillosis but without fungal ball It can be cause local infection of the skin, assays based on molecular PCR technology were employed to detect the presence of *Talaromyces islandicus* using the ITS4 genesequence is about 1,330 bp long and is composed of both variable and conserved regions. The gene is large enough, with sufficient polymorphisms of ITS4 gene, 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 5990-bp region or at the end of the whole sequence, and the sequence of the variable region in between is used for the comparative taxonomy (1-3). The current study aimed to Molecular identification of *Talaromyces islandicus* as unknown sample using ITS4 gene.

MATERIALS AND METHODS

Sample collection and DNA Extraction

During June, 2018, sample were taken from paients have infection with aspergillosis had been incubated 7 day, 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 (G- spin dna extraction kit , intron biotechnology , cat.no. 17045) according to manufacture instructions (4). Specific primers were designed for amplification by using a forward primer (ITS4 *DNA* F: 5′- AGA TGG CTC AG -3′) and a reverse primer (ITS4 *DNA* R:5′ GGT G ACT T -3′) (5). 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) 2mM MgCl2, 2µl of (10 Pmol\ µl) of each primer, 2µl of D W. Amplification program was 1 cycle at 94°C for 0.5 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 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) 5 (,6,7).

RESULTS AND DISCUSSION

The specimen unknown were directly inoculated onto plates DNA extracted successfully from *Talaromyces islandicus* as show in figure 1 to use it in polymerase chain reaction (PCR) application. The concentration and purity of total DNA isolates in the samples were measured spectrophotometrically at wavelengths of A260 and A280. It was performed in a Nano Drop machine (Thermo Scientific). The yield of the DNA extracted

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Fig 1: Electrophoresis gel for genomic DNA of *Talaromyces islandicus* isolates by electrophoresis on 1% agarose gel stained with ethidium bromide

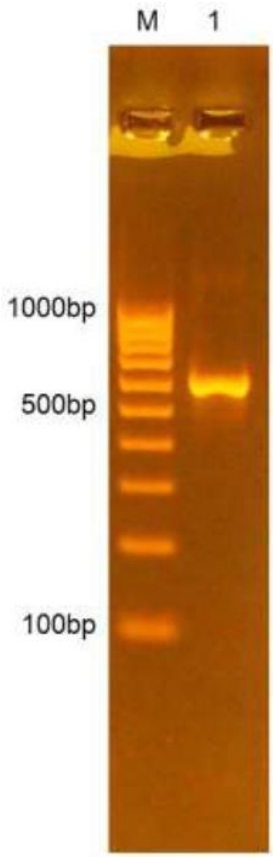

Fig 2: PCR Result Results of the presence of ITS gene of Unknown Fungal species were fractionated on 1% agarose gel electrophoresis stained with Eth.Br. Lane1:100bp DNA marker.

from *Talaromyces islandicus* isolates was in range of (216) ng/µl with purity of (1.5).

All the processes of DNA amplification were performed with the use of ITS4 gene for the confirmation of *Talaromyces islandicus* following the procedure published by (8). *Talaromyces islandicus* DNA gene was successfully amplified using specific PCR primer amplification of 16srDNA gene

Figure (2) appeared that molecular weight of ITS4 gene in the PCR product of *Talaromyces islandicus* strains was exclusively used to proceed for the sequencing analysis assay to confirm the identification of *Talaromyces islandicus* and also detect the polymorphism in gene content.

Sequencing of ITS gene was performed to confirm the identification of *Talaromyces islandicus* strains based on species level (9)

Data Analysis

Sequencing of ITS gene was performed to confirm the identification of *Talaromyces islandicus* strain isolated during the current study, Sequences alignment using BLAST and BioEdit showed that the strain *Talaromyces islandicus* accession number : LN998.1, 100% compatibility with NCBI, score 2026 and expect 0.0 of the ITS gene the compatibility of *Staphylococcus haemolyticus* strain with the strain mammoth-14 isolated in France,

Types of substitution detected in partial ITS gene in *Talaromyces islandicus* strains 1 Transversion and 1 transition substitution location (10)

The ITS4 geneis used as the standard for classification and identification of microbes, because it is present in most microbes and shows proper changes. Type strains of ITS4 genesequences 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 16S rRNA sequences are widely used(11)

Talaromyces islandicus strain EA111 ITS4 gene, partial sequence

GenBank: K530.1, LOCUS KY\0 180 bp DNA linear BCT 12-JUN-2017, DEFINITION *Talaromyces islandicus* strain EA111 16S ribosomal RNA gene, partial sequence. ACCESSION KY938530 VERSION KY938530.1 KEYWORDS. SOURCE *Talaromyces islandicus* ORGANISM *Talaromyces islandicus* REFERENCE 1 (bases 1 to 180) AUTHORS wafaa

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