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Rapid Identification of *Aspergillus Fumigatus* Using *Beta-Tubulin* and *RodletA* Genes

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Abstract

AIM: The main purpose of the present study was to test the β-tubulin and *rodletA* genes for rapid identification of *Aspergillus fumigatus*.

MATERIALS AND METHODS: Fifty-one *A. fumigatus* strains including environmental, clinical and reference isolates were tested in this research. PCR was carried out based on βtub and *rodA* partial gene sequences.

RESULTS: A 198 bp DNA fragment was obtained using ***βtub*** gene. PCR amplification of the *rodA* gene resulted in a 313 bp band. The ***βtub*** and *rodA* genes PCR products exhibited a 100% homology with the associated sequences in the GenBank.

CONCLUSION: In the present study, we used a PCR approach that was able to discriminate *A. fumigatus* from other related species within the section Fumigati.

Introduction

Aspergillus fumigatus is an environmental filamentous fungus and is the main causal agent of the aspergillosis. Increasing the use of immunosuppressive therapy for treating many human diseases, the incidence of mortality rate of invasive aspergillosis (IA) is raised between 30–95% [1].

Teleomorphic species of the *Aspergillus* section *Fumigati* (AsF) belong to the Neosartorya genus. *Aspergillus* section *Fumigati* (AsF) is an economically important fungus and teleomorphic species of this section belong to the genus *Neosartorya*. Seventeen species of *Neosartorya* and 8 strictly mitotic species are typically recognized [2].

Aspergillus section Fumigati and also its teleomorph Neosartorya are significantly important in clinic because they are able to pathogenic or allergenic to human [3-5], responsible for food spoilage and producing mycotoxins [6, 7].

The clinical isolates of *Aspergillus* species are not necessary morphological the same and wrong recognitions of species with morphological characteristics have frequently happened. With the intention of development the diagnostic method, including DNA detection it is important to elucidate intra and interspecies variety in *A. fumigatus* and closely related species.

Misrecognition of species within the section Fumigati has been frequently reported by clinical laboratories. Species, for example, *Aspergillus viridinutans, Aspergillus lentulus, Aspergillus fumisynnematus, Aspergillus fumigatiaffinis, Neosartorya pseudofischeri, Neosartorya udagawae* and *Neosartorya hiratsukae*, are commonly reported as *A. fumigatus* [8, 9].

A number of biochemical and molecular approaches have been performed for identification of *A. fumigstus* and related species. Sequencing of genes, for example, *ITS, calmodulin, actin, βtubulin (βtub)* and *rodlet A (rodA),* has been used to discrimination *A. fumigatus* from related species [10, 11]. In the current study, the βtub and rodA gene were tested for identification of Aspergillus section Fumigati.

Materials and Methods

Microorganisms

A total of 51 *A. fumigatus* strains including environmental, clinical and reference isolates were used in this study. The following strains of *A. fumigatus* were used as a reference: IBRC-M 30033, IBRC-M 30040, IBRC-M 30048. Eight clinical isolates were kindly provided by Dr Mojtaba Taghizadeh (Mazandaran University of Medical Sciences, Mazandaran, Iran). The environmental isolates were recovered from soil or air. The strains were incubated on Sabouraud dextrose agar (Merck KGaA, Darmstadt, Germany) at 37°C. All *A. fumigatus* isolates were recognized by morphology. For getting a pure culture, the isolates were subcultured three times and then stained with lactophenol aniline blue. Phenotypic techniques including colony morphology, conidial arrangement, philiades, vesicles and conidiophores were considerated for identification.

DNA extraction

One ml thick spore suspension from each isolate was transferred to an Erlenmeyer flask containig 50 ml yeast extract peptone dextrose medium (Merck KGaA).

The flasks were incubated at 200 rpm under agitation at 27˚C for 72 h to allow for mycelium growth.

The harvested mycelia were washed with 0.5 M ethylenediamine tetraacetic acid (EDTA) and sterile distilled water (dH2O) and freeze-dried at -70˚C for DNA extraction. Then, the mycelia were ground into a fine powder with a pestle and mortar. The DNA was extracted using the GF-1 Plant DNA Extraction Kit (vivantis, Malysia).

PCR amplification

In our study, PCR was performed based on *βtub* and *rodA* partial gene sequences. The primer sets, βtub-F (5'- TGACGGGTGATTGGGATCTC-3') and βtub-R (5'- CGTCCGCTTCTTCCTTGTTT-3') was used to amplify a 198bp DNA fragment of the *βtub* gene. The primer sets, *rodA* -F (5'-ACATTGACGAGGGCATCCTT -3') and *rodA* -R (5'-ATGAGGGAACCGCTCTGATG -3') was used to amplify a 313bp DNA fragment of the *βtub* gene. The PCR reactions were prepared to a final volume of 30 µl, comprised of 3 µl 10X reaction buffer, 2.2 mM MgCl2, 200 µM of each dNTP, 2.5 unit of Taq DNA polymerase (CinnaGen, Karaj, Iran), a 30 ng DNA template and 50 pmol of each primer.

An initial denaturation for 5 min at 94˚C was followed by 35 cycles of denaturation at 94˚C for 1 min, annealing at 68˚C for 1 min and extension at 68˚C for 2 min. The PCR products were electrophoresed on 2% agarose gel in TAE buffer at 50 V for 2 h and stained with ethidium bromide.

Sequencing

A number of *βtub* and *rodA* genes amplicons were submitted for sequencing (Bioneer Corporation, Daejeon, South Korea). Searching in the NCBI database (http://www.ncbi.nlm.nih.gov/) showed that sequences had 100% identity with *A. fumigatus* β*tub* and *rodA* genes. The MEGA5 software package (http://www.megasoftware.net) was employed for alignment of sequences.

Results

Fifty moulds, all formerly recognized as *A. fumigatus* by morphology, were screened by the PCR method to identify *A. fumigatus* isolates. The *βtub* and *rodA* genes were considered as genes markers.

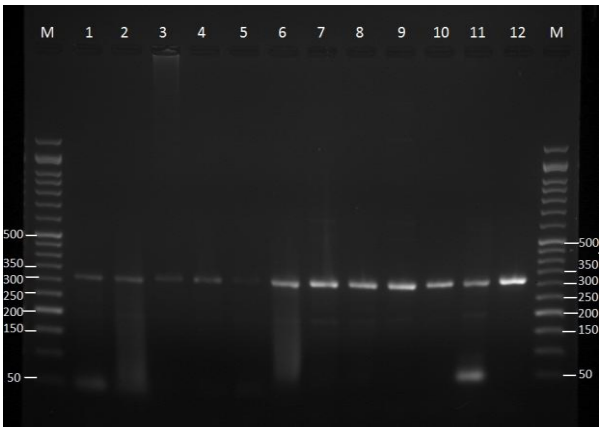

Figure 1: Agarose gel electrophoresis of rodA gene products (313 bp) of the Aspergillus fumigatus (lane 1, 2 references strains; lanes 3-5, clinical isolates; lane 6-12, environmental isolates. Lane M, 50-bp ladder; lane 1, IBRC-M 30040 lane 2, IBRC-M 30048; lane 3, MF6; lane 4, MF30; lane 5, MF34; lane 6, MF13; lane 7, MF17; lane 8, MF35; lane 9, MF39; line 10, MF42; line 11, MF46; line 12, MF53

PCR amplification of the *rodA* gene for all 51 isolates with primers *rodA* -F and *rodA* -R resulted in a 313 bp band (Fig. 1). PCR amplification of the *rodA* gene for all 51 isolates with primers βtub-F and βtub-R resulted in a 198 bp band (Fig. 2).

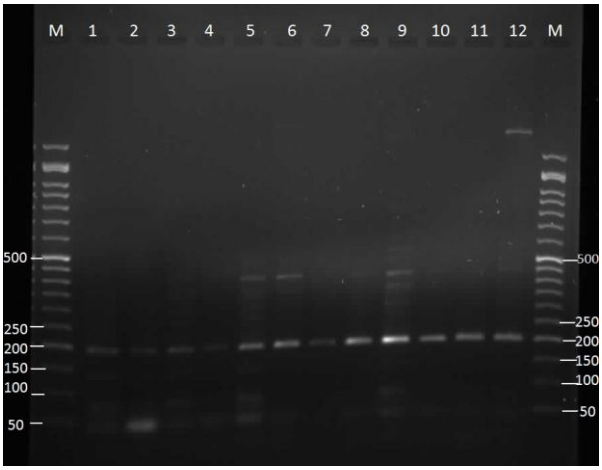

Figure 2: Agarose gel electrophoresis of βtub gene products (198 bp) of the Aspergillus fumigatus (lane 1, 2 references strains; lanes 3-5, clinical isolates; lane 6-12, environmental isolates. Lane M, 50-bp ladder; lane 1, IBRC-M 30040 lane 2, IBRC-M 30033; lane 3, MF6; lane 4, MF30; lane 5, MF34; lane 6, MF13; lane 7, MF17; lane 8, MF35; lane 9, MF39; line 10, MF42; line 11, MF46; line 12, MF53

The *βtub* and *rodA* genes products were sequenced for several isolates, including the reference strains. A Basic Local Alignment Search Tool (BLAST) search demonstrated that the *βtub* and *rodA* genes PCR products exhibited a 100% homology with the associated sequences in the GenBank.

Discussion

Identification of filamentous fungi dissimilar to bacteria, rely mostly on morphological characterizations, however, limitations of phenotypic typing of pathogenic fungi are being progressively more recognized. The new fungal species have recently been recognized within the section Fumigati, Some of them have been associated in severe cases of aspergillosis including pulmonary, cerebral, liver, cutaneous and trabecular bone invasions.

In regard to the *A. fumigatus* may shows a significant part of all aspergillosis clinical cases, molecular description is important for the accurate detection of species within the section of Fumigati.

Hong et al (2005) reported the variability within *A. fumigatus* section in Korea by morphology, growth temperature, extrolite patterns and DNA analyses of the partial *β*-tubulin, actin and calmidulin gene and they suggested two new species which were *A. fumigatiaffinis*, *A. novofumigatus* [12].

The phylogenetic associations between *A. fumigatus* and related species has also been analysed by partial sequencing of cytochrome b gene [13].

Identification of *A. fumigatus* is vital because this fungus is one of the most significant fungal pathogens. The recognition of *Aspergillus* spp. isolated from clinical samples depends mostly on morphological features. However, morphology is not enough for the detection of some clinical isolates because of the occurrence of polymorphism and the deprived development of reproductive structure. Consequently, several additional techniques have been used performed in the study of *A. fumigatus* [10, 14-16]. Burnie et al. employed restriction fragment length polymorphism analysis (RFLP) to discriminate the clinical isolates of *A. fumigatus*. They were succeeded classify 21 isolates into six types with *Xba*I digestion [17].

In the present study, we used a PCR approach that was able to discriminate *A. fumigatus* from other related species within the section Fumigati.

Sequence analysis of PCR products the *benA* and *rodA* genes of several isolates revealed that this approach accurately differentiated the non-*A.fumigatus* from the *A. fumigatus* isolates.

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