هفدهمین کنفرانس سراسری و پنجمین کنفرانس بین المللی زیست شناسی ایران

Phylogenetic relationship of some imperfect fungi using ITS and EF1-α

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Abstract

Anamorphic fungi are a controversial group among fungi and their classification criteria has undergone many changes over the years. The study was conducted to investigate phylogenetic relationships of some imperfect fungi. For this purpose, selected isolates from Plant tissues and soil samples were collected from several provinces of Iran. Then, their total DNA were amplified by ITS and TEF primers. Phylogenetic relationship which was conducted by neighbor joining method revealed that ITS cant separate *Alternaria*, *Ulocladium* and some *Cladosporium* species but EF-1α is suitable for breaking them up. Results obtained from both ITS and EF-1α was in congruent with each other and with previously described phylogenies and morphology based classification schemes.

Key words: anamorphic fungi, imperfect fungi, phylogeny, ITS, EF1- α

Introduction

Anamorphic or imperfect fungi are a group of fungi, which their sexual reproduction absolutely don't exist or haven't seen or known and formed in nature, rarely. We often faced their anamorphic forms. Most of them are asexual forms of Ascomycota and a few of them are Basidiomycota asexual forms(Alexopoulos CJ 1996). Most of these fungi are terrestrial and some live in fresh and salt water. Many of them are parasites that are dangerous due to disease in plants and animals, including humans, are considered important. Some are involved in commercial production of chemicals such as antibiotics and a number directly or indirectly by producing toxins, food poisoning can cause harm.

Anamorphic fungi are a controversial group among fungi and their classification criteria has undergone many changes over the years. Preliminary classification of incomplete fungi and the key to their identification is based on a system that shown by (Saccardo 1886) in Sylloge fungorum, and later (Lindu 1900), Saccardo (1906) and others made modifications to the system.

Over the years, lots of dissatisfaction was made towards Saccardo classification, especially the criteria used to classify Hyphomycetes.

Many critics, like (Vuillemin 1910) and (Mason 1925) in older articles and Hughes (1953), (Goos 1956), (Tubaki 1955), Subramanian (1962) and (Luttrell 1963; Luttrell 1964) in more recent articles, criticized this system and considered it an artificial classification.
Molecular revolution in fungi taxonomy started in first decade of 1990 which was associated with amplification of rRNA genes by PCR. Nowadays, molecular systematics of fungi have the maturity of the arrangements and in it a multi-gene data banks of a broad samples of taxa and strong and specific analysis methods of them have been standardized.

According to the latest rankings provided by (Hibbett, Binder et al. 2007) using a molecular phylogeny of fungi, the anamorph of Ascomycetes and Basidiomycetes , placed in the orders, and a family that owned it is the sexual form.

The study was conducted, to determine real taxonomic position of some taxa in this group.
We tried to use isolates which were collected from different geographical regions of Iran. Then the results were compared with GenBank sequences.

Materials and methods

Seventy fresh specimens of some saprophytic and pathogenic of imperfect fungi were collected from different provinces of Iran, during 2010 to 2011 and returned to laboratory for examination. Then, 25 representative of the isolate were selected for molecular analysis, which their hosts and geographic origin are listed in Table 1.
Total genomic DNA of pure isolate was extracted by using modified CTAB protocol and was subjected to polymerase chain reaction in PCR Thermal Cycler (What man-Biometra, Goettingen, Germany).
PCR primers used in the amplification reactions for Internal transcribed spacers and 5.8S region were ITS4 (TCCTCCgCTTATTgATATgC) and ITS5 (ggAAgTAAAAgTCgTAACAAgg) (White, Bruns et al. 1990).
FRPB2-5f (gAYgAYMgWgATCAYTTYgg) and FRPB2-7CR (CCCATRgCTTgYTTRCCCAT) primers (Vilgalys, Hopple et al. 1994) were used to amplify a piece of translation elongation factor encoding gene.
After the amplification of the ITS region of the rDNA and EF1-α, each product was purified using the QIA quick PCR Purification Kit using manufacturer protocol (Qiagen Inc., Valencia, CA, USA). The purified PCR product was send by overnight mail to DNA Sequencing Facility at BioNeer in Korea where isolates were sequenced.
Sequences were aligned using BioEdit(Hall 2004). Then, alignments were manually edited by inserting gaps for optimization using Se-Al (Rambaut, 1996). Phylogenetic analysis were performed by neighbor joining and Maximum Likelihood methods (The bootstrap settings were 1,000 replicates and retaining groups with frequency >50 %) and Phylogenetic trees were constructed using MEGA 5. Gaps were treated as missing data and uninformative characters were excluded from the analysis.

Results

The ITS 4 and ITS 5 primers directed the amplification of a single product, approximately 550 bp from all isolates except two. DNA sequencing revealed that these fragments ranged in size from 530 to 570 bp (including primer sequences) except in *Tritirachium* which amplified a 735 bp and *Paecilomyces tenuis* that length of amplified fragment in it was 1052 bp, nearly doubled length of the same part in other taxa used in this study. Dendrogram was constructed by neighbor joining method is as shown in figure 1.
Approximate length of fragments amplified by TEF primers on agarose gel, was approximately 400 bp in most fungi and in *Paecilomyces tenuis* (P5-4 isolate) was 550 bp. It should be noted that these primer amplified two fragments of different molecular weight in the isolated fungus *Cladosporium tenuissimum* (CL4-6), that the first one was the same as other fungi, approximately 400 bp and the second fragment was 1050 bp. Picture of agarose gel has been shown in figure 2.
After extraction from the gel pieces with larger length, was sequenced.
Compared with other sequences revealed that 20 nucleotides of beginning of sequence is less than the other tested isolates. Then, the sequence was very similar to the others and additional nucleotides were after number 390.

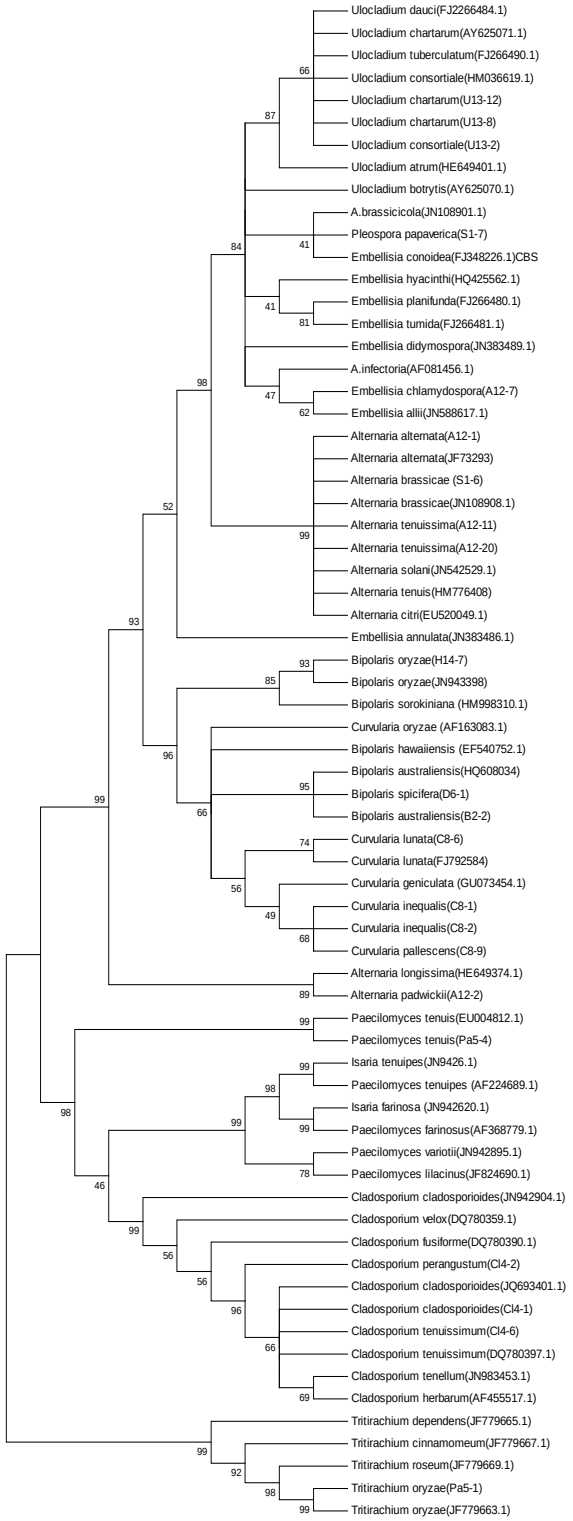

Figure 1) Phylogram generated from the neighbor joining analysis based on ITS sequence of selected isolate. Bootstrap support values >40% are shown below or above the branch.

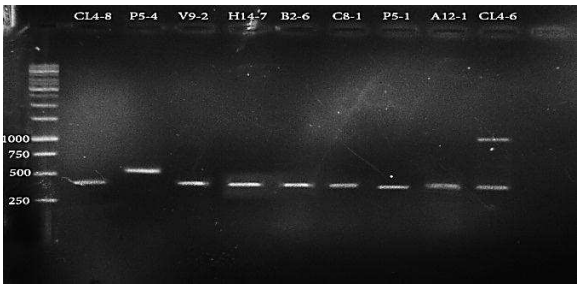

Figure 2) Agarose gel picture of amplified fragment using TEF primer in different isolates

At last, dendrogram was constructed by neighbor joining method, which is shown in figure 3.

Discussion

Results obtained from analyzing of ITS dendrogram revealed that sequences from this region of ribosomal RNA aren't able to separate small spore *Alternaria*, *Ulocladium* and some *Cladosporium* species from each other.
Bipolaris and *Curvularia* which both are anamorphs of *Cochliobolus* placed in a same clade.
Alternaria padwickii and *A. longisima* which belong to big spore group of *Alternaria*, placed togather and far from other *Alternaria* species. While, *Alternaria infectoria* placed in a clade near two species of *Embellisia. Paecilomyces* species placed in a same clade with their sexual form, *Isaria*. It seems that *Tritirachium* compose a group that is far from the other studied groups and have lees phylogenetic relationship with them.

This piece of protein coding gene of EF1-α was able to separate *Cladosporium* species. But, our data wasn't enough for proving this about *Alternaria* and *Ulocladium* species.

In both dendrogram, *Bipolaris* and *Curvularia* placed togather and *Cochliobolus* anamorphs were separated into two distinct groups. But, in dendrogram obtained from TEF sequences,

Alternaria Ulocladium clade, placed between these two clusters.

Considering that, teleomorph of studied taxa placed in different orders, it seems that both ITS and TEF are appropriate for survey of phylogenetic relationships in this group of fungi. Even if EF1-α is more suitable in some cases which was explained before.

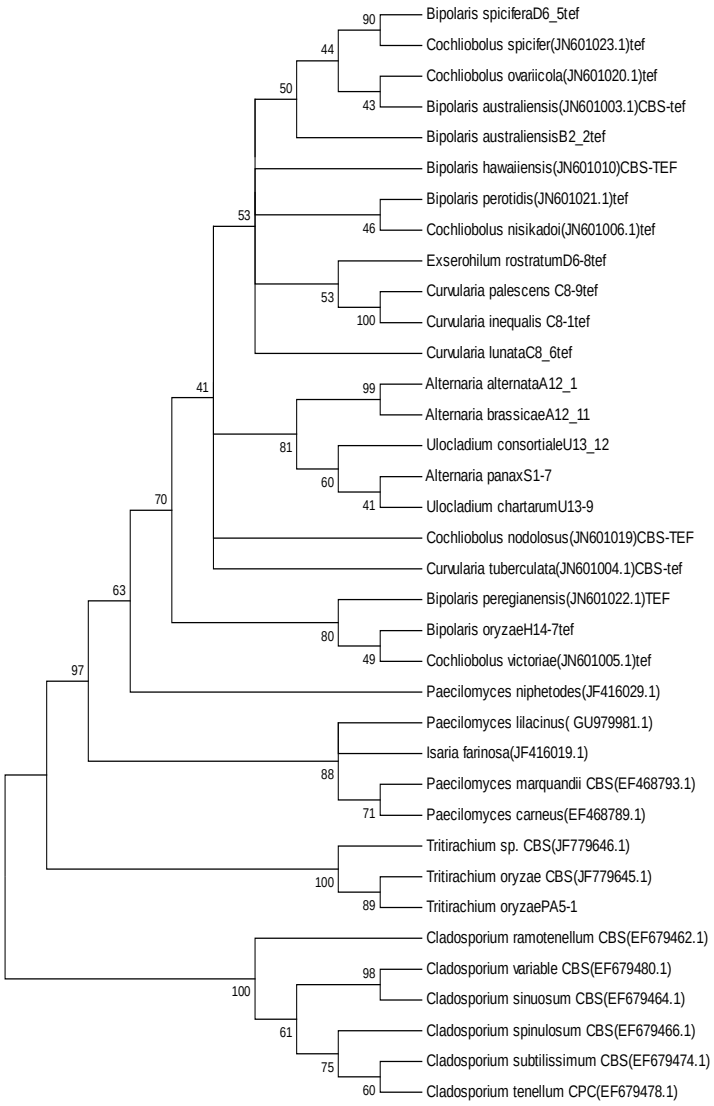

Figure 3) Phylogram generated from the neighbor joining analysis based on TEF sequence of selected isolate. Bootstrap support values >40% are shown below or above the branch.

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