Molecular identification of *Ganoderma lucidum* from Iran

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Abstract

Medicinal mushroom *Ganoderma lucidum* has been used in East Asia for centuries in order to prevent and treat a variety of diseases such as hepatitis, immunological disorders and cancers. In fact, this fungus contains a vast source of polysaccharides, proteins and secondary metabolites with anti-tumor and immuno-modulatory properties. Up to now, a variety of drug metabolites extracted from *G. lucidum* have reached the stage of commercial production. Recently, in addition to China, Korea and Japan, this valuable fungus has been identified in different parts of the world such as Pakistan, Malaysia and Turkey. In 2007, *G. lucidum* has been identified in northern forests of Iran; however, molecular identification of this fungus has not been reported yet. The aim of this study was, therefore, to identify this fungus collected from northern Iran by analyzing ITS-5.8S rDNA sequence. The result of this experiment confirmed that the collected specimen were *G. lucidum*.

Keywords: Anti-tumor, fungus, ITS, North of Iran, taxonomy
**Introduction**

*Ganoderma lucidum* is one of the most important medicinal fungi discovered until now. This fungus has a vast source of polysaccharides, proteins and secondary metabolites with therapeutic properties, such as anti-cancer properties, preventing chronic disease, enhancing memory and immuno-modulating properties. Different kinds of commercial *G. lucidum* products are also available (Cheng & Sliva 2015). Furthermore, *G. lucidum* is one of the most valuable fungus that has many applications in traditional Chinese medicine for more than 2000 years in order to prevent and treatment of various diseases (Pawlik et al. 2015). In addition, to application of this fungus in East Asia, medicinal properties of *Ganoderma lucidum* have been identified throughout the world including Turkey, Malaysia and Pakistan in recent years (Guzeldag & Colak 2007, Zakaria et al. 2009, Nasim et al. 2010). In 2007, *G. lucidum* was found in Shir-darreh which is located in North of Iran (Moradali et al. 2007). This fungus was characterized based on its morphological and physiological features such as color, size and spores (Moradali et al. 2007). In addition, further investigations on *G. lucidum* from Iran indicated that, this fungus is capable to produce worthwhile secondary metabolite with the same characteristics as the *G. lucidum* from China. (Keypour et al. 2010). Moreover, growth condition of Iranian *G. lucidum* was optimized in order to produce anticancer metabolite (Heydarian et al. 2015).

Traditionally, identification and taxonomy of basidiomycetes were based on morphological feathers; however, it is not a reliable method due to some problems such as their plasticity, absence of basidiocarps during certain times and also similar characteristics of some species which is difficult to recognition (Gottlieb & Wright 1999, Pilotti et al. 2004). Consequently, molecular techniques have overcome these difficulties and are recognized as a trustworthy method. Some of these methods for fungi analysis are DNA sequence of ribosomal RNA (rRNA) genes, certain ribosomal elongation factors, genes from the nuclear and the mitochondrial genomes (Tan & Niessen 2003, Moreau et al. 2006). Moreover, the non-coding Internal Transcribed Spacer (ITS1 and ITS2) regions located in ribosomal DNA (rDNA) are highly variable in length and sequences in comparison with their close related spises and consequently are a significant genetic marker in order to determine taxonomic identity of fungi (Gallego & Galián 2001). Nowadays, the PCR amplification and analysis of the ribosomal region (ITS1 and ITS2) and also the 5.8S rRNA has been accepted as a powerful technique for species identification and epidemiological tracing in mycology (Korabecna 2007). In this study, therefore, for the first time we used this method in order to genetically analyze and identify newly discovered *G. lucidum* from Iran. In fact, this work is a complementary to prior studies on physical and visual physiological characteristics of *G. lucidum*.

**Materials and Methods**

- **Collection**

  The strains of *Ganoderma lucidum* were collected from Dohezar forest, Tonekabon, Mazandaran, Iran, growing on *Carpinus betulus* L. (*Corylaceae*) (Fig. 1). Faculty of Bioscience, Shahid Beheshti University, Tehran, Iran (Sp. GIRAN17).
- Culture condition

The strain of *Ganoderma lucidum* was maintained aseptically on potato dextrose agar (PDA) plates supplemented with streptomycin sulphate and incubated at 25°C in the dark for 5 days. Then the colonies were aseptically transferred to fresh PDA media without streptomycin and incubated for 14 days. The 250 ml shaken flasks included 75 ml broth culture. The optimum conditions followed for maximal cell growth were temperature (25°C), aeration (130 rpm) and initial pH (6.5) (Heydarian et al. 2015).

- DNA isolation and PCR amplification

After freeze drying the biomasses at −70°C for 3 days, the total DNA was extracted according to previous protocols (Guzeldag & Colak 2007). To amplify 5.8S and ITS regions, the primer set used was 5’ GTACACACCGCCCGTCG 3’ and 5’ GGTTGGTTTTCTTTCTCT 3’ and the process was done according to previous protocols. (White et al. 1990) finally, the band which includes 800 base pairs was eluted from the gel and was recovered by GTP recovery kit.

- DNA sequencing

The PCR products were purified by a QIAquick PCR Purification Kit (QIAGEN, Germany) according to its instruction. Then the PCR products were sequenced in both directions with the same set of primers which used for PCR. The sequencing of the recovered PCR product was performed by Gene Fanavaran Company, Iran.

- DNA sequencing analysis

Sequence from the ITS regions were compared with others using the BLASTn (Basic Local Alignment Search Tool for nucleotides) as an alignment tool. The results of BLASTn searches showed the highest similarity 99% between new sequence and those in GenBank and EMBL.

**Results and Discussion**

DNA sequence analysis of the ribosomal DNA (rDNA) region is a reliable method to determine the taxonomy in fungi. Furthermore, based on the multiple nucleotide alignments of the ITS 1 and ITS 2 region sequences, the 5.8S rDNA gene between the ITS 1 and 2 regions was 99% identical to other spices of *G. lucidum*.

We also applied blast program to search for identical sequence in GenBank database. The results demonstrated that this sequence is belonged to Basidiomycota, Hymenomycetes, Homobasidiomycetes, Aphyllophorales, Ganodermataceae, and *Ganoderma lucidum* (GenBank accession number: KX765192). Since different strains produce wide variety of biochemical compounds, characterization of *G. lucidum* from various geographical distributions is really important especially in terms of their metabolites production. Prior studies on *Ganoderma*...
**Ganoderma lucidum** from Iran examined the production of range of active compounds and also antibacterial activity (Keypour et al. 2008, 2010). However, for the first time, the molecular identification of *G. lucidum* was performed and consequently we could approve that the isolated fungus from north of Iran is *G. lucidum*. On the other hand, investigations on pharmaceutical mushrooms and their metabolites are increasing in recent years in Iran. Since this fungus is very precious fungus which has many applications in drug development, cell and tissue engineering and also bioremediation (Mesa Ospina et al. 2015), discovery of the native species of these mushrooms can lead researchers to enhance their studies. Moreover, further investigations are required to investigate the medicinal properties of this valuable mushroom.

**References**


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