

## Molecular identification of *Ganoderma lucidum* from Iran

Received: 11.09.2016 / Accepted: 28.12.2016

**Motaharehsadat Heydarian:** MSc Graduate, Microbial Biotechnology, Department of Life Science Engineering, Faculty of New Sciences and Technologies, University of Tehran, 14395-1561, Tehran, Iran

**Ashrafalsadat Hatamian-Zarmi**✉: Assistant Prof., Department of Life Science Engineering, Faculty of New Sciences and Technologies, University of Tehran, 14395-1561, Tehran, Iran (hatamian\_a@ut.ac.ir)

### 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

## شناسایی مولکولی *Ganoderma lucidum* در ایران

دریافت: ۱۳۹۵/۰۶/۲۱ / پذیرش: ۱۳۹۵/۱۰/۰۸

**مطهره سادات حیدریان:** دانش‌آموخته کارشناسی ارشد بیوتکنولوژی میکروبی، گروه مهندسی علوم زیستی، دانشکده علوم و فنون نوین، دانشگاه تهران، تهران، ایران  
**اشرف‌السادات حاتمیان زارمی**✉: استادیار گروه مهندسی علوم زیستی، دانشکده علوم و فنون نوین، دانشگاه تهران، تهران، ایران (hatamian\_a@ut.ac.ir)

### خلاصه

قارچ دارویی *Ganoderma lucidum*، قرن‌ها به منظور پیشگیری و درمان انواع بیماری‌ها از جمله هپاتیت، اختلالات ایمنولوژیک و سرطان در شرق آسیا مورد استفاده قرار گرفته است. در واقع، این قارچ شامل منبع عظیمی از پلی‌ساکاریدها، پروتئین‌ها و متابولیت‌های ثانویه با خواص ضدتومور و تنظیم‌کنندگی سیستم ایمنی است. تا به حال، انواع متابولیت‌های دارویی از این قارچ استخراج و به مرحله تولید تجاری رسیده است. در سال‌های اخیر، این قارچ ارزشمند علاوه بر چین، کره و ژاپن، در نقاط مختلف جهان از جمله پاکستان، مالزی و ترکیه شناسایی شده است. در سال ۲۰۰۷، برای نخستین بار قارچ مذکور در جنگل‌های شمال ایران شناسایی گردید ولی با این حال، تا کنون هویت مولکولی آن گزارش نشده است. هدف از این مطالعه، تایید هویت مولکولی قارچ جمع‌آوری شده از شمال ایران به کمک تجزیه و تحلیل توالی ITS-5.8S، DNA ریبوزومی است.

**واژه‌های کلیدی:** رده‌بندی، شمال ایران، ضد تومور، قارچ، ITS

## 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 features; however, it is not a reliable method due to some problems such as their plasticity, absence of basidiocaps

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 species 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).



Fig. 1. *Ganoderma lucidum* growing on *Carpinus betulus* L.

#### - 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' GGTGGTTTCTTTTCCT 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 witch 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*, *Aphylophorales*, *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*

*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.

## References

- Cheng, S. & Sliva, D. 2015. *Ganoderma lucidum* for Cancer Treatment: We are close but still not there. *Integrative Cancer Therapies* 14(3): 249–257.
- Gallego, D. & Galián, J. 2001. The internal transcribed spacers (ITS1 and ITS2) of the rDNA differentiates the bark beetle forest pests *Tomicus destruens* and *T. piniperda*. *Insect Molecular Biology* 10(5): 415–420.
- Gottlieb, A.M. & Wright, J.E. 1999. Taxonomy of *Ganoderma* from southern South America: subgenus *Ganoderma*. *Mycological Research* 103(6): 661–673.
- Guzeldag, G. & Colak, O. 2007. Molecular identification of *Ganoderma lucidum* from Turkey. *International Journal of Agriculture and Biology* 9(6): 767–770.
- Heydarian, M., Hatamian, A., Amoabediny, G. & Yazdian, F. 2015. Native medicinal fungus: investigation and optimization important factors on growth of *Ganoderma lucidum* in order to product anticancer metabolite. 15th Iranian National Congress of Chemical Engineering. Tehran, Iran.
- Heydarian, M., Hatamian, A., Amoabediny, G., Yazdian, F. & Doryab, A. 2015. Synergistic effect of elicitors in enhancement of ganoderic acid production: Optimization and gene expression studies. *Applied Food Biotechnology* 2(3): 57–62.
- Keypour, S., Rafati, H., Riahi, H., Mirzajani, F. & Moradali, M.F. 2010. Qualitative analysis of ganoderic acids in *Ganoderma lucidum* from Iran and China by RP-HPLC and electrospray ionisation-mass spectrometry (ESI-MS). *Food Chemistry* 119(4): 1704–1708.
- 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.
- Keypour, S., Riahi, H., Moradali, M.-F. & Rafati, H. 2008. Investigation of the antibacterial activity of a chloroform extract of Ling Zhi or Reishi medicinal mushroom, *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst. (*Aphyllphoromycetideae*), from Iran. *International Journal of Medicinal Mushrooms* 10(4): 345–349.
- Korabecna, M. 2007. The variability in the fungal ribosomal DNA (ITS1, ITS2, and 5.8 S rRNA gene): its biological meaning and application in medical mycology. *Communicating Current Research and Educational Topics and Trends in Applied Microbiology* 105: 783–787.
- Mesa Ospina, N., Ospina Alvarez, S.P., Escobar Sierra, D.M., Rojas Vahos, D.F., Zapata Ocampo, P.A. & Ossa Orozco, C.P. 2015. Isolation of chitosan from *Ganoderma lucidum* mushroom for biomedical applications. *Journal of Materials Science: Materials in Medicine* 26(3): 135.
- Moradali, M.F., Hedjaroude, G.A., Mostafavi, H., Abbasi, M., Ghods, S. & Sharifi-Tehrani, A. (2007) The genus *Ganoderma* (*Basidiomycota*) in Iran. *Mycotaxon* 99: 251–269.
- Moreau, P.-A., Peintner, U. & Gardes, M. 2006. Phylogeny of the ectomycorrhizal mushroom genus *Ahnicola* (*Basidiomycota*, *Cortinariaceae*) based on rDNA sequences with special emphasis on host specificity and morphological characters. *Molecular Phylogenetics and Evolution* 38(3): 794–807.
- Nasim, G., Ali, M. & Mehmood, N. 2010. Molecular analysis of *Ganoderma lucidum* isolates from Lahore. *Pakistan Journal of Botany* 42(5): 3307–3315.

- Pawlik, A., Janusz, G., Dłuska, I., Siwulski, M., Frąć, M. & Rogalski, J. 2015. Genetic and metabolic intraspecific biodiversity of *Ganoderma lucidum*. *BioMed Research International* 2015: 1–13.
- Pilotti, C.A., Sanderson, F.R., Aitken, E.A.B. & Armstrong, W. 2004. Morphological variation and host range of two *Ganoderma* species from Papua New Guinea. *Mycopathologia* 158(2): 251–265.
- Tan, M.-K. & Niessen, L.M. 2003. Analysis of rDNA ITS sequences to determine genetic relationships among, and provide a basis for simplified diagnosis of, *Fusarium* species causing crown rot and head blight of cereals. *Mycological Research* 107: 811–821.
- White, T.J., Bruns, T., Lee, S. & Taylor, J.W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: A Guide to Methods and Applications* 18: 315–322.
- Zakaria, L., Ali, N.S., Salleh, B. & Zakaria, M. 2009. Molecular analysis of *Ganoderma* species from different hosts in Peninsula Malaysia. *Journal of Biological Sciences* 9(1): 12–20.

ROSTANIHA