Molecular Identification of Two Strains of *Phellinus* sp. by Internal Transcribed Spacer Sequence Analysis

Kwang-Soo Shin*

*Department of Microbiology and Biotechnology, Daejeon University, Daejeon 300-716, Korea*

(Received November 1, 2011. Revised November 3, 2011. Accepted November 7, 2011)

Two species of cultivated *Phellinus* sp. were identified as *P. baumii* by internal transcribed spacer (ITS) sequence analysis. The fruit bodies of the examined strains were similar to those of naturally occurring strains, having a bracket-like form, yellow-to-orange color, and poroid hymenial surfaces. The DNA sequences of ITS region of both strains showed a homology of 99% with ITS1 to ITS2 sequences of *P. (Inonotus) baumii* strain PB0806.

**KEYWORDS:** Identification, ITS region, Molecular phylogeny, *Phellinus*

*Phellinus* belongs to a genus of pore fungi that have important medicinal value; many species in this and related genera have been used as folk medicines because of their biochemical or pharmaceutical actions [1]. *P. linteus* also has anti-carcinogenic properties [2-4], and polysaccharides purified from *P. linteus* mycelium strongly stimulate B-lymphocyte production [4], cell-mediated and humoral immunity [5], and inhibit tumor growth and metastasis [6]. Aqueous extracts from *P. rhabarbarinus* potently affect human immunodeficiency virus [7] and extracts of *P. gilvus* and *P. baumii* may be useful in preventing acute pulmonary inflammation in human diseases [8].

These benefits have made cultivation of the fruit body of *Phellinus* sp. popular in Korea. However, clear identification of cultivated *Phellinus* to the species level has not been reported, which hinders their use in pharmaceutical therapy. In the present study, the most popularly used *Phellinus* were identified on the basis of molecular biological properties.

The specimens were purchased from herbal medicine markets in Daejeon (KM-5) and Geumsan (KM-6). Total DNA was extracted from the specimen using genomic DNA prep kit (Solgent, Daejeon, Korea) and internal transcribed spacer (ITS) regions including the 3’ flanking region of nuclear small subunit ribosomal DNA and the 5’ flanking region of nuclear large subunit ribosomal DNA were amplified by PCR using primers NS7 [9] and LW2 [10]. An amplicon was used as the template for a second PCR using ITS1 and ITS4 as the primers (Fig. 1). The reaction involved denaturation for 15 min at 95°C, 35 cycles of 20 sec at 95°C, 40 sec at 50°C, and 1 min at 72°C, with final extension of 3 min at 72°C. The purified PCR product was sequenced and homologous sequences were found by a BLAST Search. The sequences determined in this study were deposited in GenBank under accession number JN887691 (KM-5) and JN887692 (KM-6). ITS sequences were aligned using the CLUSTALX program [11] and phylogenetic relationships were estimated from the aligned sequences for each data set using PAUP*4.0b4a [12]. Neighbor-joining method with distance option and parsimony method with heuristic option were applied for phylogenetic analyses. Bootstrap values were determined to support individual branches (1,000 replications) [13, 14].

The basidiocarps of the examined strains showed typical *Phellinus* characteristics found naturally. The fruit bodies were bracket-like, broadly attached to the substrate, yellow-to-orange, and the hymenial surfaces were poroid (Fig. 2). The amplicon size of strain KM-5 and KM-6 was 811 bp and 812 bp, respectively. Their sequence homology was 99.4%. The ITS region sequences of KM-5 and KM-6

---

*Corresponding author  <E-mail: shinks@dju.ac.kr>*

© This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

299
showed 99% similarity with a sequences *P. (Inonotus) baumii* strain PB0806 (AY436629). The maximum parsimony cladogram is presented in Fig. 3 along with the bootstrap values. *Phellinus* was separated in three groups: *baumii* group, *igniarius* group, and *johnsonianus-linteus-rhabarbarinus* group. This result was slightly different to previous results obtained on the basis of combined data of ITS and mitochondrial SSU rDNA sequences analyses. In the previous study the groups were: group A (*P. linteus, P. baumii, P. johnsonianus, P. rhabarbarinus, P. tropicalis*), group B (*P. igniarius, P. nigricans*), and group C (*P. hispidus, P. pini, and P. hartigii*) [15]. From these results, *Phellinus* sp. KM-5 and KM-6 were identified as *P. baumii*. *P. baumii* has previously been demonstrated to have several pharmaceutical activities *in vitro* and *in vivo*. Extracts of *P. baumii* showed anti-obesity effects in high-fat diet-fed mice [16], anti-oxidant and free radical scavenging activity [17], and inhibited pulmonary inflammation in rats [8]. Further investigations for other pharmaceutical activities by this artificially cultivated mushroom are clearly warranted.

References

1. Jeong SC, Cho SP, Yang BK, Jeong YT, Ra KS, Song CH. Immnomodulating activity of the exopolymer from submerged mycelial culture of *Phellinus pini*. J Microbiol Biotechnol 2004;14:15-21.
2. Ikekawa J, Nakanimish M, Uehara N, Chihara G, Fukuoka F. Antitumor action of some basidiomycetes, especially *Phellinus linteus*. Gann 1968;59:155-7.
3. Lee JH, Cho SM, Kim HM, Hong ND, Yoo ID. Immunostimulating activity of polysaccharides from mycelia of *Phellinus linteus* grown under different culture conditions. J Microbiol Biotechnol 1996;6:52-5.
4. Song KS, Cho SM, Lee JH, Kim HM, Han SB, Ko KS, Yoo ID. B-lymphocyte-stimulating polysaccharide from mushroom *Phellinus linteus*. Chem Pharm Bull (Tokyo) 1995;43:2105-8.
5. Kim HM, Han SB, Oh GT, Kim YH, Hong DH, Hong ND, Yoo ID. Stimulation of humoral and cell mediated immunity by polysaccharide from mushroom *Phellinus linteus*. Int J Immunopharmacol 1996;18:295-303.
6. Han SB, Lee CW, Jeon YJ, Hong ND, Yoo ID, Yang KH, Kim HM. The inhibitory effect of polysaccharides isolated from *Phellinus linteus* on tumor growth and metastasis. Immunopharmacology 1999;41:157-64.
7. Walder R, Kalvatchev Z, Garzaro D, Barrios M. Natural products from the tropical rain forest of Venezuela as inhibitors of HIV-1 replication. Acta Cient Venez 1995;46:110-4.
8. Jang BS, Kim JC, Bae JS, Ree MH, Jang KH, Song JC, Kwon OD, Park SC. Extracts of *Phellinus gigovus* and *Phellinus baumii* inhibit pulmonary inflammation induced by lipopolysaccharide in rats. Biotechnol Lett 2004;26:31-3.
9. White TJ, Bruns TD, Lee SB, Taylor JW. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: a guide to methods and applications. San Diego: Academic Press; 1990. p. 315-22.
10. Jeong WJ. Phylogenic analysis of *Phellinus* based on internal transcribed spacer and mitochondrial small subunit ribosomal sequences [dissertation]. Seoul: Seoul National University; 1999.
11. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The Clustal_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 1997;25:4876-82.
12. Swofford DL. PAUP*4.0b: phylogenetic analysis using parsimony (* and other method). Version 4. Sunderland: Sinauer Associates; 2002.
13. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 1985;39:783-91.
14. Hillis DM, Bull JJ. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst Biol 1993;42:182-92.
15. Jeong WJ, Lim YW, Lee JS, Jung HS. Phylogeny of *Phellinus* and related genera inferred from combined data of ITS and mitochondrial SSU rDNA sequences. J Microbiol Biotechnol 2005;15:1028-38.
16. Noh JR, Lee IK, Yang KJ, Gang GT, Kim YH, Hwang JH, Yun BS, Lee CH. A *Phellinus baumii* extract reduces obesity in high-fat diet-fed mice and absorption of triglyceride in lipid-loaded mice. J Med Food 2011;14:209-18.
17. Shon MY, Kim TH, Sung NJ. Antioxidants and free radical scavenging activity of *Phellinus baumii* (*Phellinus of Hymenochaetaeae*) extracts. Food Chem 2003;82:593-7.