RESEARCH NOTE

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

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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].

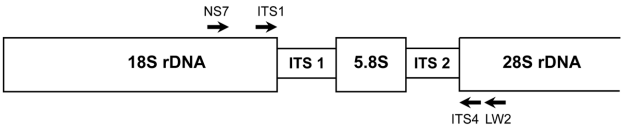

Fig. 1. Location of primers for the amplification of internal transcribed spacer (ITS) region. Primers for the first PCR were NS7 and LW2, and for the second PCR were ITS1 and ITS4, respectively.

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

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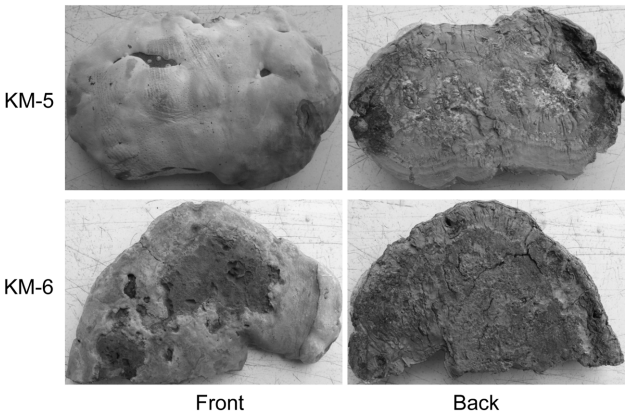

Fig. 2. Photographs of basidiocarps of two strains of *Phellinus* sp.: KM-5 and KM-6.

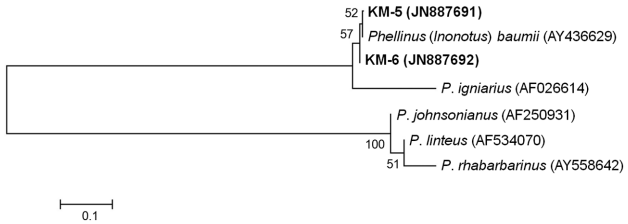

Fig. 3. Phylogenetic tree using ITS sequences of *Phellinus* sp. KM-5 and KM-6 and its allied species. The tree was constructed by the neighbor joining method and bar indicated number of nucleotide substitutions per site.

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 small subunit ribosomal DNA 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.

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