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First Note of *Hypoxylon truncatum* sensu Miller in Korea

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국내에서 발견된 *Hypoxylon truncatum* sensu Miller의 형태 및 유전적 특징

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ABSTRACT: Occurrence of *Hypoxylon truncatum*, known as a symbiont of white jelly mushroom, was first noted and described in Korea. Two stromatal forms reported in North American collections of *H. truncatum* sensu Miller were also observed in Korean collections. Based on evidences from other studies, polymorphic patterns seen in six enzyme digestions of nuclear Internal Transcribed Spacer (ITS) regions of Korean isolates indicated that the two stromatal forms were genetically distinct. Because there was a clear association of stromatal morphology with genetic differences, the different stromatal forms might be different species. In addition, clear species concept on the species *H. truncatum* would provide aids in selecting proper strain for cultivation of white jelly mushrooms.

KEYWORDS: *Hypoxylon truncatum*, stromata, PCR-RFLPs, white jelly mushroom

Hypoxylon truncatum (Schw.: Fr.) Mill. sensu Miller is a cosmopolitan fungus, occurring principally on hardwoods (Miller, 1961). It is especially common on *Quercus* species (Farr *et al.*, 1989), and in Asia it causes economic losses to production of shiitake mushrooms [*Lentinus edodes* (Berk.) Sing.] on *Quercus* logs by antagonistic growth (Abe, 1989). However, *Hypoxylon* species in recent years attract increasing attention due to its beneficial effects on production of white jelly mushrooms, *Tremella fusiformis* Berk. in Asia (Huang, 1982; Huang, 1986).

Despite of its beneficial effects on white jelly mushroom, *H. truncatum* has not been used by Korean mushroom growers. This seems to stem from the fact that this species has not been recorded in Korea, and species concept of this fungus has not been established yet. In order for this fungus to be properly used in mushroom production, a clear species concept of this fungus should be established with extensive investigation of its occurrence and distribution in Korea.

H. truncatum exhibits the xylaria centrum type (Jong and Rogers, 1972). In the sense of Miller (1961), stromata of this species are quite variable, ranging from very small tuberculate to hemispherical, to applanatae and widely effused. Shear (1945) considered the different stromatal types to be stable features with taxonomic significance. Miller (1961), however, believed that different forms of stromata can not be indicative of different

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species beccause stromata are variable due to different environmental factors. In order to clarify these different views on the taxonomic significance of different stromatal forms, Yoon and Glawe (1993) examined Random Amplified Polymorphic DNA (RAPD) markers on North American isolates of *H. truncatum* sensu Miller. Their results showed that a clear association existed between RAPD markers and two stromatal types in the North American isolates studied, and suggested that it would be useful to designate the different stromatal forms as different species. However, Yoon and Glawe (1992) used only single molecular technique to resolve such a taxonomic problem. In addition, data analyzed from RAPD markers have not been widely accepted due to the lack of reproducibility of RAPD markers.

As reported herein, we first recorded occurrence of *H. truncatum* complex in Korea, with description of morphological and molecular characteristics. We employed Polymerase Chain Reaction (PCR)-Restriction Fragment Length Polymorphisms (RFLPs) of nuclear ITS regions to examine genetic relationship of two stromatal forms. Results of this study were compared with those of the previous study (Yoon and Glawe, 1992) from RAPD analyses. Besides the practical considerations regarding the taxonomic aspect described above, this study was also initiated to help mushroom growers in producing fruiting bodies of the white jelly fungus by providing a clear species concept of its symbiont, *H. truncatum*.

Materials and Methods

Collections and cultures

Each of the two stromatal forms of *H. truncatum* sensu Miller was collected on different dead *Quercus* sp. limbs at Haenam, Jeonnam Province in 1995. Each collection fitted the description of *H. truncatum* given by Miller (1961). Cultures were started by soaking stromata in water for 2-3 h, blotting them dry on paper towels and then placing them in inverted petri plates containing Difco potato dextrose agar (PDA) for 1-2 days. Ascospores were discharged upward onto the agar. For mass-ascospore isolates, groups of ascospores were transferred to new plates. Two mass-ascospore isolates, each from two stromatal forms, were prepared.

Preparation of genomic DNA

To produce mycelia for DNA isolation, isolates were transferred to flasks containing 5 g Difco yeast extract and 20 g glucose/L of tap water. Isolates were grown in shake cultures at 25°C for approximately one week. Mycelia were harvested by filtration, blotted on paper towels, and stored at −20°C.

DNA isolation was performed as described previously (Yoon *et al.*, 1991). DNA solutions containing 100 μg of RNase A/m*l* final concentration were incubated at 37°C for 2 h to digest RNA. Concentrations of DNA in samples were estimated prior to PCR amplification by comparing the intensity of DNA bands in 0.8% agarose gels with a series of λDNA dilutions, and by viewing them under UV light (310 nm) after staining with ethidium bromide.

PCR amplification and digestions

PCR was used to amplify nuclear ITS1-5.8S-ITS2 rDNA regions in an MJ Research DNA thermal cycler (model PTC-150). Primers ITS1 and ITS4 (Bioneer) were used for PCR amplification and primer sequences were listed in White *et al.* (1990). Amplification reaction mixtures were 100 μ*l* in volume and contained 10 mM Tris-HCl (pH 9.0 at 25°C), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 200 μM each of dATP, dCTP, dGTP and TTP (Perkin-Elmer-Cetus), 50

ρmol of each primer, 2.5 units of *Taq* polymerase and 25 ng of genomic DNA. The amplification program consisted of pre-denaturation at 94°C for 5 min; 35 cycles of 94°C/1 min, 50°C/1 min, and 72°C/2 min; a final incubation at 72°C for 5 min to complete the last extension. Amplified PCR fragments were digested with each of six restriction endonucleases (*Hae*III, *Alu*I, *Hpa*II, *Cfo*I, *Hsp* 92II and *Mbo*I) according to the manufacturer's protocols (Promega Biotech). Digested DNA fragments were resolved by electrophoresis (7 V/cm) for 3 h in a gel composed of 1.5% NuSieve GTG agarose (FMC Bioproducts) and 1.5% ultra pure agarose (Sigma), and detected by UV transillumination. A 100 bp DNA ladder (Gibco BRL) was also run on the gel to serve as a size marker.

Results and Discussion

Morphological observations

Each collection generally included approximately 10 stromata. One collection included only hemispherical stromata that lacked apparent perithecial elevations, whereas the other collection from different *Quercus* limb included effused stromata with readily apparent perithecial elevations (Fig. 1). Effused stromata superficially resembled the hemispherical form due to their large size, but there were clear differences in the more apparent perithecial elevations. The size of hemispherical stromata was more variable than effused stromata, ranging from 1 mm to 6 cm across. In addition to differences in stromatal types, there was a subtle difference in color between the two forms of stromata. The color of hemispherical form was usually black at maturity but that of effused form appeared to be dark-brown. Ostioles of both stromatal forms were acutely papillate from the center of stromata. The asci were cylindrical and unitunicate. Ascospores of both forms were di-

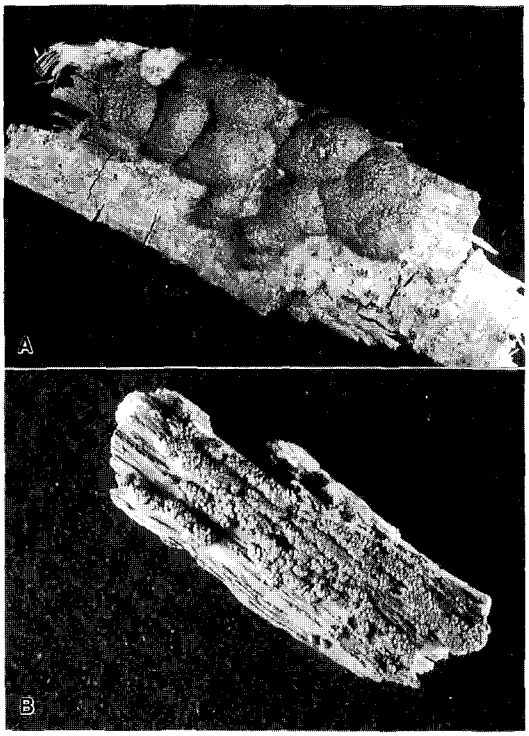

Fig. 1. Different stromatal forms of *Hypoxylon truncatum* sensu Miller collected at Haenam, Jeonnam Province. Hemispherical stromata lacking apparent perithecial elevations (A), and effused pulvinate stromata with readily apparent perithecial elevations (B).

agonally uniseriate and phaseoliform (Fig. 2). The color of ascospores was dark brown at maturity.

These results are consistent with those of the previous study (Yoon and Glawe, 1993) on North American isolates of *H. truncatum* sensu Miller. In that study, 54 isolates collected at various geographical origins were clearly separated into the two stromatal types; hemispherical and effused pulvinate forms. Their results indicated that differences in stromatal type and color are associated with genetic differences rather than place of origins. However, their collections were restricted to North America. In the present study, which involved working with original collec-

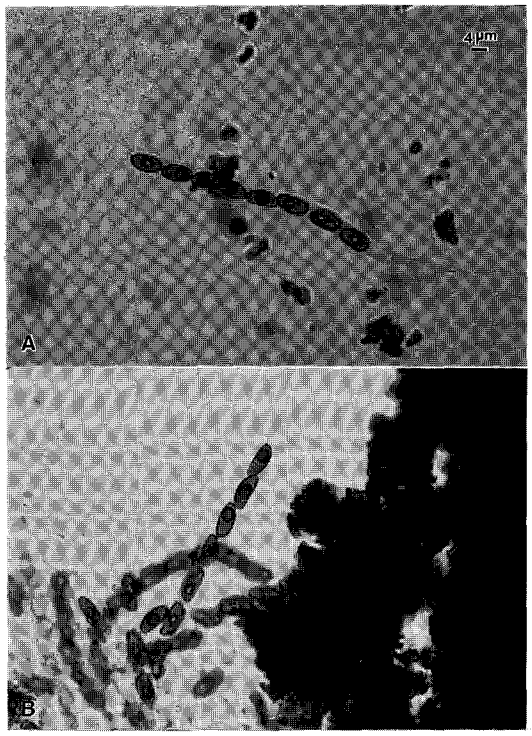

Fig. 2. Asci and ascospores of hemispherical and effused pulvinate stromatal forms of *Hypoxylon truncatum* sensu Miller collected at Haenam, Jeonnam Province. Cylindrical, unitunicate asci, and diagonally uniseriate, phaseoliform ascospores (8-9 μm × 3-5 μm) of hemispherical form (A), Cylindrical, unitunicate asci, and diagonally uniseriate, phaseoliform ascospores (8-9 μm × 3-5 μm) of effused pulvinate form (B).

tions made in Korea, two stromatal types were also observed in Korea and they were not found to intergrade within single collections. This finding supports assertions of Shear (1945), and Yoon and Glawe (1993) that *H. truncatum* sensu Miller includes more than one taxonomic entity. Because intermediate forms of stromata were not observed in the previous study (Yoon and Glawe, 1993) and the present study, the results did not support Miller's assertion that stromatal morphology is variable in this species.

PCR products and restriction patterns

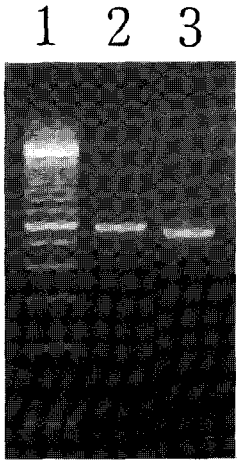

Fig. 3. PCR products of nuclear ITS regions electrophoresed 3% agarose and stained with ethidium bromide.. Lane 1: 100 bp ladder DNA as standard; Lane 2: hemispherical form. Estimated size of fragment was about 580 bp; Lane 3: effused pulvinate form. Estimated size of fragment was about 560 bp.

ITS1-5.8S-ITS2 fragments amplified from each of the two stromatal forms were different from each other (Fig. 3). The size of fragment from hemispherical form was approximately 580 bp, whereas that from pulvinate form was estimated to be 560 bp. These results indicate that genetic differences exist between the two forms in the region amplified.

RFLP phenotypes for the PCR products from six enzyme digests are shown in Fig. 4. In general, there was good agreement between size estimates for the undigested PCR products and the sums of fragment sizes. Six endonucleases (*Hae*III, *Alu*I, *Hpa*II, *Cfo*I, *Hsp*92II and *Mbo*I) generated polymorphic phenotypes sufficient to distinguish genetic difference between the two stromatal forms. Among restriction fragments generated from six digests, none of fragments was shared by the two stromatal forms. Restriction patterns were completely different from each other. The results are clearly in agreement with the

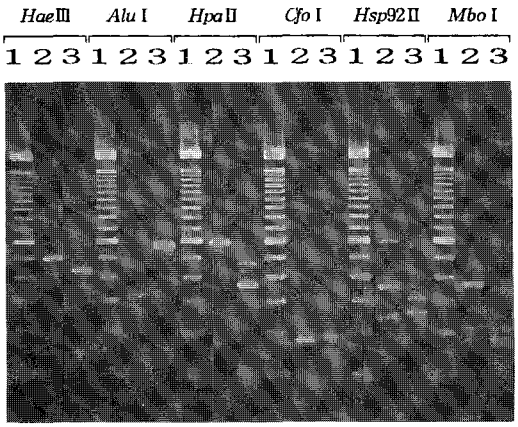

Fig. 4. 3% agarose-gel electrophoresis of restriction fragments from six enzyme digests. Lane 1s: 100 bp ladder DNA as standard; Lane 2s: hemispherical form.; Lane 3s: effused pulvinate form.

previous study (Yoon and Glawe, 1993) that genetic rather than environmental causes are responsible for the different stromatal types. In that study, phenetic analyses of 99 different RAPD markers separated 54 collections made at various geographical distances into the two groups according to different stromatal types.

Even though the results in the previous study (Yoon and Glawe, 1993) indicated that the two stromatal forms are genetically distinct taxa, it was not certain that if the two groups observed in their analyses were completely due to environmental factors specific to North America or less reproducible RAPD markers. If genetically distinct groups were restricted to North American isolates due to special environmental factors or due to less reproducible RAPD markers, data derived from PCR-RFLPs of nuclear ITS1-5.8S-ITS2 of Korean isolates would show similar restriction phenotypes for the two stromatal forms. In fact, the two stromatal forms did not share any of restriction fragments generated by six endonucleases. This result indicates that differences in stromatal type are associated with genetic differences rather than place of origin. Thus, the results from this study, and those from the previous study (Yoon and Glawe, 1993), suggest that fungi with different stromatal types might be distinct species. For a clear answer to how these two forms should be designated, and how synonymy should be revised, critical examination of type material will be necessary.

In conclusion, even though *H. truncatum* has been known to have beneficial effect on production of white jelly mushrooms, occurrence of this fungus was never reported in Korea. Herein, we first recorded and described occurrence of *H. truncatum* in Korea.

Two stromatal forms reported in North American collections of *H. truncatum* sensu Miller were also observed in Korean collections. Based on evidence from other studies, polymorphic patterns seen in six enzyme digestions of nuclear ITS regions of Korean isolates indicate that the two stromatal forms are genetically distinct. Because there is a clear association of stromatal morphology with genetic differences, it would be useful to designate the different stromatal forms as different species. In addition, clear species concept on the species *H. truncatum* would provide aids in selecting proper strain for cultivation of white jelly mushrooms.

摘 要

국내에서 처음으로 *Hypoxylon truncatum*을 채집하여 형태 및 유전적 특징을 관찰하였다. 채집된 균주의 형태적 특징은 문헌에 기록된 외국의 기존 균주의 특징과 일치하였으며 각기 다른 두 형태의 stromata를 지니고 있었다. 대상균주로부터 DNA를 추출한 후 ITS 부분에 대한 PCR-RFLPs를 이용하여 분석을 하였다. 그 결과, 총 6종의 제한효소로부터 유래된 절편들 중 대상균주간에는 서로 공통되는 절편이 관찰되지 않았다. 이러한 결과를 볼 때 다른 형태의 stromata를 지니고 있는 균주간에는 상당한 유전적 거리가 있다고 할 수 있다. 그러므로 다른 형태의 stromata를 지니고 있는 *H.*

truncatum 균주는 다른 종일 것이라 사료된다. 또한 *H. truncatum*은 백목이 버섯의 인공재배에 있어서 아주 중요한 역할을 할 수 있으므로 본 균주를 사용하기 전에, 이 균에 대한 정확한 분류학적 개념 확립은 백목이 버섯 인공재배를 위한 정확한 균주 선발에 큰 도움이 되리라 생각된다.

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