

Morphological characteristics of fruit bodies and basidiospores of *Wolfiporia extensa*

Woo-Sik Jo^{1,*}, Sung-Hak Lee, Jinmo Koo, Songyi Ryu, Min-Gu Kang, Soon-Young Lim, and Seung-Chun Park²

¹Gyeongbuk Province Agricultural Technology Administration, Daegu 41404, Korea

²College of Veterinary Medicine, Kyungpook National University, Daegu 41566, Korea

ABSTRACT: *Wolfiporia cocos* is a well-known traditional medicine in China, Japan, Korea, and other Asian countries owing to its numerous therapeutic properties. With the aim to determine the morphology and genetic characteristics of *W. cocosten* strains of *W. cocos* were cultivated *in vitro*, and subsequently, rapid amplification of polymorphic DNA was performed. To the best of our knowledge, this is the first study to examine the morphology of fruit bodies of *W. cocos* in Korea. *W. cocos* were cultured on PDA agar at different temperatures (12, 16, 20, 24, and 28°C) under 12-hour light (600 Lux) / 12-hour dark photoperiod condition for 1 month. Appearance of fruit body was the highest at 28°C condition in all the strains investigated. Honeycomb-like structure on sclerotia was observed in Andong 01, Andong 02, Andong 03, KFRI 1104, KFRI 1105, KFRI 1106, KFRI 1107, KFRI 1108, and ASI 13007 strains of . The KFRI 1103 strain formed cosmos petal-like structure on sclerotia. The average size of basidiospores was recorded as 7.55 μm in height and 3.35 μ in width.

KEYWORDS: Basidiospores, Fruit bodies, *Wolfiporia extensa*

Wolfiporia extensa (Schwein.) Ryvarden & Gilb. [syn *Poria cocos* (Schw.) Wolf] is one of the most precious and widely used medicinal fungi belonging to the Polyporaceae family of the Basidiomycetes (Dai *et al.* 2009). The dried sclerotia have been used as a sedative, stomachic, and diuretic in traditional Chinese herbal medicine for thousands of years, and has been commercially applied to formulate nutraceuticals or functional foods (Wang *et al.* 2013). Several reports demonstrated that polysaccharides of *W. cocos* have positive anti-tumor, anti-inflammatory, anti-oxidant, and anti-aging effects (Choi, 2016; Hung *et al.*, 2007; Jang and Lee, 2015). Furthermore, *W. extensa* can be used as natural substance for cosmetics which are safe in antioxidant and anti-aging effects (Wang *et al.*, 2013; Choi,

2016; Haung *et al.*, 2007). This study is focused on determination of the morphology and genetic characteristics of 10 strains of *W. extensa* in Korea. Customary cultivation of *W. cocos* is progressed in underground (Wang *et al.*, 2013; Fu *et al.* 2002). On this account, it is very difficult to observe the real-time morphology of *W. extensa* in the course of growth cycle, including production of fruit bodies and basidiospores. In this regard, we cultured *W. extensa* *in vitro* in order to have a detailed observation of its fruit body and basidiospore formation. We also did a phylogenetic analysis based on internal transcribed spacer (ITS) region of rDNA, including the 5.8S region in 10 strains of cultured *W. extensa*.

Fungal Isolates

The isolates of 10 strains of *W. extensa* species used in this study are listed in Table 1. *W. extensa* ASI 13007 was obtained from Rural Development Administration (RDA) in south Korea. KFRI 1103, KFRI 1104, KFRI 1105, KFRI 1106, KFRI 1107, and KFRI 1108 were obtained from Korea Froest Service. Andong 01, Andong 02, and Andong 03 were obtained from Ryu Chunghyeon medicinal mushroom. Co., Ltd. Isolates were maintained on Potato Dextrose Agar (PDA) medium.


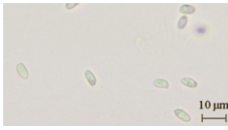

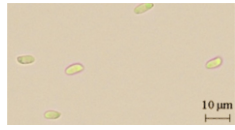



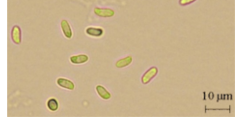

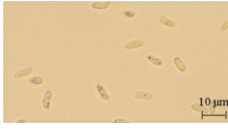

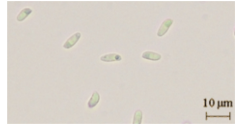

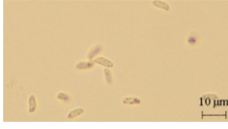

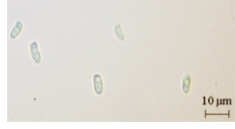

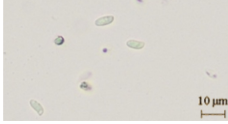

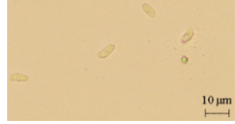
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*Corresponding author
E-mail : jws67@korea.kr
Tel : +, Fax : +82-53-320-0295

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Table 1. Morphology of fruit bodies and basidiospores of *W. extensa*

No.	Strain	Fruit Bodies	Basidiospores	No.	Strain	Fruit Bodies	Basidiospores
1	ASI13007			2	Andong1		
3	Andong2			4	Andong3		
5	KFRI1103			6	KFRI1104		
7	KFRI1105			8	KFRI1106		
9	KFRI1107			10	KFRI1108		

Fruit Body Induction

PDA media were sterilized for 30 minutes at 121°C and aseptically poured into plastic petridish (90×15 mm). Mycelia of 10 strains were separately inoculated on a Potato Dextrose Agar medium (PDA) and incubated at 25 °C in darkness for 5 days. They were then transferred to different temperatures (12, 16, 20, 24, 28°C) with 12-hour light (600 Lux) / 12-hour dark photoperiod condition for 1 month for fruit body formation. Fruit body formation rate was checked every 1 week.

When cultured in different temperatures (12, 16, 20, 24, 28°C), all 10 strains of *W. extensa* were induced to form fruit bodies successfully under the condition of 28 °C. Only 5 strains (Andong 01, KFRI 1105, KFRI 1106, KFRI 1108 and ASI 13007) induced fruit bodies in 20 and 24 °C. Fruit body was not induced in 12 and 16 °C. There were two kind of fruit body structures, one is a honeycomb-like structure and the another is a cosmos petal-like structure. Andong 01, Andong 02, Andong 03, KFRI 1104, KFRI 1105, KFRI 1106, KFRI 1107, KFRI 1108, and ASI 13007 formed honeycomb-like structure on sclerotia. KFRI 1103 formed cosmos petal-like

structure on sclerotia (Table 1). The fruit bodies' color was light-yellow.

Single-spore Isolation and Optical Microscopy

The fruit bodies were picked out and attached onto a petri dish bottom. The plates were placed upside down and incubated at 25 °C, in order that spores could shed onto the lid of the petri dish. The spore suspension was obtained by adding 1 mL of sterilized water to the spore print and using a sequential gradient dilution with sterile water until a concentration of 1×10^3 spores/mL is achieved. The spores were observed by a optical microscopy using Nikon Eclipse 50i (Nikon Corp., Tokyo, Japan). The spore size (length and width) was measured by i-solution program (iMT echnology, Daejeon, Korea).

Basidiospores were observed under optical microscope. Spore size was 7.55 μm in height and 3.35 μm in width on average. The size of spores were different upto strains, Andong 01 (Hight : 8.05 ± 0.65 , width : 3.51 ± 0.25), Andong 02 (Hight : 7.45 ± 0.52 , width : 3.49 ± 0.30), Andong 03 (Hight : 7.22 ± 0.20 , width : 3.25 ± 0.22), KFRI1103 (Hight

: 7.24±0.61, width : 2.95±0.24), KFRI1104 (Hight : 7.98±0.65, width : 3.42±0.22), KFRI1105 (Hight : 7.06±0.56, width : 2.86±0.35), KFRI1106 (7.73±1.00, width : 3.38±0.27), KFRI1107 (Hight : 7.48±0.47, width : 3.37±0.42), KFRI1108 (Hight : 7.85±0.79, width : 3.43±0.30), ASI13007 (length : 7.39±0.47, width : 3.79±0.33) (Table 2).

Phylogenetic analysis

Fungal DNA was extracted using Qiagen kit (Qiagen Korea Ltd, Seoul, Korea) according to the manufacturer's protocols. PCR was performed to amplify the internal transcribed spacer (ITS) region of rDNA, including the 5.8S region, with the primers ITS1F and ITS4[100] under the following conditions: 94°C for 5 min followed by 30 cycles of 94°C for 30 sec, 50°C for 30 sec, 72°C for 1 min, and final extension at 72°C for 5 min. PCR products were sequenced by Solgent (Daejeon, Korea). The sequence was deposited in NCBI GenBank (accession number

KX868076) and compared with those available in GenBank via the BLAST search function.

Phylogenetic analysis was conducted using the maximum likelihood (ML) method in MEGA7, with the Tamura-Nei substitution model [101]. All characters were equally weighted and unordered. Gaps and missing data were treated as complete deletions. Support for specific nodes on the ML tree was estimated by bootstrapping 1,000 replications. The sequence for *W. extensa* was used as an outgroup.

Phylogenetic analysis revealed that Andong 01 was the most genetically closest strain to the Korean traditional *W. extensa* strain, ASI13007 and KFRI1106 was the most genetically furthest strain among 10 strains of *W. extensa*.

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References

- Choi SH. 2016. Comparison of ingredients and antioxidant activity of the domestic regional *wolfiporia extensa*. *Kor J Mycol.* 44: 23-30.
- Dai YC, Yang ZL, Cui BK, Yu CJ, Zhou LW. 2009. Species diversity and utilization of medicinal mushrooms and fungi in china (review). *Int J Med Mushrooms.* 11: 287-302.
- Fu J, Wang KQ, Shu W, Fang H, Deng F. 2002. Review of Chinese traditional medical fungus: *Wolfiporia cocos*. *Res Inf Tradit Chin Med.* 4: 16-17.
- Huang QL, Jin Y, Zhang LN, Cheung PCK, Kennedy JF. 2007. Structure, molecular size and antitumor activities of polysaccharides from *Poria cocos* mycelia produced in fermenter. *Carbohydr Res.* 70:324-333.
- Jang YA, Lee JT. 2015. The evaluation of antioxidant, antiinflammatory, and anti-aging of extract solvent and *poria cocos* by parts. *Kor J Aesthet Cosmetol.* 13:377-383.
- Wang YZ, Zhang J, Zhao YL, Li T, Shen T, Li JQ, Li WY, Liu HG. 2013. Mycology, cultivation, traditional uses, phytochemistry and pharmacology of *Wolfiporia cocos* (Schwein.) Ryvarden et Gilb.: A review. *J Ethnopharmacol.* 147:265-276.

Table 2. Size of basidiospores of *W. cocos*

Strain	Hight(μm)	Width(μm)
Andong 1	8.05±0.65 ^a	3.51±0.25 ^b
Andong 2	7.45±0.52 ^{abcd}	3.49±0.30 ^b
Andong 3	7.22±0.20 ^{cd}	3.25±0.22 ^b
KFRI1103	7.24±0.61 ^{cd}	2.95±0.24 ^c
KFRI1104	7.98±0.65 ^{ab}	3.42±0.22 ^b
KFRI1105	7.06±0.56 ^d	2.86±0.35 ^c
KFRI1106	7.73±1.00 ^{abc}	3.38±0.27 ^b
KFRI1107	7.48±0.47 ^{abcd}	3.37±0.42 ^b
KFRI1108	7.85±0.79 ^{abc}	3.43±0.30 ^b
ASI13007	7.39±0.47 ^{abcd}	3.79±0.33 ^a

^{a-d} : Value in the same line with different literal at Duncan's multiple range test(P<0.05) and results are mean ± standard deviation of three replicates

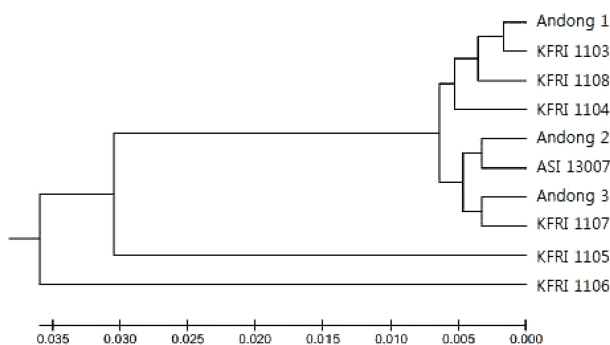


Fig. 1. Phylogenetic analysis of *W. cocos*