

Mycelial Properties of *Tremella fuciformis* and *Hypoxylon* sp.

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흰목이와 *Hypoxylon* sp.균의 균사적 특성

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ABSTRACT: Two strains, each of *Tremella fuciformis* and *Hypoxylon* sp. were isolated and their mycelial properties were investigated. *T. fuciformis* produced yeast-like conidia and dikaryotic mycelia from white to yellow color. The diameter of the hyphae was 1.5 to 3.0 μm , and septa with clamp connections were present. Secondary mycelia with clamp connections could produce fruitbodies on sawdust medium when the environmental conditions were suitable for fructification. The symbiotic fungus, *Hypoxylon* sp. produced white feather-like mycelia. But the color of old mycelia was changed to light yellow or light brown and pigmented the culture medium from light brown to brown or very dark green. Generally it did not produce conidia, but in a special case it produced conidia of which color was yellow green to grass green and of which shape was sub-elliptical with the size of approximately 3 to 5 μm .

KEYWORDS: *Hypoxylon* sp., Symbiotic fungus, *T. fuciformis*, Yeast-like conidia

The white jelly mushroom, *Tremella fuciformis*, belongs to the family Tremellaceae of the order Tremellales in the class Hymenozymycetes of the division Basidiomycota (Lowy, 1971). The so-called jelly fungi are a group of species which form gelatinous basidiocarps.

Many jelly fungi were brightly colored and were shaded yellow, orange, or red. On the other hand, the fruitbody of *T. fuciformis* is pure white in color. The majority of jelly fungi was saprophytes on wood, but some were parasitic on plants. The important characteristics of the genus *Tremella* were fructification, firm to extremely tough, gelatinous when fresh, cerebriform to foliose, brainlike or tubercular, drying horny (Kawamura, 1909; Rea, 1922; Whelden, 1934; Olive, 1948). *T. fuciformis* is one of approximately 40 species in the genus *Tremella*. The members of the

genus were widely distributed, being essentially worldwide. It is found mainly in subtropical regions, but it has also been reported from tropical and temperate regions and even frigid zones. It grows on the wood of hardwood trees. It usually appears in rainy seasons in mountain areas and also grows on the dead trunk or branches of hardwood. The purpose of this paper is to report the possible morphological characteristics of the fungus.

Materials and Methods

The stromatal forms of *Hypoxylon* sp. and the mycelia of *T. fuciformis* were collected from dead *Quercus* limbs at Haenam, Jeonnam province in 1995. For the identification of these two fungi, mycelial properties and microscopic features were investigated by naked eye, stereomicroscopy and compound microscopy.

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Hypoxylon sp. was isolated from dead *Quercus* limbs grown white jelly fungus. It was successfully isolated from spore suspensions (yeast-like conidia) to use as a spawn for the observation of *Tremella* mycelium and fruitbody.

The isolation procedure of *Tremella* mycelium by spores is as follows; ① Washing the fruitbody with sterilized water, ② Suction of water on the surface of the fruitbody with absorbent cotton, ③ Preparation of sterilized agar plate (petri-dish), ④ Taping the fruitbody beneath petri-dish cap in clean bench, ⑤ Turning round at intervals of 8 hours 4 times, ⑥ Isolation of spores at the 4th turning intervals.

Results

Hyphae, conidia of *T. fuciformis*

For the result of tissue and spore isolation in *T. fuciformis*, the contamination rate was approximately 90%, the mycelia type was hymenia in tissue isolation, and 0% in spore and wood log isolation respectively, and the mycelial types were basidiospores and dikaryon (Table 1). The hyphae obtained by suspending freshly collected single fruitbody over agar media was colorless and hyaline, 2~3.5 μm in diameter, and provided with clamp connections.

The basidiospores produced on the hymenium germinated tubes on basidiospores and budded out forming round or ovoid conidia or budded again like yeasts to produce conidia or secondary basidiospores which were called

Table 1. Contamination rate of *T. fuciformis* according to isolation method

Isolation method	Isolation numbers	Mycelia type	Contamination rate (%)
Tissue	10	hymenia	90
Spore	10	basidiospores	0
Wood log	10	dikaryon	0

Table 2. Description of *T. fuciformis* hyphae

Hyphae		Basidiocarp		Basidiospore	
Diameter	Colour	Size	Style	Size	Style
2~3.5 μm	hyaline	7.2 μm	obovate	5.3~6.8 μm	obovate

yeast-like conidia (Table 2).

Fruitbody

T. fuciformis varies from nearly translucent to opaque white in color, firmly gelatinous, agarlike at first, becoming soft and mucilaginous, often acquiring a sordid tint with age.

The hymenium spreads over the whole surface of the fruitbody and is very rarely papillate. The probasidia were spherical or hemispherical type and had cross septa. The epibasidia were ovoid to clavate.

In cultivation, there were two kinds of fruitbodies such as nut-gall type and cock's comb type (Fig. 1). The nut-gall type was larger and thicker in blade than the cock's comb type, and it had higher economic value.

Basidia, Basidiospore

Basidia were obovate, 7.2 μm in size and closely packed to form the hymenium longitudinally divided into four cells. Epibasidia were white, rarely yellowish, subglobose to obovate, 8~10 \times 1.5~2 μm , narrow toward one end, usually with granules inside. Basidiospores were obovate or hemispherical, 5.3~6.8 μm in size. The basidiospores bud out small conidia.

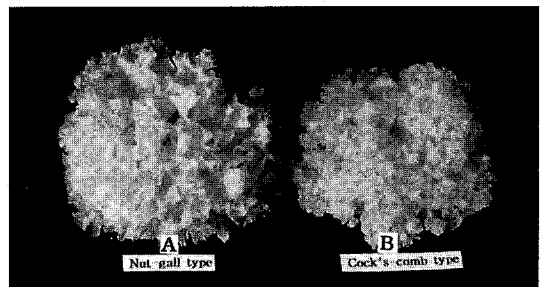


Fig. 1. Fruiting body type of *T. fuciformis* (A: Nut-gall type B: Cock's comb type).



Fig. 2. Yeast-like mycelia isolated from spore of *T. fuciformis*.

The mycelium sometimes was produced from the bud conidia (Fig. 2, 3, 4, 5).

Isolation rate of *Hypoxylon* sp.

Isolation rate of *Hypoxylon* sp. was 62% or 18% in *T. fuciformis*, 8% in *Trichoderma viridae*, 4% in *Pseudomonas* sp., 3% in *Strepto-*

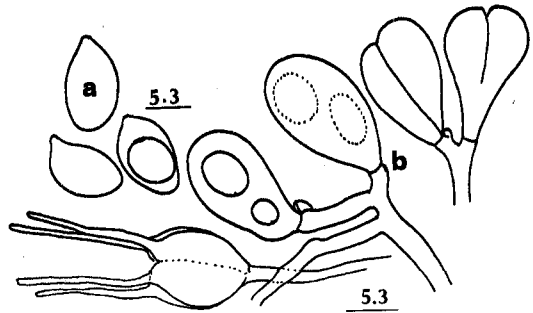


Fig. 3. Spore(a) and basidia(b) of *T. fuciformis*.

myces sp. and 1% in each *Aspergillus* sp., *Penicillium* sp., *Saccharomyces* sp., *Rizopus* sp. and *Monilia* sp.. These 8 kinds of fungi were isolated from oak wood log collected from Haenam, Jeonnam province (Table 3).

Mycelium, conidia of *Hypoxylon* sp.

The fungus of *Hypoxylon* sp. was identified as ascomycetes. It was the role of the biological factor or the mycelium to help *Tremella* in the digestion of the wood and to provide some residual nutrition. The feather like

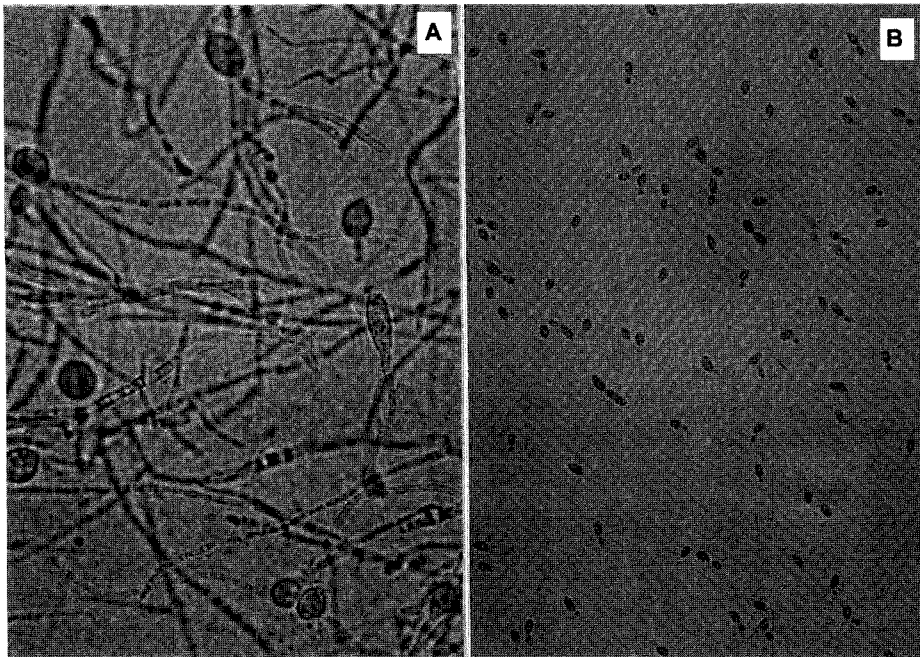


Fig. 4. Hymenia(A) and basidiospores(B) of *T. fuciformis* ($\times 600$).

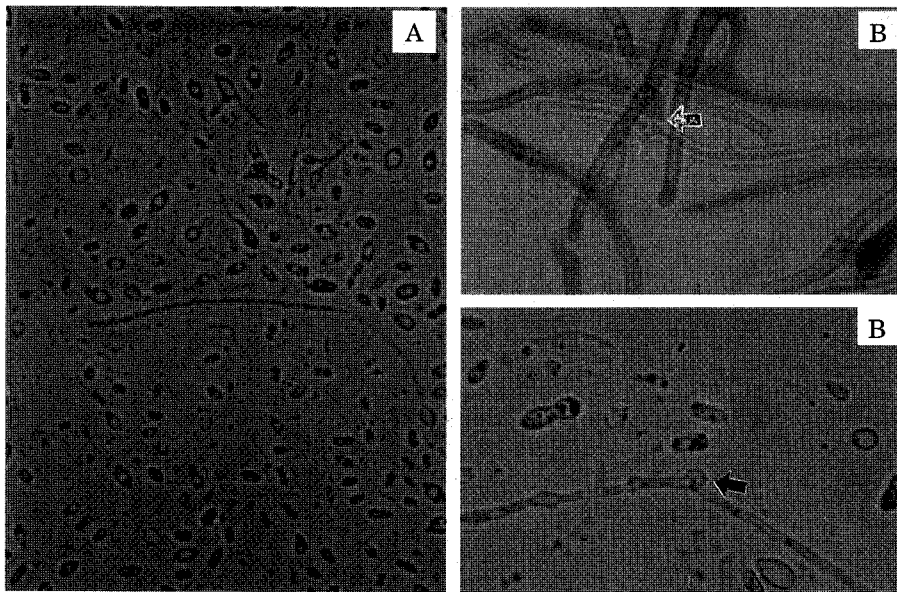


Fig. 5. Budding yeast-like conidia(A) and secondary hyphae with clamp connections(B) ($\times 650$).

Table 3. Microorganisms isolated from the wood log (*Quercus serrata*) growing *T. fuciformis*

Microorganisms	Isolation frequency(%)	Collection site
<i>Hypoxyylon</i> sp.	62	Jeonnam Haenam Gun
<i>Tremella fuciformis</i>	18	
<i>Trichoderma viridae</i>	8	
<i>Pseudomonas</i> sp.	4	
<i>Streptomyces</i> sp.	3	
<i>Aspergillus</i> sp.	1	
<i>Penicillium</i> sp.	1	
<i>Saccharomyces</i> sp.	1	
<i>Rizopus</i> sp.	1	
<i>Monilia</i> sp.	1	

hyphae of the ascomycete led the way into the wood log, and presumably provided

some nutrition to support the growth of *Tremella*. First, the mycelial color of *Hypoxyylon* sp. was white with feather shape and feather-like side branches. The old mycelium was the light yellow or light brown, and the culture medium has been gradually changed from light brown to black or very dark green. Aerial hyphae were gray-white and thin. The mycelium has a velvety surface. Usually, there were no conidia. They were a yellow-green to grass-green, sub-elliptical in shape and approximately 3 to 5 μm in size (Table 4, Fig. 6).

Stroma

Generally, each collection strain included 5 to 10 pieces of stromata. One of these strains had only hemispherical stromata that lacked apparent perithecial elevations, whereas the other collection from different oak wood log

Table 4. Description of *Hypoxyylon* sp.

Shape		Colour				Size (μm)	
Mycelium	Conidia	Hyphae	Aerial	Medium	Conidia	Hyphae	Conidia
Feather like side branches	Sub-elliptical	Light-yellow	Gray-white	Blackish brown	Yellow-green	5~6	3~5

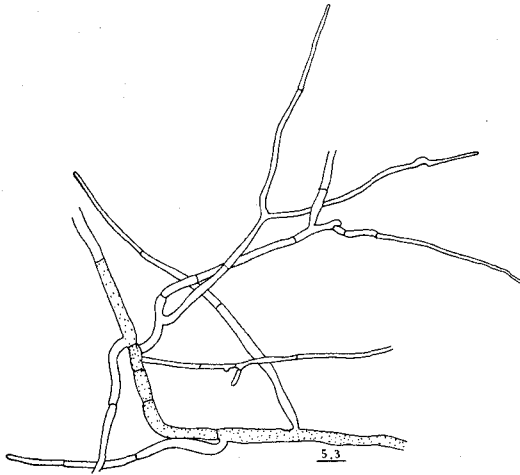


Fig. 6. Mycelium of *Hypoxylon* sp. ($\times 650$).

included effused stromata with readily apparent perithecial elevation. Effused stromata superficially resembled the hemispherical form due to their large size, but there was clear difference in the more apparent perithecial elevations. The size of hemispherical stromata was more variable than effused stromata, ranging from 1 mm to 1 cm across. In addition to difference 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. Ostiole of both stromatal forms were acutely papillate from the center of stromata. The asci were cylindrical and unitunicate. Ascospores of both forms were diagonally uniseriate and phaseoliform. The color of ascospores was dark brown at maturity (Fig. 7).

Discussion

The germination of basidiospores in the Heterobasidiomycetes was known to have four types, i.e., germination by long germ tubes, by repetition, by conidial formation, and by yeast-like budding (Kobayashi and Tubake, 1965). In this investigation, the basidiospores of *T. fuciformis* germinated two ways—germ tubing and yeast-like budding. The yeast-like budding may be important in pursuing the phylogenetical relations between the fungus and the yeast. This type of basidial germination was the same as *Tremella frondosa* (Whelden, 1934). Whelden

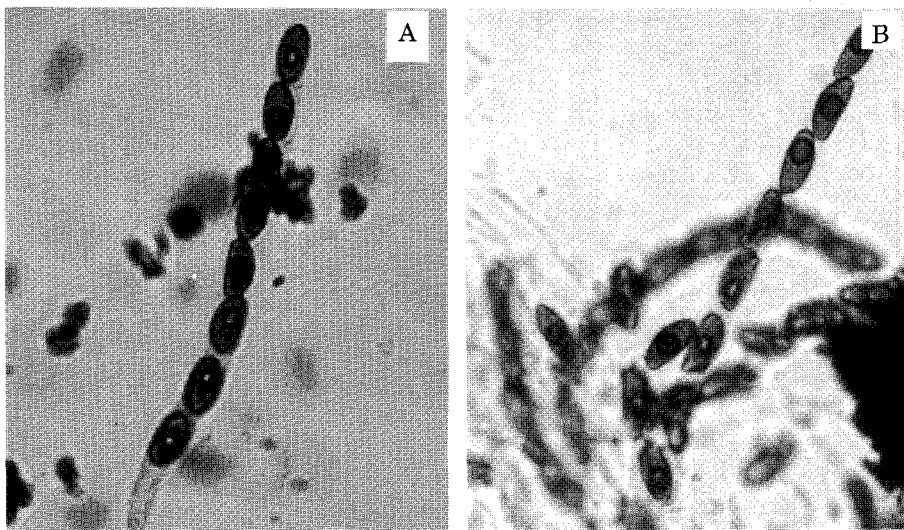


Fig. 7. Mycelia form, asci and ascospores of hemispherical and effused pulvinate stromatal forms of *Hypoxylon* sp. (A). Mycelia form and ascospores of effused pulvinate form (B).

(1934) found that when the basidiospores of *Tremella frondosa* germinated in water, they developed from one to four chains with two to three cells each, budded off from the primary spores.

The basidiospores may germinate in either of two ways. One type of germination was typical of that of basidiospores of other members of the class in that they form germ tubes, and then the germlings develop into mycelia.

The other type of germination of basidiospores involves a budding to form yeast-like conidia. These kinds of conidia looked like *Penicillium*. This investigation shows that the penicillium-like conidia were either monokaryon or dikaryon.

Two kinds of fruit bodies were regarded the cock's comb type as a morphological variety of the nut-gall type and named it *T. fuciformis* Berk. f. *formiculata* Kobayasi (Ito, 1955; Hiroe, 1958). Kobayasi (1939) found a white jelly mushroom that has black sparse ciliate at the base of the fruit body and was considered as a new genus. It was given as a new name, *Nakaiomyces nipponicus* (Ito, 1955; Hiroe, 1958). However, Olive (1958) considered that *Nakaiomyces* was a "nomen confusum" based upon a parasite on *Tremella* and was therefore, not a valid genus.

적 요

흰목이 2핵균사는 색택이 흰색과 노란색의 중간이며 균사의 직경은 1.5~3.0 μm 이고 균사의 격막이 있는 곳에 클렘프 연결체가 있다. *Hypoxylon* sp.(공생균)의 균사는 흰색이며 갓과 같은 모양이고 길고 가는 주요균사는 갓과 같은 가지를 친 균사를

가진다. 오래된 균사는 연노랑 혹은 연갈색이며 배양배지가 점차 연갈색에서 갈색이나 진한 녹색으로 변한다. 보통 분생자는 잘 생성되지 않으나 생성될 경우 분생자는 노랑~녹색에서 연두색이며 형태는 반타원형이며 약 3~5 μm 의 크기이다. 흰목이의 자실층 발아에 의해 생성되는 담자포자는 발아관과 효모상 분열자의 두가지 방법으로 생성되며 담자포자와 효모상 분열자는 둘다 1핵체이며 발아후 1차균사를 생성한다. 1차균사의 융합후 담자기는 클렘프를 갖는 2차균사로 생성한다. 효모상 분생자는 발아하여 두꺼운 벽을 가진 젤라틴질 균사로 자라며 재배배지에서는 노란균사의 집단으로 발달한다. 2차균사는 환경조건이 알맞으면 톱밥배지에서 자실체를 형성한다.

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