

# Germ Tube Formation of Ascospores of Two Terrestrial Higher Ascomycetes, *Hypoxylon mammatum* and *H. truncatum*\*<sup>1</sup>

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## ABSTRACT

Two wood decay ascomycetes fungi identified as *Hypoxylon mammatum* and *H. truncatum* were isolated from backyard of Korea Research Institute of Chemical Technology (KRICT) in Korea. *Hypoxylon truncatum* is newly recorded as a wood degrader in Korea. Unusual germination mechanisms of ascospores in *H. mammatum* and *H. truncatum* are described and illustrated. The differences between two species were noticed on the process of germ tube formation. In the process of germ tube formation, the fast movement to pigmented ascospores activated from their perispores was termed as spore eclosion that was only found in *H. mammatum*. This sophisticated recognition mechanism indicated the existence of specific eclosion and germ tube formation due to the composition of cell wall layers and their preferable host derive, based on examined two species under a genus. The observation on present study postulates different composition of wall layers of ascospore and different nutrient composition for germination.

*Keywords* : Ascospore, eclosion, germ tube, *Hypoxylon mammatum*, *H. truncatum*

## 1. INTRODUCTION

Great parts of the works in mycology have been done on the germination of fungal spores. Since all fungus biodegradation must begin with infection, the environmental condition for ascospore germination or inhibition is one of the great practical importances. *Hypoxylon truncatum* has been well known as an inhibitor to *Lentinus edodes* (Berk.) Sing. and *H. mam-*

*matum* (Wahl.) Miller (*H. pruinautum*) has been known to cause *Hypoxylon* canker in a serious impact on quaking aspen (French & Manion, 1975). However, little is known of the mechanism of germination and fungal attachment into wood elements. Recently Lee (1997) presented the mathematical data of some higher ascomycetes fungi in germination rate and its relationship with habitat. In ascospore morphological shape, Bartnicki-Goucia (1984) consid-

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ered three main steps of germination, such as activation, spherical growth, and germ tube formation. Chapela and Petrini (1990) reported an unusual ascospore germination of *H. fragiforme*, focusing on the first step in establishment of an endophytic symbiosis. They also examined on ascospore germ tube formation with the percent germination due to substratum in microscopic study. Furthermore, Read *et al.* (1992) studied germination and development of attachment structures by aquatic fungi with a scanning electron microscope study.

Microscopical observation on the fast movement during germ tube formation is necessary for the study of the initial step of germination. This study was undertaken to observe the ascospore eclosion and its germ tube formation on wood substratum under the light microscopy for examining the points of attachment of ascospores and its adhesive capability to wood surface.

## 2. MATERIALS and METHODS

### 2.1 Isolation of fungal species

Decayed wood (hardwood) by *H. mammatum* and *H. truncatum* were collected from backyard of Korea Research Institute of Chemical Technology (KRICT) in Taejon. Morphological and molecular characteristics were used for fungal identification (Lee, 1999). Fresh ascospores were isolated and used for germination observation with light microscope. Axenic cultures of wide range ascospores were prepared and used in the methods of Chapela *et al.* (1990).

### 2.2 Methodology of fungal identification

Each description was based on macro- and microscopical analysis of the materials collected. Stromata and perithecium were observed by stereomicroscopy. Squash slides were made from fresh ascomata and then ascospores and asci were observed by light microscopy. Dirty particles on the surface of stromata were removed by 1% KOH, and apical apparatus of the ascus was stained with the Melzer's reagent. Samples of 20 fully mature ascospores and asci were measured.

### 2.3 Preparation for light microscopic observation

Discharged ascospores of *H. mammatum* and *H. truncatum* were freshly obtained from stromata stored at 15°C at refrigerator. Germination rates were measured on the surface of wood section; a drop of ascospore suspension ( $2 \times 10^4$  spores/ml) dropped on the wood thin section (20-30  $\mu\text{m}$  thickness) of longitudinal direction ( $1 \times 1 \text{ cm}^2$ ). Ascospore suspensions on wood section in agar plates were incubated at 25°C for 24 hours. A drop of ascospores suspension ( $2 \times 10^4$  spores/ml) dropped on a microscope slide and transferred to moist petri dish sealed and was incubated at 25°C for 24 hours for the observation of germination. Incubated ascospore suspensions on the slides were observed for 24 hours.

## 3. RESULTS

### 3.1 Characteristic of new fungus

*Hypoxyton truncatum* (Schwein: Fr.) J.H. Miller, Trans. Brit. Mycol. Soc. 17: 130. 1932. (Plate 1).

type disc, 0.3-0.6 mm; Asci not found; Ascospores brown to dark brown, uniseriate, ellipsoid-inequilateral, with obtuse ends, 9.0-11.5 × 4.0-5.5 μm, with full straight germ slit.

Known distribution: Southern United States, Mexico, and Japan.

Known habitat: on *Quercus* and on many kinds of dicotyledonous wood.

Known collection in Korea: Mt. Naejang (Lee, not reported).

Specimen collected: Yusong, Taejon in Korea.

Note: *Hypoxylon truncatum* is a complex of species with wide variation in stromatal shape (Abe and Liu, 1995). This fungus is a common member of the genus *Hypoxylon* in Japan and China. Very small annulate disc around the ostiole is the distinct character of *H. truncatum* (Abe, 1984). This collection would be the first record of description in Korea.

Plate 1. Scanning electronic photomicrographs of *H. truncatum*. a. ascospore with round end (bar: 1 10 μm). b. cross section of perithecium wall (bar: 10 μm).

*Sphaeria truncata* Scriften Naturf. Ges. Leipzig 1: 44.

*Hypoxylon annulatum* (Schwein.: Fr.) Mont. var. *truncata* (Schwein.: Fr.) Starb., Bih. Kongl. Svenska Vetensk.-Akad. Handl. 27(3): 8. 1901.

*Sphaeria truncatula* Schwein., Trans Amer. Philos. Soc. n. ser., 4: 210. 1832; non Fr.: Fr., 1832.

Stromata hemispheric to effused-pulvinate, erumpent, 1-1.5 cm × 1-2 mm thick, dark brackish brown with 1% KOH greenish brown extraction, shiny blackish layer beneath surface; Perthecia hemiglobose, elevated very clearly; Ostioles papillate at the center of a truncatum-

### 3.2 Germination rate of *H. mammatum* and *H. truncatum*

Growth responses to temperature in time interval were similar in the two species of *Hypoxylon*. All species started to germinate within 6-12 hours on wood section, even though they germinated within 12-18 hours on CMA media. Two groups were identified on the basis of germinating time at which significant germ tube formation was recorded. However, these groups can be overlapped by the media influence. Higher germ tube formation was recorded for *H. mammatum* on two different media. Therefore two species on wood section also had the higher germ tube formation which was indicated that natural preferable host was better than artificial nutrient media was for fungal germination was.

### 3.3 Germination of *H. mammatum* in vitro (Plate 2. a-d)

When ascospores of *H. mammatum* were inoculated onto glass surfaces and incubated in a moist petri dish for 24 hours, they germinated to produce more than 90% in a stage of eclosion or germ tube formation. The features of eclosion of *H. mammatum* ascospores were dormant spore stages, spore activation, rupturing of wall layer (Plate 2. a-b) and exiting of germ tube (Plate 2. c-d). Within 30 minutes to 2 hours, spores dramatically changed in color into very dark brown and shaped into oval to cubic. The rupturing of spore wall layers caused the change of color and shape. After dormant spore stage, the spores at first were swollen slightly and the fissure of outer layer started to open their wall layer. The colorless outer wall layer (perispore) opened in equator direction along with the germ slit. Removing outer wall layer also caused dark

color ascospores during activation of spore wall. Continued distention of wall layer until germ fissure on equator line was separated into an arc of about 90-150°. Finally, perispores were forced away from the dark and swollen ascospores and germ tube emerged to one side or even different direction.

### 3.4 Germination of *Hypoxylon mammatum* in situ (Plate 2. e-f)

When ascospores of *H. mammatum* were inoculated onto wood sections and incubated in a moist petri dish for 24 hours, the culture conditions might influence the ascospore germ tube formation. More than 90% of eclosion were produced within 12-16 hours. However, the trace of perispore was not fully observed on wood sections, because the color of wood elements disturbed light transmission for light microscope observation. The opening of individual spores and forming germ tube included two different distinctive stages; swelling and activation of fissure of ascospores and opening of valves of pigmented wall layers due to liberated out colorless

Plate 2. Light photomicrographs of various stages in the eclosion and germ tube formation of *H. mammatum*. a-b. eclosion of pigmented and swollen ascospore (s) with perispore (w). c-d. germ tube (g) formation with changed ascospore with sound ascospore (s). e-f. hypha growth on wood section and pigmented and swollen ascospore (S). Longitudinal length of ascospore: 10  $\mu\text{m}$ .

layer. Strong correlation between eclosion and germ tube formation was observed, and apparently eclosion was a necessary requirement for germination of ascospores.

### 3.5 Germination of *Hypoxylon truncatum* in vitro (Plate 3. a-c)

After 2 hours inoculation on the glass slide and incubated at 25°C, none of ascospores were germinated, and even the majority of the ascospores showed no evidence of fissure activation. Within 2 to 6 hours incubation, spores had dramatically changed into very dark black color and opening of fissure along with germ slit in trough shape. Three main stages can be grouped and observed with light microscope as following: dormant spore stages, activation of wall layers, and exiting of germ tube. After dormant spore stage, the ascospores were swollen slightly and pressed the fissure of outer layer along with germ slit to become opening of wall layer. It was hardly noticed that there was no evidence of liberation of outer wall layer and relation of perispores during germ tube formation. Germ tubes come throughout the rumen of ascospores in trough shape of opening ascospores. Polar germ tubes were observed at the begging of germination of *H. truncatum*.

## 4. DISCUSSION

In general it was found that germ tube germination and hyphal germination were more rapid on natural substrata than synthetic substrata. It was also observed that more germ tube formation was present on the wood sections than on the glass slides. Therefore most of the micrographs here were taken from the *Quercus* species and water. General germination rates of two

Plate 3. Light photomicrographs of ascospores of *H. truncatum*. a. ascus containing of ascospores with apical apparatus of *Hypoxylon* sp. b-c. gaping of germ fissure (arrow) and trough shape of ascospore (w) that produce germ tube to one direction with sound paraphysis (P). Longitudinal length of ascospore: 12  $\mu$ m.

fungal species of fresh ascospores were slow in water compared to on wood sections. However, the most interests in present study were the mechanism of spore activation and germination of the ascospores when they discharged onto host material.

The observation presented here can be interpreted in relation to eclosion and germ tube for-

mation. A clear relationship between eclosion and germ tube formation has been reported, considering eclosion was a necessary requirement for germination (Chapela *et al.*, 1990). However, there were possibility to be either eclosion or germ tube formation without the other part. The present data revealed that certain species have their specific pathways to germinate. Child (1929) and Chapela *et al.* (1990) found that *H. fragiforme* and *D. concentrica* ascospores possess an endogenous dormancy mechanism and germinate only in the presence of potential host material without inhibitors on nonnutrients medium. It can be postulate that the same pathway of eclosion and germ tube formation stages would be occurred to *H. mammatum* and *H. truncatum*. The difference of two species was noticed at each stage that was strongly influenced by the natural inheritance (Plate 2, 3). Even though there were distinct differences between two species, 3 main stages of dormant stage, activation of spores and exiting germ tube from spores have been observed. The stages in eclosion and germ tube formation of *H. mammatum* followed as 1) dormant stage: spores were dormant, 2) spore activation: spores were slightly swelling and starting to open the germ fissure in visible, 3) germ fissure formation: outer wall layer ruptured along with equatorial seam and gaping of germ fissure, 4) pigmentation of spore wall: pigmented wall of spore opened along the germ fissure, 5) formation of ghost shell: exiting germinating the shell formed by the outer wall layer, 6) further distention composed of wall layer, and 7) germ tube initiation and the shape changing: the shape of spore changed and initiated germ tube from the shape changed spore at this early stage of germination. However, *H. truncatum* showed different pathway of each eclosion and germ tube formation stage, as following: 1) dormant spore, 2) spore activation: slightly distinctive cell wall

layers in visible but not swelling under a light microscope, 3) rupturing of each side of ascospore along with germ slit and gaping of ascospore, 4) continuing distention of the pigmented thick wall, 5) separation of both side in pigmented ascospores in trough shape, 6) further separation of germ fissure but not change in shape, 7) germ tube initiation but not changing the shape of ascospore.

Child (1929), Beckett (1976a, b) and Lee (1997) reported the difference of eclosion mechanisms and germ tube formation of *H. fragiforme* to *D. concentrica* as follows: 1) the speed of germ fissure separation of the ascospores of *H. fragiforme* during the eclosion process was wide and extremely fast and 2) *H. fragiforme* ascospores possess an endogenous dormancy mechanism and germinate only in the presence of potential host material. Present study also noticed *H. truncatum* and *H. mammatum* were similar to the germination type of *H. fragiforme*. After rupturing of outer wall layer and gaping of germ fissure by arc 90-150° the pigmented ascospores of *H. mammatum* formed germ tubes from trough shape ascospore, separated of both sides in pigmented ascospores. Also during early stage of tube formation, themselves changed the shapes of ascospore due to nutrient consumption (Plate 2). However, *H. truncatum* did not show any change outer layer, W1 and W2 wall layer.

## 5. CONCLUSION

Ascospores settled on natural substrata activated and formed germ tube more rapidly than those on artificial substrata did (Lee, 1997). The differences on mechanisms of germ tube process were found in *H. mammatum* and *H. truncatum*, which showed their own specific way. However, their *Hypoxylon mammatum* showed better germination rates on CMA and wood sections than

*H. truncatum*, while both species tested had better germination on the wood sections than on CMA. Future work should concentrate on the chemical inducers for ascospore germination under the various environmental conditions.

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