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Syzygites megalocarpus (Mucorales): A necrotrophic mycoparasite of *Tricholoma matsutake*

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이분지털곰팡이(털곰팡이목): 송이의 괴사성 균기생균

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ABSTRACT: ***Tricholoma matsutake*, an ectomycorrhizal fungus, is the most expensive mushroom at the region of northeastern Asia. Since the mushroom is edible, it is very serious to have a parasitic fungus on the surface of fruit-body. We discovered a necrotrophic mycoparasite of *T. matsutake*. We tried to illustrate the feature of mycoparasite on *T. matsutake* in detail, and it was identified as *Syzygites megalocarpus*.**

KEYWORDS: ***Syzygites megalocarpus, Tricholoma matsutake***

The mycoparasites of fruit-body are largely known to edible mushroom (Carlile and Watkinson, 1994; Singer and Harris, 1987). The mycoparasites are divided into two classes such as biotrophic mycoparasite and necrotrophic mycoparasite based on their trophic modes (Barnett and Binder, 1973). The biotrophic mycoparasite has limited host ranges and obtained nutrients from living host cells. The necrotrophic mycoparasites have broad host ranges and usually being characterized by relatively rapid growth without any special requirement of nutrients from other organisms.

Recently, the necrotrophic parasites became an important issue in cultivation of edible mushrooms. The attacked mushrooms discoloured and softened under the influence of the parasites, which result in the final putrefaction of the mushroom. *Agaricus bisporus* was reported to have three important mycopathogens; *Verticillium fungicola* which caused 'dry bubble' disease, *Mycogone perniciosa* causing 'wet bubble' disease, and *Hypomyces rosellus*, responsible for 'cobweb' disease (Jeffries and Young, 1994). *Lentinula edodes* was also reported to have *Hyphozyma* synanamorph of *Eleutheromyces subulatus* which causes black spot in Japan (Tsuneda *et al.*, 1997). *Syzygites megalocarpus* has been reported from several cultivars of *Lentinus edodes* which are cultivated widely throughout tropical Asia (Jeffries and Young, 1994). *Sepedonium chrysospermum* is known to cause some diseases on wildlives and mushroom cultivars, but also reported to infect fruiting bodies of *Suillus* and *Agaricus*, and some of Zygomycota including *Spinellus, Syzygites, Dicranophora* (Jeffries, 1985).

As the value of mushroom is strongly acquainted to general consumers, there are plenty of farmers want to cultivate mushrooms. In addition, the air temperature is getting higher and higher, which may results in wide-spread of tropical mycoparasites. However, there are only few reports for the concern of mycoparasites in Korea. *Nyctalis asterophora* was found to grow on the pileus of *Russula*, and *Cordyceps ophioglossoides* was reported to parasitize *Elaphomycetes* in Korea (Lee and Hong, 1985; Sung, 1996).

We found a mycoparasite on the pilei of *Tricholoma matsutake*, which is the most expensive mushroom at the region of northeastern Asia. In this paper, we illustrated the symptom of infection, and tried to identify the necrotrophic fungus of *T. matsutake* with detailed description of it.

Materials and Methods

The necrotrophic fungus of *T. matsutake* was collected from fruiting bodies of *T. matsutake* at an experimental site in Hongcheon, Kangwon-do on September, 1998. Fungal mycelium was obtained by sporangiospore germination and maintained on PDA at 25°C. Specimens for light microscopy (Leica, DMRE) and stereo microscopy (Zeiss,

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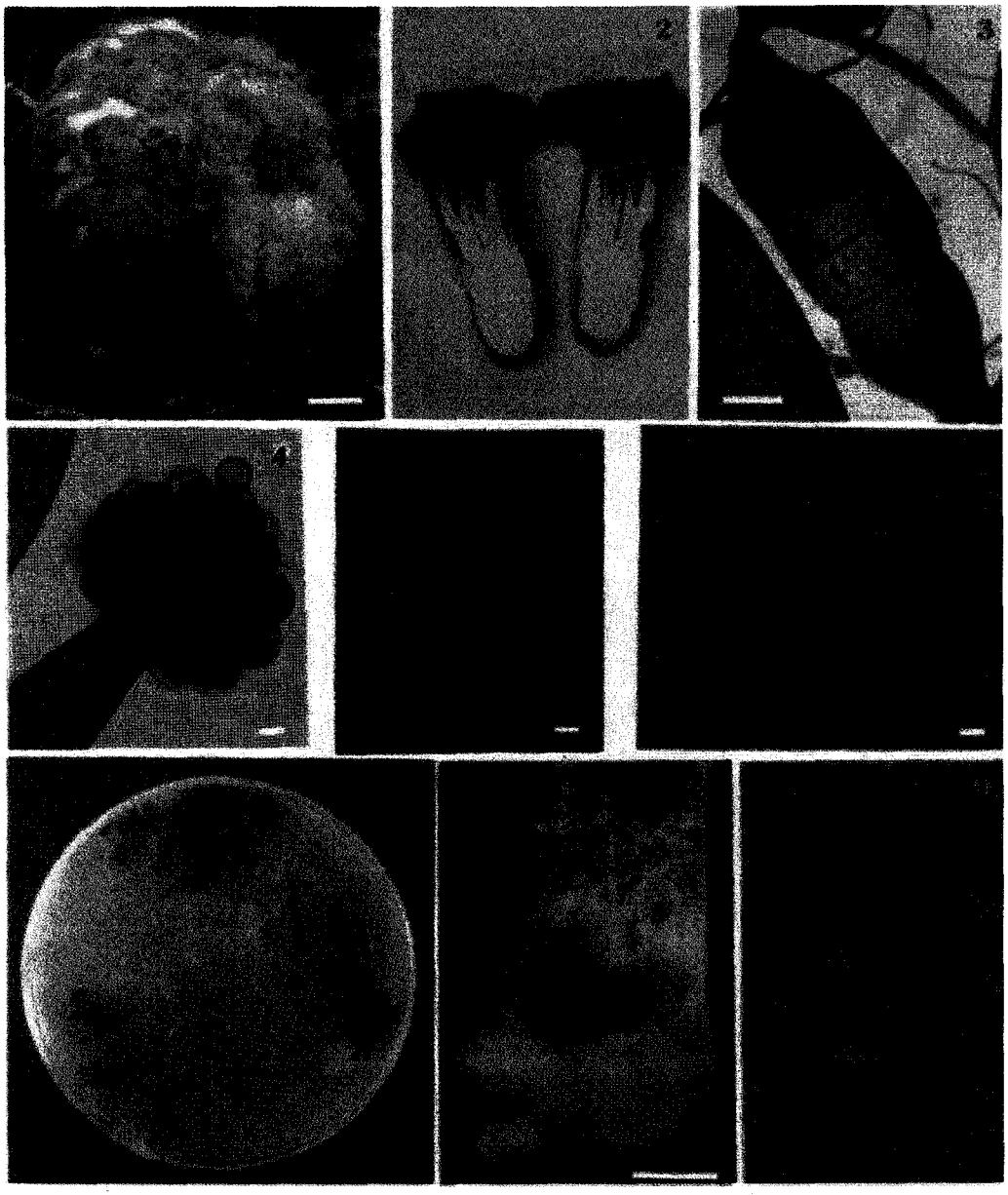
STEMI SV-8) were prepared for sporangiophores, sporangiospores and zygospores from the mycoparasites on *T. matsutake* and growing mycelia of them on PDA at 25°C. The specimens were fixed with 5% formalin or formalin-70% ethanol -acetic acid (Agerer, 1991) and stained by cotton blue (Clark, 1981).

We tried to identify the mycopathogens by morphological characteristics on the basis of species descriptions from several taxonomical reports (Arx, 1981.; Dodge, 1928; Fuller, 1978; Hesseltine, 1957; Hesseltine and Ellis, 1973; O'Donnell, 1979; Zycha, 1935).

Results and Discussion

Description of the species

The isolate was identified as *Syzygites megalocarpus* Ehrenberg ex Fries in Dicranophoraceae, Mucorales, Zygomycota (Alexopoulos *et al.*, 1996; Arx, 1981. Dodge, 1928; Fuller, 1978; Hesseltine, 1957; Hesseltine and Ellis, 1973; O'Donnell, 1979; Zycha, 1935).



Figs. 1~9. 1. Mycoparasite on fruit-body of *Tricholoma matsutake*. Bar = 1 cm. 2. Necrosis of fruit-body infected with *Syzygites megalocarpus*. 3. Gametangia borne between opposed suspensors. Bar = 100 μm. 4. Sporangiophore with sporangia. Sporangiophore turns into blue color in cotton blue. Bar = 10 μm. 5. A mature zygosporangium and suspensors. Bar = 100 μm) 6. Dichotomously branched sporangiophores and sporangiospores. Bar = 100 μm. 7. Mycelia and zygosporangia (black spots) on PDA plate. 8. Formation of sporangiophores with sporangia and zygosporangia (black spots) on PD-broth. Bar = 1 cm. 9. Mature zygosporangia on PDA plate.

Syzygites megalocarpus Ehrenberg ex Fries. Syst. Myc.3: 329, 1832 이분지털곰팡이(신칭)

= *Sporodinia grandis* Link. 1824. Spec. Plant. 6(1):94. (see Hesseltine, 1957)

= *Sporodinia megalocarpus* (Fries) Lind. 1913. Danish Fungi, p.72.
(see Hesseltine, 1957)

= *Syzygites megalocarpus* Ehrenberg ex Fries. Lloydia 20(4):228, 1957

A. Field samples

The mycoparasite occurred on the edge of pileus at first, and spreaded fast to adjacent parts. Mycelia were white at first and changed into yellow tint to grey, which finally turned into dark grey (Fig. 1). Meanwhile, the inner part of mushroom started to show necrosis. Within 2 or 3 days of the infection, about 1/3~1/2 of the fruit-body showed necrosis (Fig. 2).

Sporangiophores dropped up to 5 cm, dichotomously branched, smooth-walled, and reached 30~55 μm in diameter. turned into blue colour in cotton blue. **Sporangium** was yellow, then changed into light to dark grey, visible in naked eyes. **Sporangiospores**, developed from the branched terminal of sporangiophores, globose, 15~18 μm in diameter, hyaline to light brown, not colour change at mature stage in cotton blue. Hyphae, gametangia and sporangiospores turned into blue by cotton blue at young stage (Figs. 3, 4). Sporangiospores germinated very fast at the room temperature.

Zygospores were formed between the opposed suspensors, typically Mucor-like, involving the formation of progametangia, then homogametangia, followed by their subsequent fusion. They were hyaline to white at first, but changed into reddish brown to dark brown when they mature, and formed clusters of zygospores with the shape of globose to subglobose. They were 160~230 μm × 190~270 μm ein verrucose wall which was up to 5 μm in thickness (Fig. 5).

B. Culture of the isolates

Colonies on PDA were fluffy, at growing to the lid of a petri-dish, completely filling a petri-dish within 2 to 3 days at 23~25°C. They were pale yellowish brown and showed aerial mycelia on the agar. **Sporangiophores** formed very dichotomous hyphae, which was yellow tint to yellowish brown and changed into grey to dark brown (Fig. 6). They often made an aerial hyphae with sporangiospores after 3 days. **Sporangiospores** showed hyaline to yellow tint or yellowish grey to brownish grey. **Zygospores** were formed occasionally between the opposed suspensors after 2 days on PDA and PDB at 25°C (Figs 7, 8). Mature zygospores were dark brown to black while that of field samples was reddish brown to dark brown (Fig. 9). The optimal temperature of the isolate was 22~25°C on PDA. No growth was observed at over 30°C. Growth of the isolate was optimal between pH 5 to pH 7; growth is restricted at below pH 5 and slows above pH 7.

Habitat: On living fruit-body of *T. matsutake* (Ito et Imai.) Singer.

Notes: *Syzygites megalocarpus* was given a large numbers of scientific names by many researchers (Hesseltine, 1957). Hesseltine (1957) proposed *Syzygites* as a generic name instead of his many different names based upon detailed account of the history of the genus and in accordance with the International Code of Botanical Nomenclature.

There are four facultative parasitic genera within the family of Dicranophoraceae; genus *Sporodiniella, Dicranophora, Spinellus* and *Syzygites* (Alexopoulos *et al.*, 1996). *Syzygites* was reported to be found easily almost anywhere on mushrooms (Jeffries and Young, 1994). However, we found a few mushrooms which has the mycoparate in Hongcheon experimental site, and it was not thought as a serious problem in Korea up to now.

In upper temperature limit on the growth of the fungus, we have obtained similar result to that of Hesseltine (1957) which had found no growth above 32°C. Ekpo and Young (1979) found the spinose ornamentation of sporangiospores with SEM. However, we did not show that of fungal spores at the light microscopy.

적 요

외생균근성 버섯인 송이는 동북아시아지역에서 가장 비싼 버섯이다. 이 버섯은 식용버섯이므로 자실체 표면에 병원균이 발생한다면 매우 심각한 문제가 아닐 수 없다. 송이 자실체의 기생균을 발견하였으므로 이에 대하여 자세히 설명하고 *Syzygites megalocarpus* Ehrenberg ex Fries 로 동정하고 이분지털곰팡이로 명명 하였음을 보고한다.

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